REVIEW ARTICLE

Small Interfering RNA (siRNA) and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR): Emerging Molecular Tools for Genetic Manipulation

Simon I. Okekpa[1,2], Rabiatul Basria S.M.N. Mydin[1,3], Munirah Mohd Nor[4], Emmanuel Jairaj Moses[5]

[1] Oncological and Radiological Sciences Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Bertam, Kepala Batas, Pulau Pinang Malaysia.
[2] Department of Medical Laboratory Science, Faculty of Health Sciences, Ebonyi State University, Abakaliki, 840001 Ebonyi state, Nigeria.
[3] Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, 117543 Singapore
[4] Kolej Matrikulasi, Jalan Pongsu Seribu, 13220 Kepala Batas Pulau Pinang, Malaysia
[5] Regenerative Medicine Cluster, Advanced Medical and Dental Institute, Universiti Sains Malaysia, 13200 Bertam, Kepala Batas, Pulau Pinang, Malaysia

ABSTRACT

Gene manipulation tools have transformed biomedical research and improved the possibilities of their uses for therapeutic purposes. These tools have aided effective genomic modification in many organisms and have been successfully applied in biomedical engineering, biotechnology and biomedicine. They also shown a potential for therapeutic applications to alleviate genetic and non-genetic diseases. Small interfering RNA (siRNA) and clustered regularly inter-spaced short-palindromic repeat/associated-protein system (CRISPR/Cas) are two of the tools applied in genetic manipulation. This review aims to evaluate the molecular influence of siRNA and CRISPR/Cas as novel tools for genetic manipulations. This review discusses the molecular mechanism of siRNA and CRISPR/Cas, and the advantages and disadvantages of siRNA and CRISPR/Cas. This review also presents comparison between siRNA and CRISPR/Cas as potential tools for gene therapy. siRNA therapeutic applications occur through protein knockout without causing damage to cells. siRNA knocks down gene expression at the mRNA level, whereas CRISPR/Cas knocks out gene permanently at the DNA level. Inconclusion, gene manipulation tools have potential for applications that improve therapeutic strategies and plant-derived products, but ethical standards must be established before the clinical application of gene editing.

Keywords: Genetic manipulation tools, Small interfering RNA, Clustered regularly inter-spaced short-palindromic repeat/associated-protein system, Gene editing, Gene therapy.

Corresponding Author:
Rabiatul Basria S.M.N. Mydin, PhD
Email: rabiatulbasria@usm.my
Tel: +604-5622351

INTRODUCTION

Gene manipulation is a scientific technology used to modify an organism's characteristics by manipulating genetic materials. Current available tools for gene manipulation are classified into; gene editing technology, gene targeting technique and random gene combination technique. Gene editing technology is performed using artificial nucleases/ribozymes and RNA interference (RNAi) such as small interfering RNA (siRNA) to manipulate genetic materials. In gene targeting techniques, clustered regularly inter-spaced short-palindromic repeat (CRISPR) /CRISPR associated-protein system (CRISPR/Cas) and transcription activator-like effector nucleases (TALENs) are applied as tools to manipulate genetic materials. Random gene combination is carried out via restriction enzyme mediated-integration, Agrobacterium-mediated transformation, and transposon-arrayed gene knockout (1). Gene editing must adhere to the following accepted standards: pre-clinical standard evidence must be established, gene modification must be accurate, practitioners competency must be assessed, standard professional behaviour must be enforced, and ethical standards must be established to protect the welfare of research subjects (2). This review focuses on two of the most current techniques, namely, "siRNA and CRISPR/Cas".

siRNA

Advances in RNA molecular biology have provided a platform for the discovery of small non-coding RNAs known as siRNA with a length of 20–25 nucleotides(nt) which regulates genes expression (3,4). In mammalian

cells, siRNAs regulate the endo-nucleolytic cleavage of mRNAs in a sequence-dependent manner (5). siRNAs have become an important tool in genetic modification which has been applied to treat cancers and other incurable diseases (6,7).

Molecular mechanism of siRNA

siRNA obstructs the expression of genes that possess complementary nucleotide sequences, via post-transcriptional mRNA degradation, thereby, inhibiting translation. siRNA is a double-stranded RNA molecule, that operates within the RNAi pathway. RNAi processes and cleaves long double-stranded RNA (dsRNA) into siRNAs, with 2-nucleotide overhang on the 3'end of each strand (8). This process is activated by the enzyme RNase-III, which is also identified as Dicer. Dicer degrades long dsRNA into small effector molecules called siRNAs. siRNAs are attached by a multi-protein complex known as an RNA-induced silencing complex (RISC). Once the RISC forms a complex with siRNA, siRNA strands begins to separate into strands with a stable 5'-end which is then changed into an active RISC complex (8). The interaction of the RISC complex with a target mRNA is directed by an antisense single-stranded siRNA component through the activities of the catalytic RISC-protein of the argonaute family (Ago2), which cleaves the target RNA (9). An interaction with the RISC complex induces more mRNA to be targeted. thereby, amplifying the gene silencing effects (Fig. 1). In summary, the siRNA action mechanism works via the introduction of dsRNA into a cell. dsRNA is then reduced to one strand by Dicer to generate siRNA. The generated siRNA binds to the RISC complex to cause unwinding. An antisense RNA forms a complex with the RISC and then binds to the corresponding mRNA that later becomes inactivated via cleavage by an enzyme slicer (10).

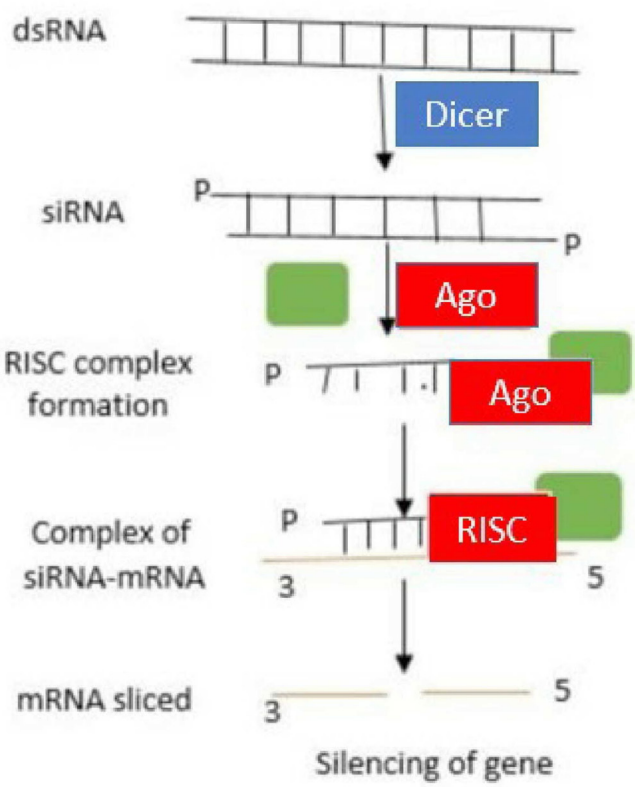

Figure 1: Molecular mechanism of siRNA. Dicer cleaves and cut long dsRNA, thereby forming siRNA. This cleavage enables siRNA to enter cells causing the formation of RISC. siRNA starts unwinding once the RISC complex is formed to produce single-stranded siRNA. The formed single-stranded siRNA then binds to the target mRNA and induces mRNA cleavage. The mRNA is cut and degraded, thereby preventing translation and silencing the mRNA-encoding gene.

Application of siRNA

Genome-wide screening

siRNA, a tool for genome screenings involves fundamental processes such as cell division, apoptosis and metabolism (8). A previous study on genome-wide siRNA screening in embryonic stem (ES) cells of mice determined that the downregulation of 148 genes is essential for cell differentiation triggered self-renewal (11). The siRNA knockdown of a specific gene has revealed interesting possibilities in functional genomic research (12). A previous study on the functional analysis of 19,427 predicted Caenorhabditis elegans genes, via RNAi revealed that 1,722 genes have mutant phenotypes (12). siRNA mechanism can be used for genome imprinting through which chromosomal condensation patterns are assessed on the basis of parent origin. This mechanism can also explain the hybrid dysgenesis phenomenon in a situation wherein large maternal siRNA pools, do not match the polymorphic repeats of paternal chromosomes, thereby resulting in transposon immobilisation and consequent chromosomal aberration (12).

Validation of potential drug targets

siRNA is beneficial to the authentication of an identified potential drug targets by using cDNA microarrays (13). siRNA provides valid targets for conventional therapeutic applications, in the form of monoclonal antibody inhibitors, and drug target validator (14). A previous study suggested that the siRNA-mediated downregulation of the pro-angiogenic genes VEGF and VEGF receptor 2 (VEGFR2) in xenograft tumour models showed a significant antitumour efficiency (14). The researcher's observation reaffirmed that proangiogenic factors and siRNA inhibitors perform anticancer drug activities (14).

Therapeutic applications

siRNA has reformed biology research and drug target discovery, via the rapid identification and authentication of gene function (12). Diseases can be prevented at the time points of transcription, posttranscription, and post-translation intervention (12). Before the siRNA discovery, drug targets were mostly proteins, and involved post translational mechanisms. The promising action of siRNA in therapeutic applications is due to a

gene's specific characteristics and the ability of siRNA to knockout proteins without damaging cells (12). siRNA inhibitory activities, such as cancer protection, HIV protection and hepatitis protection have been applied against various diseases. siRNA protected mice from fulminant hepatitis, sepsis and tumour growth (15-17). siRNA acts through the exploitation of the RNAi endogenous pathway, thereby exerting the reduction of specific disease-associated genes, especially genes with complementary sequences (18). The justification of the siRNA-mediated gene therapeutic, requires knowledge on the genetic identity of cancer. A previous investigation on the therapeutic effect of siRNAs on the silencing of targeted molecules revealed that tumour host interactions are crucial for the chemotherapeutic or radiotherapeutic resistance of tumours (18). The use of siRNA to silence a critical cancer-associated target protein yields substantial anti-proliferative and apoptotic effects.

Genetic improvement of crop plants

Efforts have been devoted to creating plants that can disrupt and consequently destroy the gene expression of insects. Crops responding to gene silencing, appear to be genetically modified with toxic protein products (12). Few researchers argued that crops with an siRNA-silenced gene is safer with less unintended effects than those with genetic modification via other techniques. siRNA can improve the quality of crop plants. For example, siRNA was previously applied in RNAi to improve rice plants and found that glutenin levels in rice decreases to produce a rice variety termed low-glutenin content1(LGC-1). The LGC-1 mutant rice was the first useful cultivar commercially produced through RNAi (12, 19). This rice cultivar has a low protein content and is beneficial to patients with kidney disease and restricted protein intake.

Hindrances in siRNA application

Limitations and uncertainty

siRNA application is limited by various factors; including difficulty in determining the effective cellular uptake, sustenance of its effectiveness in cells and quick clearance from the body. Naked siRNA usually undergoes rapid filtration from the circulatory system and then degrades, resulting in the initiation of immune responses (20). Understanding the true endogenous function of the siRNA molecule in vivo is difficult. These issues are caused by insufficient siRNA data available to the public and different data categories due to variations in data generation techniques.

Incomplete and inaccurate knockdown

siRNA has varied and incomplete knockdowns and potential non-specificity of reagents (21). Incomplete and inaccurate siRNA knockdown occurs when the targeted mRNA is incompletely hydrolysised and unable to protect the suppression of untargeted genes. This condition occurs when a selected siRNA has an average low silencing activity (62%). Inaccurate knockdown also occurs when many siRNAs are applied at once, leading to RISC saturation and untargeted genes suppression (22).

Required systemic delivery approaches

The therapeutic strategies of siRNA application to a targeted gene for silencing its expression in tissues requires an effective systemic delivery (23). siRNAs are quickly and promptly cleared from the circulatory system, but difficult to filter in the renal system because of an abnormal threshold size for filtration. Therefore, siRNA complexes and their delivery media remain in the circulatory system for a long period because their size exceeds renal clearance pores or the conveyance reagent enhances its alliance with serum proteins (23, 24). The negative charge and hydrophilicity of siRNA molecules hinders them from crossing the plasma membrane (25). Delivery vehicles protect and conceal siRNA, thereby facilitating its transportation to the targeted cytoplasm of target cells (26).

Difficulty in depleting activities.

siRNAs experience difficulty in fully depleting the targeted mRNA. Therefore, various siRNAs should be screened to determine the most effective siRNA (8). Rapidly dividing cell culture takes between three to five days to achieve an efficient transient siRNA. Thus, the application of transient siRNA to cells with slow division may cause difficulty in depleting a stable protein by an effective siRNA (8).

Off-target effect

The disadvantage of siRNA is its off-target effect. It is often triggered by the incomplete complementarity of sense or antisense strands to an unintended target. siRNA off-targets can be evidently decreased by treating cells with a relatively low dose of siRNA that is sufficient for the effective silencing of the intended target (27). However, many modifying chemicals to reduce siRNAs, but they should be further studied to obtain siRNA forms with better abilities (27). The optimisation of siRNA design, chemical modifications and concentrations, has drastically reduced the off-target effects on siRNA.

CRISPR/Cas

CRISPR is a technology that edits genes with the help of two important components known as guide RNA (gRNA) and CRISPR-associated protein. A gRNA functions to match the intended target gene, and CRISPR-associated protein functions as an endonuclease that induces double-strand break (DSB), thereby modifying the genome (28). CRISPR/Cas is classified into two categories, with six major (types I–VI) subdivisions (29, 30). The class 2 type II Cas possesses a single effector protein component called Cas9, whereas the class 2 type VI system, contains a single effector-protein Cas13 with different features from the presently known CRISPR/Cas (31, 32). Cas13 protein has four families, namely as

Cas13a, Cas13b, Cas13c, and Cas13d proteins (32-34). The Cas13 single effector-protein is nonhomologous with any DNA-nuclease domain but possesses double higher-eukaryote and prokaryote-nucleotide binding (HEPN) domains, that collectively form the active site of ribonuclease, which permits it to exert an RNA guided RNA-target effector function of CRISPR/Cas (35-38). Cas13 has two components, namely, single effector RNA-guided RNase-Cas13, which is programmable, and 64–66-nt CRISPR-RNA (crRNA), which uses a protospacer flanking site to recognise the 24–30 nt sequence of the targeted RNA (37).

Molecular mechanism of CRISPR/Cas

CRISPR/Cas specifically acts in programmable nucleases guided by RNA to degrade the DNA or RNA of predominant exogenous nucleic acids through the control of the molecular genetic memory of previous infections (39). At the molecular stage, adaptive response requires three different processes, that is; adaptation/adjustment, crRNA biogenesis and interference. Adaptation/adjustment updates the molecular memory bank on recent infection which requires foreign DNA sequences to be encoded into a CRISPR array. This CRISPR array consists of a replicate genomic sequence with diverse copies of short DNA segments which replaces semi palindromic repeats and spacer sequences that are inserted by integrase-like Cas proteins (38). Cas-proteins and host factors also transcribe CRISPR array into pre-crRNA which is subsequently processed nucleolytically to form mature crRNAs. Mature crRNAs form a complex with a subset of Cas proteins to produce a complex of effectors or induce interference that allows crRNA-guided scanning for the existence of nucleic acids in cells. The identified complementarity base pair between a crRNA spacer and a target by the effector complex of Cas exerts the cleavage and degradation of target nucleic acids thereby preventing infections.

In Cas13a pre-crRNA, the processing mechanism occurs via the mutation of an arginine residue into alanine (R1079A) within the HEPN2 domain of Lbu-Cas13a to abrogate the processing activity of pre-crRNA without altering the HEPN domain-mediated RNA cleavage (39). Thus, the catalytic mechanism of pre-crRNA has one turnover with the mature crRNA still attached after cleavage. Pre-crRNA processing liberates individual crRNA from the restrictions of CRISPR-array transcript, thereby preventing the constraints of RNA folding and the steric hindrance of crRNA-spacer species when crRNA is being loaded and targeting ssRNA (39).

In adaptive immune systems, the cell membrane is infected when the phage of a virus attaches onto the cell surface, thereby injecting the viral DNA into cells. If this cell has an active CRISPR system, it integrates the foreign DNA into the CRISPR locus through adaptation. This newly acquired sequence provides the genetic information of virus infection (25). Cells become transcribed across the CRISPR locus to make more RNA molecules. RNA molecules fold back, thereby forming RNA tags in cells and indicating the presence of CRISPR RNA in cells (39). RNAs become cleaved to generate individual RNA molecules possessing a virus sequence, which forms effector complexes with proteins encoded by the Cas gene.

These effector complexes are surveillance complexes that search for nucleic acid sequences that match the sequences of the CRISPR RNA in cells (39). The effector complex is recruited to nucleic acids via base pairing, thereby allowing the cleavage of the viral DNA by the associated Cas proteins. CRISPR systems have diverse enzymes that process foreign DNA because gRNAs are necessary to drive the functions of endonucleases. The only CRISPR protein needed for genome editing is Cas9 endonuclease (Fig. 2). The essential individual proteins included as CRISPR components have the following functions:

1) Bind to a gRNA

A gRNA directs Cas9 to cut a specific gene locus amongst numerous possible loci. Cas9 alone cannot cut the gene unless it attaches to gRNA. In the type II CRISPR-Cas system, Cas9 is guided to target sites by crRNA and tracrRNA which act as a Cas9 genomic target and a scaffold that link crRNA to Cas9, thereby facilitating the processing of pre-crRNAs into mature crRNAs from CRISPR arrays (40). These crRNA and tracrRNA are condensed into gRNA or single gRNA (sgRNA) in most CRISPR-mediated genome-editing processes (40). This gRNA contains a nucleotide target sequence that directs Cas9 to a specific gene locus and a scaffolding sequence for Cas9 binding.

2) Bind to target DNA in the presence of a gRNA provided that the target is the upstream of a protospacer adjacent motif

The attachment of Cas9 endonuclease to a target gene locus is facilitated by target sequence within the gRNA and protospacer adjacent motif (PAM) (40). The PAM sequence must be contained immediately at the downstream site of the gRNA-targeted gene to enable dsDNA by Cas9 (40). Without either a gRNA or PAM sequence, Cas9 does not bind or cut targets. Cas9 homologues or mutants developed by researchers require PAM assortments. These PAM assortments allow researchers to target numerous genomic loci.

3) Cleave target DNA and cause DSB

Cas9 has n-terminal RuvC and HNH-like nuclease domains near the protein centre (39). During target binding, Cas9 undergoes a conformational change to enable nuclease domains to cleave the opposite strands of target DNA (40). Thus, Cas9-mediated DNA damage results in a DSB within the target DNA at approximately three to four nucleotides before the PAM sequence upstream.

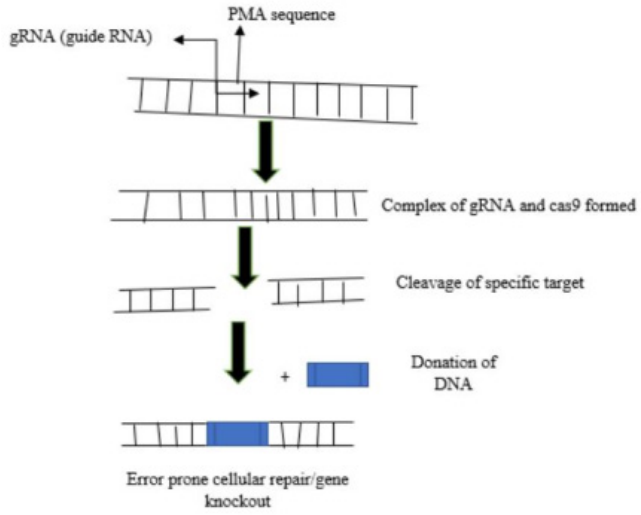

Figure 2: Pathway of gene disruption mediated by CRISPR. A gRNA consists of a crRNA sequence, which is specific to a DNA target, and a tracrRNA sequence which interacts with Cas9 protein. This interaction causes gRNA to bind to Cas9 protein possessing a DNA endonuclease activity. The complex formed causes the cleavage of the target specific double-stranded-DNA. The cleaved sites are repaired by nonhomologous end-joining, which is error-prone and may lead to insertions or deletions that disrupts gene function.

ADVANTAGES OF CRISPR/Cas

CRISPR/Cas is extremely efficient

CRISPR/Cas is more efficient than other genome editing technologies (41). Thus, CRISPR/Cas knocks out gene via RNA-mediated Cas9 nucleases. A CRISPR-Cas9 system modifies DNA with better precision than other technologies such as TALEN do (41). Genome editing with CRISPR/Cas occurs when genetically engineered nucleases possess a domain that contains a non-specific nuclease that binds to a sequence-specific DNA domain to cut the target gene, but these breaks can be repaired via non-homologous end-joining (NHEJ) or homology-directed recombination (42).

CRISPR/Cas9 consumes less time

The earliest genome editing techniques that use meganucleases and TALENs are labour intensive, requires tedious protocols to attain target specificity and takes a long period. By contrast, CRISPR/Cas9 has convenient execution design and method that consume less time.

Sander and Jong (41) stated that CRISPR/Cas9 reduces the target gene modification period compared with that of other gene manipulation tools through the creation of targeted DSBs in ES cells.

CRISPR/Cas9 improves bioinformatic tools

With bioinformatic tools, gRNAs can be designed with full optimisation that allows better experimental conditions that guarantee the successful introduction of cleavage at the desired target site (41). For example, researchers developed CLEAVE-Seq which predicts computational data by examining tendencies that can prevent off-target cleavage (43).

CRISPR/Cas9 enables genome targeting

A Cas9 system can target genomes based on the microinjection of a plasmid vector expressing a guide sequence, a specific promoter and Cas9 endonuclease or the co-injection of the CRISPR gRNA and mRNA of the Cas9 vector separately into the cytoplasm or pronucleus of a fertilised oocyte (44).

LIMITATION OF CRISPR/Cas

CRISPR/Cas carries additional modification deficiency of DNA repair mechanism

Additional modifications are generally present in targeted alleles (45). For example, deletions; causes incomplete or numerous combinations of a targeting vector, thereby leading to duplications, because the DNA repair system does not merge genomic DNA fragments. Yin et al., (46), observed that a CRISPR/Cas9-generated founder mice show difficulty in determining unwanted genomic alterations at the target site. CRISPR/Cas9 gene editing in the cells that is going to form sperm or egg, or early embryonic stage cells can cause germline editing whereas, introduction of genetic changes to other cells such as , liver cells will not cause germline editing in future(National Academies of Sciences) Editing human genome with CRISPR-Cas9 can cause mosaicism, resulting in daughter cells having either the CRISPR machinery showing the alteration, or not carry the CRISPR machinery thereby maintaining a complete genome (48).

CRISPR/Cas limits the identification of the desired allele

The desired event cannot be selected, thereby limiting the possibility of identifying the desired allele when it directly carries the CRISPR/Cas9 procedure on embryos (45). One important limitation of CRISPR/Cas9 is the induction of off-target cleavage spots resulting from gRNA binding with a complementary mismatched DNA target in the genome (47).

Mutational changes

Incorrect selection procedures and ineffective validation aim to detect mutations at the target spot, can lead to unexpected mutational changes at target locus-plague standard ES cells. This mutation genes can cause cancer (45). Thus, the safety of CRISPR/Cas relies on how often it makes these off-target mutations.

Off-target problem

Off-target assessment is expensive, and time consuming. Some reports have suggested higher off-target mutation frequencies in in vitro cell studies compared with those in in vivo animal trials (48). Off-target effects are cell-type specific and dependent on the integrity of NHEJ on cell type repair pathways. Variation in gRNA structures can also influence the on-target and off-target site cleavages

(48). However, off-target mutations are observed at higher frequencies than the intended mutation, which can cause instability in the genome with the subsequent disruption of the functionality of normal genes. This factor is the major concern on the application of CRISPR/Cas9 system to biomedical and clinical studies (48).

siRNA and CRISPR/Cas comparison

Gene silencing mechanism

The main difference between siRNA and CRISPR/Cas is that siRNA suppresses gene expression at the mRNA level (knockdown), whereas CRISPR/Cas completely and permanently silences genes at the DNA level (knockout). The knockouts of vital genes are deadly and provides incomplete information on the role of the gene of interest, and the consequence of the knockout cannot be studied. In such cases, incomplete gene knockdown provides an improved understanding of the effect of the gene on phenotype because it can permit studies on the effects of reduction in protein levels (49). Knockdown is reversible, thereby allowing the phenotypic effect to be verified because of the restoration of protein levels to normal levels in affected cells. Knockdown is more temporary, and safer than knockout, which permanently edits genome. Knockouts completely block protein expression, thereby eliminating any effect of protein expression as shown in knockdown (50).

Newest CRISPR/Cas-associated nuclease versions have enabled CRISPR to be applied to gene knockouts and other processes. For example; CRISPR-interference (CRISPRi) permits gene silencing without knockout. In this case a dead Cas9 nuclease is used to physically block RNA polymerase to inhibit gene transcription or edit gene regulators in moderating gene expression. Gene transcription is inhibited by CRISPRi at the DNA level as in CRISPR/Cas, but with different mechanisms (51, 52).

Specificity

Off-target effects found in gene editing can be either sequence-independent or sequence-dependent. siRNAs regulated transcripts are improved by sequences that have complementarity to the seed region of the siRNA whereas alteration in the sequence of the seed region results in the production of off-target effects in the transcripts sharing sequence complementarity with the new seed sequence (55,56). Jackson et al., (2006) stated that matching occurring between the guide strand of 5' end of siRNA and target transcript are essential for gene silencing whereas, mismatches between the guide strand of 5'end of siRNA and target transcript compromises the silencing efficacy suggesting the importance of seed region in off-target effects. A previous study on genes revealed that, many of the genes incorporate motifs that are partly identical to the 5'region of the guide strand of the siRNA and that effective silencing of gene requires matching between the target transcript and the 5'end of the siRNA (57,58). siRNA design optimisation, chemical modifications and concentrations adjustments have reduced the off-target-effects of RNAi (55).

CRISPR/Cas has sequence-specific off-target effects that have been recently resolved by researchers using efficient designing tools to find gRNAs with negligible off-target effects (53). sgRNA and additional modified sgRNAs suppresses off-target effects to a greater extent than plasmid and IVT-derived gRNAs do (54).

siRNA and sgRNA design

siRNA tools designs require sequence details and complementary gene transcripts whilst CRISPR/Cas designs need gene sequence details (56). siRNA processing is homologue dependent. Therefore, the sequences of gene-specific RNAi-targeting must be cautiously selected to evade cross-interference amongst homologous sequences (57,58). Targeting genes with CRISPR/Cas requires a customised sgRNA that comprises a targeting sequence known as a crRNA sequence plus a Cas9 nuclease sequence recruiter known as tracrRNA.

Advances in genetic manipulation

Validated GalNAc-siRNAs known as GalNAc conjugates have potential for application in targeting tissues other than liver. Hu et al., (59) reported that siRNA has been developed to be used for specific administration routes such as aerosol inhalation, intratracheal application and intravitreal application.

CRISPR/Cas proteins are programmed to bind to specific DNA or RNA sequences in the form of crRNAs or gRNAs designed to contain spacers for complementing targeted sequences. These binding proteins function as nucleases and are suitable for programmed gene editing (60). CRISPR/Cas can clear HIV by targeting the HIV-1 genome to inhibit HIV-1 infection and has been applied successfully to prevent HIV-1/AIDS in human and animal models (61).

Combination between CRISPR/Cas9 and siRNA.

Wissel et al (62), previously characterized gene function using minimal in vivo GFP-interference (miGFPi). miGFPi combines CRISPR/Cas9-oligo based approach by integrating immunetag V5 or HA with a 21 nucleotide eGFP derived sequence encoded on oligonucleotide in-frame into the coding region of a gene (62). The immunotag permits localization and biochemical studies, whereas 21 nucleotides function as the RNAi-effector sequence responsible for exerting loss of function with validated RNAi reagents that targets the sequence in a manner that is off-target free.

CONCLUSION

CRISPR/Cas and siRNA are effective tools for gene manipulations. Alteration in the cells by CRISPR/Cas9 could either cause germline editing or genetic changes. Gene knockdown by siRNA permits functional studies

on critical genes whose knockout will be lethal to cells. siRNA knockdown mimics the effects of inhibitory drug while CRISPR/Cas knockout represents the total loss of gene function. Considering current facts, we can conclude that siRNA and CRISPR/Cas are unique in their applications.

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