HIGH SCHOOL EDITION
Journal of Student Research
Volume 12 Issue 3 (2023)

CRISPR Based Gene Editing Technologies in Cancer Research and Detection via miRNA

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ABSTRACT

The CRISPR system of genetic editing has been a breakthrough in all fields of science and medicine, including that of cancer research, therapy, and diagnosis. In the last two years, research has narrowed down on CRISPRs ability to serve as a tool to detect oncogenes in patients to diagnose cancer in a quick and efficient manner. CRISPR and the three key enzymes (cas9, cas12, and cas13) are a revolutionary tool for medicine and cancer trials. MicroRNAs (miRNAs) are short noncoding RNA which regulate gene expression and gene functions, both crucial to cell functions. However, aberrant miRNA is expressed in all tumor proliferation diseases, including cancer. Studies have shown CRISPRs ability to detect cancerous levels of oncogenic MicroRNAs in human samples through a variety of methods such as CRISPR-Cas9/RNAi, CRISPR/Cas12 and RCA coupling, CRISPR/Cas13 exosome coupling, and Cas13a-Cas12a amplification. All of these methods provide extremely quick high sensitivity and high specificity detection of oncogenic miRNA in samples with concentrations below 1 fM. This paper is a synthesis of the current research on the methods mentioned above to aid in the further research of CRISPR/Cas systems in cancer diagnosis and cancer therapeutics.

Introduction

In recent decades, the technology commonly known as “CRISPR” has been on the forefront of disease therapy and detection. Within the past few years, the technology’s use in the field of cancer research has grown exponentially. The technology’s rise to prominence was a result of the invention of the CRISPR-Cas9 system. This system would be the first of many to have the ability of genome editing (Gostimskaya, 2022). CRISPR technology has many uses in cancer research, including in cancer detection, such as through the detection of miRNA indicators.

Background and Context

CRISPR, short for “Clustered Regularly Interspaced Short Palindromic Repeat ", was first identified and described in bacterial strains like E. Coli in 1987 by the Osaka University in Japan (Gostimskaya, 2022). This system was detected due to repetitive DNA fragments in bacterial cells when under attack by bacteriophages. Bacteriophages are viruses that evolved to target and spread among bacterial populations. Researchers at Osaka University would identify the CRISPR system as a natural defense exhibited by certain bacteria (Ishino et al. 2018). This system worked like memory cells of the human immune system, with it being able to store information on phages and produce an efficient defense response upon re-exposure.

CRISPR would not be further analyzed until 2007, in which advancements in technology made by the Human Genome Project (1990-2003) allowed researchers to take a deeper look at how the system worked in bacteria cells. Studies would show that CRISPR worked by the presence of Cas proteins, which had the ability to “cut” fragments of DNA as large as 20 base pairs in length (Barrangou et al.2007). This cut piece would be pasted to form CRISPR arrays

which then produced CRISPR RNAs. This CRISPR complex would then be transported to the viral DNA, which was injected into the cell by the phage, and would be cleaved, thus destroying the phage DNA and protecting the bacterial cell.

The ability of CRISPR to serve as a genetic scissor in bacteria cells would later be modified to be used on the human genome in a 2012 paper by Emmanuelle Charpentier and Jennifer Doudna through their discovery of the CRISPR/Cas9 system. Modifications to the CRISPR/Cas9 system in this study produced a CRISPR/Cas9 assay with the ability to delete a gene or to insert a different gene into the site of the previously deleted one (Dr. Pancholi, 2020). They were even able to modify the system in a way that deactivated CRISPR's DNA cleaving effect and instead allowed it to bind to a DNA sequence, deactivating or activating target genes (Dr. Pancholi, 2020). This system can modify one single nucleotide base or even large chunks of chromosomes. The modifications performed in this study would open the door to an array of cancer diagnostics, therapies, and research.

Nevertheless, there are relevant gaps in the literature regarding the use of CRISPR-Cas systems to diagnose and research cancer. In general, cancer research has gaps in literature because of issues pertaining to obtaining samples. It is difficult for researchers to obtain untreated cancer cells for studies because most cancer patients seek immediate treatment for such a lethal disease. To overcome this, some use lab grown cancer cells. However, although these cells provide a valuable resource for research, they are not a one-to-one indicator of how the technology may actually function in the human body.

Methodology

The methods utilized to perform this review included the expansive analysis of various publications and findings in the field of cancer and CRISPR research. The following electronic databases were searched, covering the time period 1980 to 2023: NCBI.nlm.nih.gov, addgene.org, NIH (genome.gov), NIH.gov, AACR.org, Oxford Academic (academic.oup.com), journals.sagepub.com., PLOS (journals.plos.org), Scitable (nature.com), PNAS.org, AR (ar.iiarjournals.org), MDPI.com, Nature.com, Frontiers (frontiersin.org), SpringerLink (link.springer.com), Cell.com, BMC.com (iologicalproceduresonline.biomedcentral.com), ACS publications (pubs.acs.org)

Selection criteria for this research can be dichotomized by specificity; general histories of cancer research were drawn upon using older articles (time range) whereas more specific research regarding CRISPR research and current and future uses were sourced from articles published in 2021 onwards.

Research articles were chosen based on the specificity and expanse of the literature review, structure of randomized and non-randomized trials, reputability of the source, ethical ramifications of the implementation of burgeoning medical practices, and research design. As this review is a synthesis of studies that use CRISPR to diagnose cancer through miRNA, This research does not evaluate articles that researched distinctly non-human samples, did not include application to miRNA, or centered on virus or phages without a discussion on Cancer diagnosis.

This review will accumulate current knowledge on regular cell development, proto-oncogene regulation, and the development of oncogenes and cancer cells as well as the ability for CRISPR to diagnose cancer through miRNA found in cells.

Review of Medical Literature

Cancer Development within the Body

Proper Cell Development: Rudolf Ludwig Carl Virchow's work in 1855 confirmed through his investigations on diseased individuals that only specific cells or areas of an organism become diseased, not the whole organism. With these findings, Virchow would observe that "every cell stems from another cell". From this idea, further research by Walther Flemming (1882) would confirm this through his discovery of the cell cycle.

The cell cycle consists of four stages, Growth Phase 1 (G1), Synthesis (S), Growth Phase 2 (G2), and Mitosis (M). The cell cycle is designed so that cells develop properly into full maturity and are able to replicate properly without issue. G1, S, and G2 all fall within the category of interphase, the phase in which the cell grows and prepares for cell division, known as mitosis. G1, also known as gap 1, is the first stage of interphase. This stage takes place starting from the start of the cell's life cycle up until the S phase. During G1, the cell grows in response to growth factors (Israels et al.2000). If the cell reaches the G1 checkpoint but has not reached adequate development, it is sent into G0, where it continues to grow. After G1, the cell enters the S phase. During the S phase, the DNA in the cell's nucleus is synthesized (replicated) and continues onto G2. G2, also referred to as gap 2, is another growth stage in which the cell continues to grow in response to growth factors, as well as checking for any mutations in the synthesized DNA (Israels et al.2000). If a cell fails to meet the requirements of the G2 checkpoint (such as unmutated DNA), cellular signals will trigger apoptosis, or cell death. The M phase of the cell cycle creates two identical daughter cells. The M phase is where cell division takes place, and thus the cell cycle repeats.

The cell cycle is catalyzed by complexes formed of cyclins and cyclin dependent kinases (CDKs). These complexes are activated at 7 unique points during the entire cell cycle. CDKs can be activated and inhibited, depending on the phosphorylation of threonine and tyrosine (Schafer, 1998). Threonine is an essential amino acid, meaning it can only be obtained from food, and is responsible for the regulation of protein balance as well as the biosynthesis of other proteins. Tyrosine is a nonessential amino acid, as it is produced by the body, and is utilized for protein synthesis. Phosphorylation of threonine 14 and of tyrosine 15 by wee1, mik1, and myt1 protein kinases inhibit CDK activity, resulting in the cell not being able to move past one of the 7 CDK-cyclin checkpoints. However, dephosphorylation of those same amino acids results in the cell moving past those checkpoints (Schafer, 1998). Cyclins have a direct impact on this function as well, as they manage the phosphorylation and dephosphorylation of CDKs. The forming of a CDK-cyclin complex is what activates CDKs. The binding of CDKs and cyclins is what allows CDKs into the nucleus because cyclins contain the proper nuclear localization signals required (Schafer, 1998). The cell enters, passes the checkpoints, or stops at one, depending on the synthesis and degradation of specific cyclins targeted by different CDKs.

Protein synthesis is regulated by a different protein, known as p53. P53 is a key transcription factor, regulating the rate of genetic information being transcribed into messenger RNAs. P53 is more present during and after the S phase of the cell cycle. This protein monitors the state of the cell's DNA and genome, ensuring no mutations are present in the replication of DNA (Israels et al.2000). In the case of abnormalities in DNA, p53 interrupts the cell cycle to repair and remove abnormalities. In the case that mutations are unrepairable, p53 triggers apoptosis. P53 is known to be a tumor suppressor with a negative feedback loop relationship with the MDM-2 protein, meaning that as p53 concentration increases, it results in the production of MDM-2, which inhibits p53. (Amaral, 2010).

Proto-Oncogenes and Oncogenes: Proto-Oncogenes are a key player in the growth and formation of cells. Proto-Oncogenes are responsible for encoding growth factors, transcription factors, tyrosine kinases, and threonine kinases to regulate and trigger cell growth during interphase (Anderson et al. 1992). Proto-Oncogenes are genes that also inhibit the process of cell differentiation, as well as inhibit signals that trigger apoptosis (Dr. Chial, 2008). Cell differentiation is the process by which cells change to acquire a specific role in the body, building up a certain tissue type when working with other cells specialized in the same function (Wu, 2011). This process of cell differentiation is regulated by enzymes which modify histones via methylation and acetylation. Research has also shown that extracellular factors such as morphogens and environmental cues impact how cells differentiate (Brun-Usan et al. 2020). Proto-Oncogenes inhibit this process during embryogenesis to ensure proper tissue and organ development, without excess growth known as tumors. Proto-Oncogenes inhibit apoptosis to avoid the unnecessary termination of cells, whether it be an accidental signal, or to stall a cell that could be repaired. The issue arises with proto-oncogenes once mutated into oncogenes, as this makes all its previous regulatory actions occur without the cell passing cellular checkpoints.

Oncogenes are mutated proto-oncogenes with a mutation to their proteins that influences their control of cell growth and cellular division. Oncogenes deregulate both processes, allowing excessive cell division and proliferation (Kontomanolis et al. 2020). When oncogenes are present instead of proto-oncogenes, cells ignore cellular checkpoints within the typical cell cycle. This means that the cell either continues past G1 without fully maturing or moves past the G2 checkpoint with damaged or mutated DNA from the S phase, producing cancerous cells during mitosis. These cancerous cells then continue to divide uncontrollably and bypass apoptosis signals because of the mutated oncogenes. Proto-Oncogenes are responsible for signals that inhibit apoptosis; thus, oncogenes are constantly inhibiting apoptosis, even in mutated cells. Oncogenes can develop because of a variety of mutations in proto-oncogenes such as point mutations and chromosomal translocation mutations. In the case of a proto-oncogene to oncogene mutation, this is a "gain-of-function" mutation, as instead of for example inhibiting the function of tumor suppressors, it instead stimulates excessive cell proliferation (Lee et al. 2010). A point mutation, also referred to as a missense mutation, is a mutation where a singular base pair in a sequence is substituted for another, producing a different amino acid after protein synthesis. An example of a point mutation is the mutation of p53 in breast cancer patients. Greater than 75% of mutations leading to oncogenic p53 are point mutations (Kato et al.2003). Once the gene for p53, known as TP53, mutates and creates mutated p53, it gains the function of suppressing the binding of Mre11 (a gene responsible for coding proteins that manage chromosome structural components and DNA break repairs) (Lee et al. 2010). This is shown by the increased concentration of mutant p53 in tumors. An example of a chromosomal translocation mutation occurs when a piece of one chromosome splits off and attaches to a different chromosome.

An example of a chromosomal translocation mutation which produces an oncogene is a mutation in the MYC gene family (Kontomanolis et al. 2020). The key three MYC genes are N-MYC, L-MYC, and C-MYC. Certain DNA-binding proteins which originate from the MYC gene play a role in regulating parts of the cell cycle. The MYC gene is also responsible for cell proliferation, transformation, and in avoiding differentiation and apoptosis. Oncogenic MYC genes occur from C-MYC deletions during nuclear translocation, which also play a role in the negative regulation of the APC gene (APC regulates the mitotic spindles to properly segregate chromosomes during mitosis) (Marandel et al. 2004). This "knockdown" of the APC gene by the oncogenic C-MYC gene affects p53 activation and reading frame checkpoints (Kontomanolis et al. 2020). The oncogenic C-MYC gene also suppresses microRNAs, which are a key indicator and player in cancer diagnosis with CRISPR.

CRISPR use in Cancer Research

How CRISPR functions regarding cancer research: CRISPR technology is extensively tested and researched for its ability to detect these oncogenes in experimental samples of cancer cells and precancerous cells. The primary CRISPR system used as a base for nearly all experiments regarding CRISPR is the original CRISPR/Cas9 system discovered by Emmanuelle Charpentier and Jennifer Doudna. Developments and further research on CRISPR has allowed researchers to utilize other systems for cancer research, notably, the CRISPR/Cas12 and CRISPR/Cas13 systems.

CRISPR/Cas9 was the first CRISPR system utilized for genome editing, requiring two RNAs to identify and cut its target (See Figure 1). CRISPR/Cas9 is used through a process known as "knockout". Knockout is the term used in genome editing and testing to refer to using gene editing tools to deactivate or remove a gene or genes (Dr. Morris, 2023). Knockout is key to determining the effect a gene has by observing what occurs in its absence. CRISPR/Cas9 can produce large libraries of knockout samples for researchers to study loss-of-function mutations. One large library of knockout genes was produced by Ophir Shalem and his team. This library is referred to as the Genome-wide CRISPR/Cas9 knockout library (GecKO) and contains 18,080 targeted human genes (Shalem et al. 2014). Experiments utilized the GecKO library in combination with a similar library containing rat genes were successfully able to identify oncogenes that result in tumor evolution, including 5 protein-coding genes and 2 miRNAs (Chen et al. 2015). In these studies, CRISPR/Cas9 arrays were used to knockout oncogenes and tumor suppressor genes to identify mutated proteins and miRNAs that result in tumor development. Another study performed on human cells using pgRNAs

for deletion of long non-coding RNA (lncRNA) segments identified about 50 lncRNAs that contribute to tumor growth. CRISPR/Cas9 is also used as the basis for the CAPTURE system, which studies show is able to isolate chromosome interactions at a single-copy genomic locus for analysis in laboratories to detect abnormalities (Zhang et al. 2021).

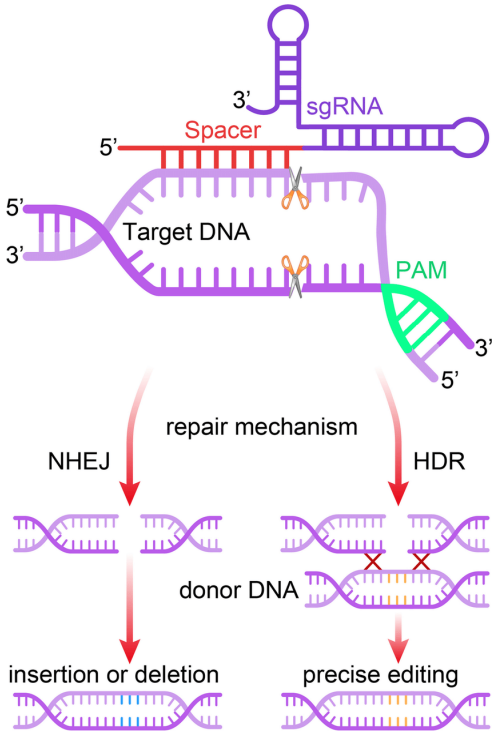

Figure 1. *Mechanism of the CRISPR/Cas9 gene editing system* (Zhang et al. 2021)

CRISPR/Cas12 was discovered in 2015, 3 years after CRISPR/Cas9. The CRISPR/Cas12 system has improvements such as processing its own guide RNA and only needing CRISPR RNA (crRNA) for targeting. CRISPR/Cas12 utilizes its own crRNA to guide it to the target site where it binds and cleaves the target sequence. The Cas12-crRNA complex used to guide the CRISPR sequence can identify both single strand DNA as well as double strand DNA, making it efficient in DNA and RNA detection and cleavage (Xu et al. 2022). Cas12 assays are utilized in cancer research for their ability to detect methylation and nucleotide base changes. Methylation is the process in which methyl groups are added to DNA molecules to change their activity without affecting the sequence itself. In a recent study performed by Wenfei Xu and his team showed the Cas12 system's ability to detect the methylated oncogene mSEPT9. Within this study, the team was able to detect the oncogenic mSEPT9 in concentrations as low as 0.01% with a 100% sensitivity and a 92.3% specificity (Xu et al. 2022). Other research with the CRISPR/Cas12 system is utilizing it through the CRISPR/Cas12 based DETECTR system, a DNA reporter (See Figure 2). The DETECTR system has effectively been used and sold for its ability to detect single nucleotide changes in cells, proving effective in the detection of virus DNA in cells as well as mutations in cancer cells (Vangah et al. 2020).

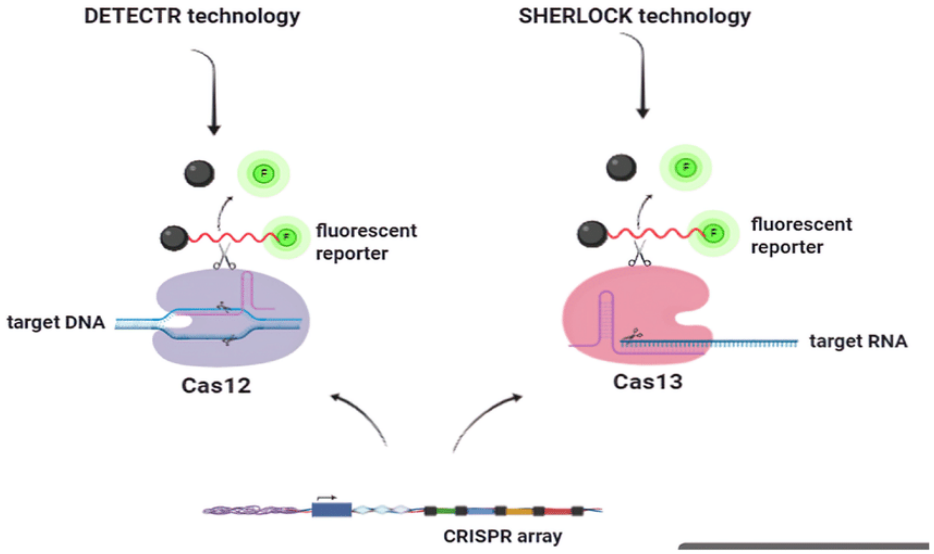

Figure 2. *Cas12 and Cas13 Cleavage Activity* (Vangah et al. 2020)

CRISPR/Cas13 was also discovered in 2015, the same year as Cas12. The CRISPR/Cas13 system is an RNA based system, being able to target RNA viruses directly. CRISPR/Cas13 works similarly to CRISPR/Cas12 (See Figure 2). CRISPR/Cas13 has the added advantage of not causing permanent genome edits, making it a valuable tool for in vivo experiments. Cas13 assays are used to study the effect of noncoding RNAs on tumor progression and cancer development. Research using CRISPR/Cas13 has identified 64% of 22 very long intergenic non-coding RNA (vlincRNA) are responsible for avoiding apoptosis and enhancing cell survival (Xu et al. 2020). CRISPR/Cas13 has also been used in conjunction with the DETECTR system, as well as the SHERLOCK system, a system similar to DETECTR that uses amplified RNA, to detect nucleotide changes (Katti et al. 2022). Both CRISPR/Cas12 and CRISPR/Cas13 are being used to detect miRNA markers in cancerous cells, a field with very promising results for cancer diagnostics.

How CRISPR can detect miRNA and diagnose cancer

Detecting cancer through MiRNA: MicroRNAs, typically called miRNA, are small cellular RNA fragments which negatively regulate gene expression. MiRNA negatively regulates gene expression by destroying messenger RNA that would have produced proteins needed for the coding or expression of that gene. MiRNA has been a recently studied indicator of cancer, as it is already an indicator of many other human diseases. These diseases such as cancer can be detected because of a change in the expression of miRNA or a change in their copy number (Betel et al., 2008). Diseases can also be detected via miRNA through detecting mutations in the miRNA itself or in its target site on the messenger RNA. Studies continue to show CRISPRs ability to detect these miRNAs in cancer and precancerous cells. To study and detect these miRNAs the system used are typically those of CRISPR/Cas13, CRISPR/Cas12, or a combination of both. CRISPR/Cas9 even has been effectively used for the detection of miRNA. Multiple studies utilizing these CRISPR systems have successfully identified a variety of miRNA in samples that lead to tumor growth and cell proliferation. These detections take advantage of Cas13's trans-cleavage activity and Cas12's cleavage of ssDNA.

CRISPR/Cas12 assays solve issues formerly present in research on the detection of miRNA for cancer diagnosis. These issues are primarily the lack of accurate, affordable, and quick assays for detection. Cas12a assays have solutions for these issues. Cas12's cleavage activity allows for exponential rolling-circle amplification of targeted sequences and its trans-cleavage activity for detection (Yan et al. 2023). CRISPR/Cas12 detection of the miRNA is visible through a fluorescent reporter system. Originally, CRISPR/Cas12 detection systems took upwards of 5 hours,

but a new method known as a padlock probe was developed to reduce the time of detection by about 4 hours (Niu et al. 2023). The padlock probe was developed with the ability to hybridize with helper DNA and be ligated by T4 DNA ligase. This probe was used after EXO I hydrolyzes DNA and after EXO III hydrolyzes nonspecific complexes (Yu-Li et al. 2017). After those processes, Cas12 cleavage activity is used for a much quicker detection of about 70 minutes (Niu et al. 2023). Cas12 can cleave miRNA for detection by the coupling of rolling circle amplification (RCA) with Cas12, known as an rRCA-Cas complex. In the research performed by Niu and his team, miRNA hybridized to the precirculization probe and was extended by phi29 DNA polymerase to trigger the RCA process. This generated an ssDNA sequence containing multiple triggers for hybridization to the Cas12-crRNA complex. After hybridization, cleavage of the ssDNA occurred and released the quencher thus increasing the fluorescent signaling (See Figure 3) (Niu et al. 2023). To detect miRNA, traces (10 nM) of miRNA-21 are added alongside the phi29 DNA polymerase. A secondary examination was performed to detect whether the fluorescent signal appeared due to the presence of miRNA-21, thus checking the method's efficiency. In this secondary examination, the previous steps were followed the same except one group contained trace amounts of miRNA-21 and the other group did not. In the group containing miRNA-21, the same result occurred as before. On the other hand, the group not containing any amount of miRNA-21, no fluorescent signal was obtained because the processes of RCA could not occur (Niu et al. 2023). Further examination also identified that as the concentration of Cas12-crRNA complex increases, so does the detection rate. Furthermore, this entire process performs best at 37 degrees Celsius, the human body temperature, showing its efficiency in detection of miRNA-21 in human samples (Niu et al. 2023).

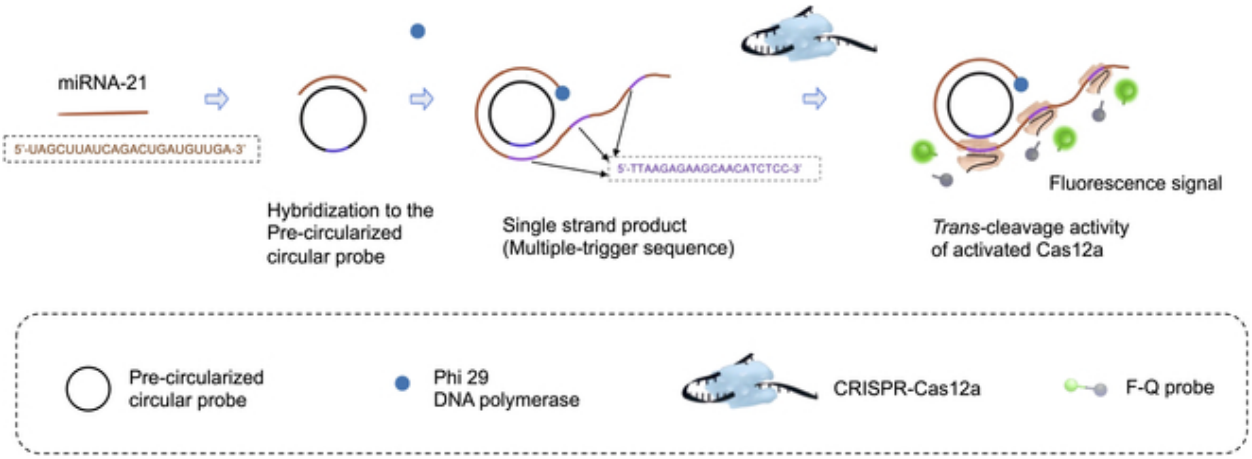

Figure 3. *Schematic of the rRCA–Cas reaction* (Niu et al. 2023)

Another study performed by He Yan et al. utilized CRISPR/Cas12 assays to detect traces of miRNA-21, miRNA- 196a, miRNA-1246, and miRNA-451a. This study used an isothermal CRISPR/Cas12a assay named "Endonucleolytically Exponentiated Rolling Circle Amplification with CRISPR–Cas12a" (EXTRA-CRISPR). This method works past some of the shortcomings of the RCA method, namely reducing the manual operations and shortening the time even more, from 70 minutes down to 20 (Yan et al. 2023). Research using the EXTRA-CRISPR system was also first conducted using miRNA-21. This system is a tri-enzyme system consisting of the cas12a enzyme, RCA, RNP, and a protospacer-adjacent motif (PAM) (See Figure 4). The study consisted of two other control groups, each missing a component of the tri-enzyme system, and each group showed no fluorescence signal. The study examined the successful detection of miRNA-21 in various concentrations by measuring the cleavage activity of Cas12a. The results maintained the general conclusion as the study performed by Niu and his team; RCA is an effective technique used alongside CRISPR to detect miRNA (Yan et al. 2023). Coupling CRISPR/Cas12's trans-cleavage and cis-cleavage activity with RCA was concluded to be an effective method of biosensing for multiple miRNA biomarker, not just the miRNA primarily documented in the study (Yan et al. 2023).

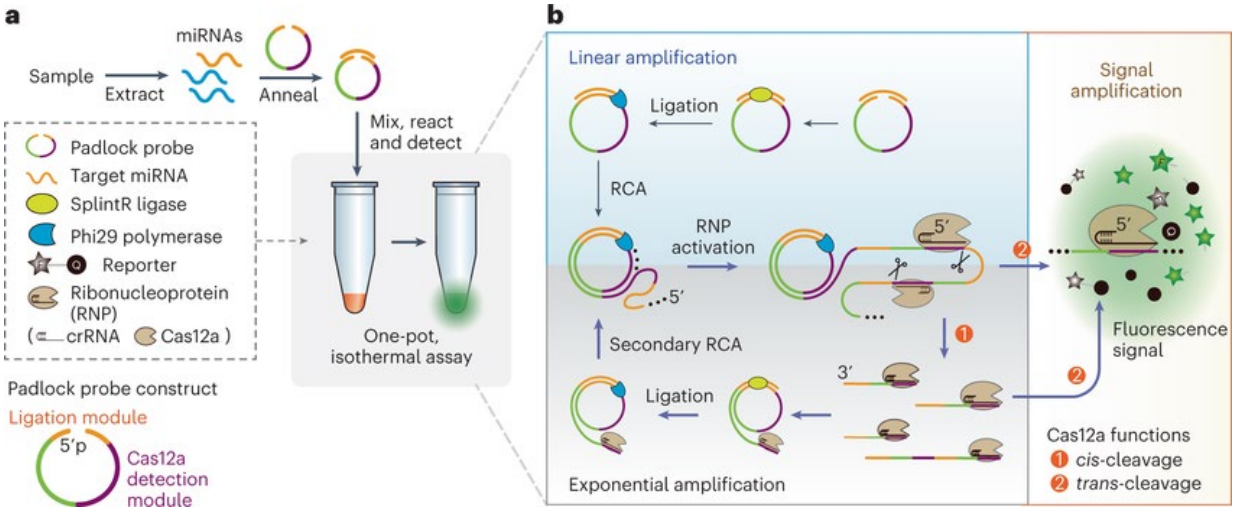

Figure 4. *The major components and workflow of EXTRA-CRISPR assay* (Yan et al. 2023)

CRISPR/Cas13 is studied to detect the tumor proliferator miRNA-21 through the use of exosomes. Exosomes are extracellular vesicles which carry proteins, DNA, and microRNAs. MiRNA-21 is known to be carried in samples with signs of cancerous or precancerous cells. Furthermore, the miRNA within exosomes is highly stable, providing credence to the idea that this is a promising noninvasive technique for early detection of cancer (Zhang et al. 2023). Zheng and his team used a liposome-mediated membrane fusion strategy (MFS) to insert the CRISPR/Cas13a strand into the exosome, creating an MFS-CRISPR complex (See Figure 5). This complex can detect miRNA concentration in plasma. Cas13a will have a guided trans-cleavage of fluorophores and labeled reporters, creating a fluorescent signal after “target-RNA-triggered" RNase activity (Zhang et al.2023). The exosome containing CRISPR/Cas13a is designed to recognize and bind to the targeted miRNA, in this study miRNA-21. This study concluded that the MFS-CRISPR complex was successfully able to discriminate between healthy samples and breast cancer samples containing the oncogenic miRNA-21 in concentrations as low as 1.2 × 103 particles/mL) in less than an hour (Zhang et al. 2023).

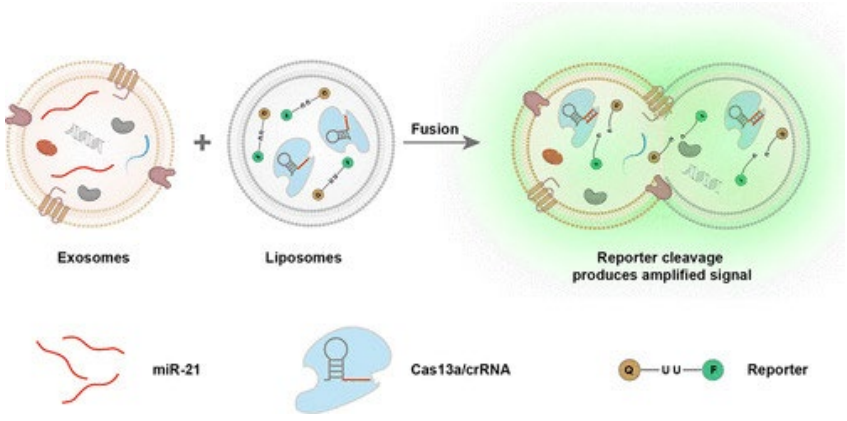

Figure 5. *Exosome CRISPR Complex* (Zhang et al. 2023)

Studies have shown that using both CRISPR/Cas12 and CRISPR/Cas13 assays in conjunction has better detection results than each one has individually. A study testing a CRISPR/Cas13a-triggered Cas 12 (Cas13a-12a amplification) showed that this system was able to detect the presence of miRNA-155, a miRNA found in abundance in multiple cancers such as breast cancer and leukemia, in concentrations as low as 0.35 fM (Zhao et al. 2023). When the Cas13a enzyme is in the presence of the target miRNA-155, trans-cleavage activity is activated to cleave the blocker strand and release a primer strand that activates the cas12a cleavage of fluorophore targeted ssDNA (Zhao et al. 2023). The final cleavage by the cas12a enzyme amplifies the fluorescent signal for detection (See Figure 6). This method takes advantage of the nucleic acid recognition of both enzymes, providing ultra-sensitive and highly specific detection for miRNAs in cancer and precancerous cells (Zhao et al. 2023).

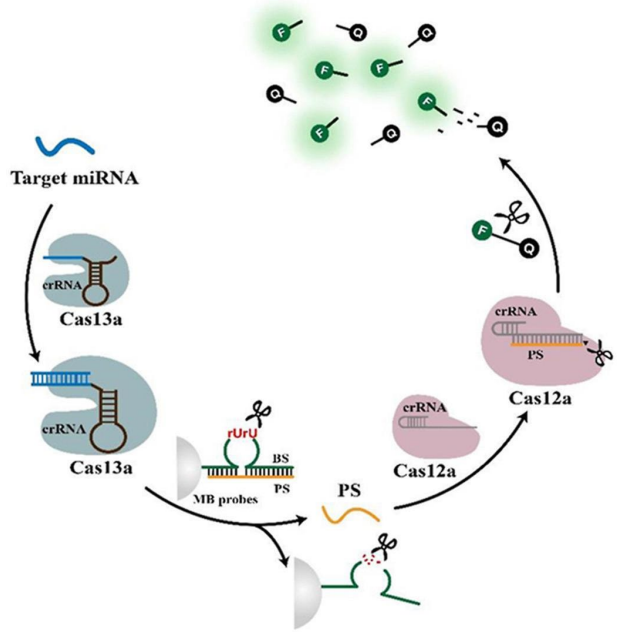

Figure 6. *Cas13a-Cas12a Amplification System* (Zhao et al. 2023)

Detection of cancerous miRNA indicators is also accurately performed by the CRISPR/Cas9 system. A study examined 73 miRNAs that could detect lung cancer and determined that 4 were oncogenic via CRISPR-CAs9/RNA interference assays (RNAi) (Ye et al. 2023). This method of miRNA detection takes a sample of isolated possible oncogenic miRNAs within the patient's samples and selects specific tumor proliferation miRNAs after a CRISPR-CAs9/RNAi assay (See Figure 7). Once the proliferation miRNAs are detected, scientists can perform studies on the genes which encode those miRNAs, known as miRs.

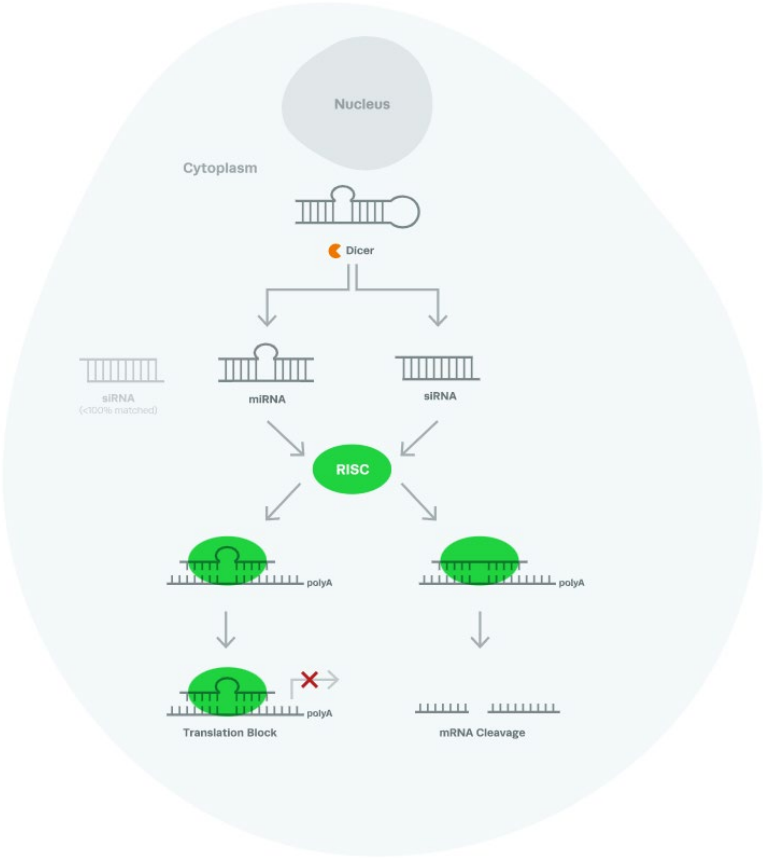

Figure 7. *Schematic of the RNAi mechanism* (Prabhune, 2023)

The method performed in this study yielded a 96.3% in the training patient cohort samples and a 92.3% accuracy in supervised classification in the validation set (Ye et al.2023). Though this method requires more steps and a longer time than the cas13 and cas12 based methods, it is still a viable method for miRNA detection, albeit inferior to the previously mentioned.

Discussion

As shown by the current research, CRISPR/Cas systems have shown a huge use in the field of medicine and cancer research in specific. CRISPR/Cas systems are studied to show a high specificity and high sensitivity detection for numerous oncogenes, such as miRNA oncogenes. Multiple studies show a success rate of higher than 90% for the detection of specific oncogenic miRNA in human samples. There have been further studies to increase the system's attributes as well as produce cheap and quick tests, all being less than an hour long to perform.

Conclusion

The development of CRISPR technologies in medicine has been extraordinary in recent years. Research on the CRISPR/Cas systems show the gene editing technology's capability for detecting diseases such as cancer. The three CRISPR/Cas enzymes presented in the current research show a high specificity and high sensitivity with quick detection rate of oncogenic miRNA. These systems have proven to be effective in detecting cancer at the precancerous

stage with success rates of more than 90% when targeting miRNAs. Continued progress in research of existing CRISPR/Cas systems can continue to shorten the detection time as well as increase the success rate of detection of cancer, as the current trend in research shows. Continuous efforts and studies to fully understand and utilize the technologies delivery and editing systems will ensure the CRISPR system's full potential will be used to benefit patients and society.

Future Research

The development of CRISPR technologies in medicine has been extraordinary in recent years. Research on the CRISPR/Cas systems show the gene editing technology's capability for detecting diseases such as cancer. The three CRISPR/Cas enzymes presented in the current research show a high specificity and high sensitivity with quick detection rate of oncogenic miRNA. These systems have proven to be effective in detecting cancer at the precancerous stage with success rates of more than 90% when targeting miRNAs. Continued progress in research of existing CRISPR/Cas systems can continue to shorten the detection time as well as increase the success rate of detection of cancer, as the current trend in research shows. Continuous efforts and studies to fully understand and utilize the technologies delivery and editing systems will ensure the CRISPR system's full potential will be used to benefit patients and society.

Limitations

It is important to address limitations that arose in the research process. The largest limitation faced was the limited time frame. The limited time frame in the sense that IRB approval could not have been given in time to include primary source data. A limited time frame in terms of the research is also a limitation, as studies on CRISPR in cancer diagnosis in general, not just miRNA detection, have only been performed in the past two years. That being said, further research is definitely on the way, with over one billion dollars being raised in venture capital funding in 2021 for gene editing (*Over $1bn raised in venture capital funding for gene editing in 2021,* 2022) and another $89 million being raised by the NIH for gene editing technologies (*NIH Awards $89 Million for Additional Projects to Advance Genome Editing,* 2022). Another limitation to acknowledge as a high school student is the limited access to full databases due to paywalls and Dual Enrollment colleges not having a valid institutional login.

Acknowledgement

I would like to thank all those researchers who provided the sources and valuable studies to create this review. I would like to acknowledge my English Language Arts 3 teacher Ms. Laura Prince, who guided me throughout the entire investigation and writing process from the beginning of the idea to the end product. Special thanks to my PLTW and science teacher Ms. Barbara Encalada, who truly sparked my interest in medicine, cancer, and research through her interest in medicine and teaching, as well by being someone to provide support and advice. Thank you to all educators in PPMH who have paved the road ahead to mold and inspired my goals. Lastly, special thanks to my family for always supporting me throughout everything.

References

Anderson, M. W., Reynolds, S. H., You, M., & Maronpot, R. M. (1992). Role of proto-oncogene activation in carcinogenesis. *Environmental Health Perspectives*, *98*, 13–24. https://ehp.niehs.nih.gov/doi/10.1289/ehp.929813

Barrangou, R., Fremaux, C., Deveau, H., Richards, M., Boyaval, P., Moineau, S., Romero, D. A., & Horvath, P. (2007). CRISPR provides acquired resistance against viruses in prokaryotes. *Science (New York, N.Y.)*, *315*(5819), 1709–1712. https://doi.org/10.1126/science.1138140

Betel, D., Wilson, M., Gabow, A., Marks, D. S., & Sander, C. (2008). The microRNA.org resource: targets and expression. *Nucleic Acids Research*, *36*(Database issue), D149-153. https://doi.org/10.1093/nar/gkm995

Brun-Usan, M., Thies, C., & Watson, R. A. (2020). How to fit in: The learning principles of cell differentiation. *PLOS Computational Biology*, *16*(4), e1006811. https://doi.org/10.1371/journal.pcbi.1006811

Chen, S., Sanjana, N., Zheng, K., Shalem, O., Lee, K., Shi, X., Scott, D., Song, J., Pan, J., Weissleder, R., Lee, H., Zhang, F., & Sharp, P. (2015, March 15). *Genome-wide CRISPR Screen in a Mouse Model of Tumor Growth and Metastasis*. Cell. https://www.cell.com/cell/fulltext/S0092-8674(15)00204-4?_returnURL=https%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS0092867415002044%3Fshowall%3Dtrue

Chial, H. (2008). *Proto-oncogenes to Oncogenes to Cancer | Learn Science at Scitable*. Www.nature.com. https://www.nature.com/scitable/topicpage/Proto-Oncongenes-to-Oncogenes-to-Cancer-883/

GlobalData Healthcare & GlobalData Healthcare. (2022, January 27). Over $1bn raised in venture capital funding for gene editing in 2021. *Pharmaceutical Technology*. https://www.pharmaceutical-technology.com/comment/venture-capital-gene-editing-funding/

Gostimskaya, I. (2022). CRISPR–Cas9: A History of Its Discovery and Ethical Considerations of Its Use in Genome Editing. *Biochemistry (Moscow)*, *87*(8), 777–788. https://doi.org/10.1134/s0006297922080090

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), e00580-17. https://doi.org/10.1128/JB.00580-17

Israels, E. D., & Israels, L. G. (2000). The Cell Cycle. *The Oncologist*, *5*(6), 510–513. https://doi.org/10.1634/theoncologist.5-6-510

Jolany vangah, S., Katalani, C., Booneh, H. A., Hajizade, A., Sijercic, A., & Ahmadian, G. (2020). CRISPR-Based Diagnosis of Infectious and Noninfectious Diseases. *Biological Procedures Online*, *22*(1). https://doi.org/10.1186/s12575-020-00135-3

Kato, S., Han, S.-Y., Liu, W., Otsuka, K., Shibata, H., Kanamaru, R., & Ishioka, C. (2003). Understanding the function–structure and function–mutation relationships of p53 tumor suppressor protein by high-resolution missense mutation analysis. *Proceedings of the National Academy of Sciences*, *100*(14), 8424–8429. https://doi.org/10.1073/pnas.1431692100

Katti, A., Diaz, B. J., Caragine, C. M., Sanjana, N. E., & Dow, L. E. (2022). CRISPR in cancer biology and therapy. *Nature Reviews Cancer*, *22*. https://doi.org/10.1038/s41568-022-00441-w

Kontomanolis, E. N., Koutras, A., Syllaios, A., Schizas, D., Mastoraki, A., Garmpis, N., Diakosavvas, M., Angelou, K., Tsatsaris, G., Pagkalos, A., Ntounis, T., & Fasoulakis, Z. (2020). Role of Oncogenes and Tumor-suppressor Genes in Carcinogenesis: A Review. *Anticancer Research*, *40*(11), 6009–6015. https://doi.org/10.21873/anticanres.14622

Lee, E. Y. H. P., & Muller, W. J. (2010). Oncogenes and Tumor Suppressor Genes. *Cold Spring Harbor Perspectives in Biology*, *2*(10), a003236–a003236. https://doi.org/10.1101/cshperspect.a003236

Li, X.-Y., Du, Y.-C., Zhang, Y.-P., & Kong, D.-M. (2017). Dual functional Phi29 DNA polymerase-triggered exponential rolling circle amplification for sequence-specific detection of target DNA embedded in long-stranded genomic DNA. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-06594-1

Morris, S. (n.d.). *Knockout*. Genome.gov. https://www.genome.gov/genetics-glossary/Knockout#:~:text=A%20knockout%2C%20as%20related%20to

NIH Awards $89 Million for Additional Projects. (2022, March 22). National Center for Advancing Translational Sciences. https://ncats.nih.gov/news/releases/2019/somatic-cells

Niu, C., Liu, J., Xing, X., & Zhang, C. (2023). Exploring the Trans-Cleavage Activity with Rolling Circle Amplification for Fast Detection of miRNA. *Biodesign Research*, *5*. https://doi.org/10.34133/bdr.0010

Pancholi, N. J. (2020, October 22). *What is CRISPR-Cas9 and How is it Used in Cancer Research?* American Association for Cancer Research (AACR). https://www.aacr.org/blog/2020/10/22/what-is-crispr-cas9-and-how-is-it-used-in-cancer-research/

Schafer, K. A. (1998). The Cell Cycle: A Review. *Veterinary Pathology*, *35*(6), 461–478. https://doi.org/10.1177/030098589803500601

Shalem, O., Sanjana, N., Hartenian, E., Shi, X., Scott, D., Mikkelsen, T., Heckl, D., Ebert, B., Root, D., Doench, J., & Zhang, F. (2013). LentiCRISPRv2 and lentiGuide-Puro: lentiviral CRISPR/Cas9 and single guide RNA. *Science*, *343*(6166). https://doi.org/10.1126/science.1247005

Wu, J. (2011). Regulating cell differentiation at different layers. *Journal of Molecular Cell Biology*, *3*(6), 319–319. https://doi.org/10.1093/jmcb/mjr036

Xu, D., Cai, Y., Tang, L., Han, X., Gao, F., Cao, H., Qi, F., & Kapranov, P. (2020). A CRISPR/Cas13-based approach demonstrates biological relevance of vlinc class of long non-coding RNAs in anticancer drug response. *Scientific Reports*, *10*(1). https://doi.org/10.1038/s41598-020-58104-5

Xu, W., Peng, J., Guo, C., Chai, Y., Zhou, H., Wang, J., & Li, X. (2023). Rapid and ultra-sensitive early detection of cervical cancer using CRISPR/Cas12-based assay based on methylated SEPT9. *Sensors and Actuators B: Chemical*, *379*, 133231. https://doi.org/10.1016/j.snb.2022.133231

Yan, H., Wen, Y., Tian, Z., Hart, N., Han, S., Hughes, S. J., & Zeng, Y. (2023). A one-pot isothermal Cas12-based assay for the sensitive detection of microRNAs. *Nature Biomedical Engineering*, 1–19. https://doi.org/10.1038/s41551-023-01033-1

Ye, Q., Raese, R., Luo, D., Cao, S., Wan, Y.-W., Qian, Y., & Guo, N. L. (2023). MicroRNA, mRNA, and Proteomics Biomarkers and Therapeutic Targets for Improving Lung Cancer Treatment Outcomes. *Cancers*, *15*(8), 2294. https://doi.org/10.3390/cancers15082294

Zhang, H., Qin, C., An, C., Zheng, X., Wen, S., Chen, W., Liu, X., Lv, Z., Yang, P., Xu, W., Gao, W., & Wu, Y. (2021). Application of the CRISPR/Cas9-based gene editing technique in basic research, diagnosis, and therapy of cancer. *Molecular Cancer*, *20*(1), 126. https://doi.org/10.1186/s12943-021-01431-6

Zhang, J., Guan, M., Ma, C., Liu, Y., Lv, M., Zhang, Z., Gao, H., & Zhang, K. (2023). Highly Effective Detection of Exosomal miRNAs in Plasma Using Liposome-Mediated Transfection CRISPR/Cas13a. *ACS Sensors*. https://doi.org/10.1021/acssensors.2c01683

Zhao, D., Tang, J., Tan, Q., Xie, X., Zhao, X., & Xing, D. (2023). CRISPR/Cas13a-triggered Cas12a biosensing method for ultrasensitive and specific miRNA detection. *Talanta*, *260*, 124582. https://doi.org/10.1016/j.talanta.2023.124582