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Nanotechnology Developments in Efficient Crispr/Cas9 Genome Editing Delivery

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Abstract

Advances in the development of genome editing technology hold promise to personalized medicine, human genetic modification and the development of new drugs; ParticularlyCRISPR/Cas9 genome editing platforms have broad impact on basic and translational research. While Genome editing technologies seems to have therapeutic potential for various incurable diseases such as cancer, genetic disorders and HIV/AIDS, to date, the delivery of the CRISPR/Cas9 system remains challenging. This review, have summarized new CRISPR/Cas9 delivery systems by using nanotechnology approaches to advance protein-based genome editing.

Keywords : Genome editing; CRISPR/Cas9; Delivery; Nanotechnology

Introduction

Recent advances in genome editing technologies have substantially improved our ability to harnesses programmable nucleases to cut and paste genetic information in a targeted manner in living cells and organisms [1]. Programmable nucleases, particularly the CRISPR/Cas system, are already revolutionizing our ability to interrogate the function of the genome and can potentially be used clinically to correct or introduce genetic mutations to treat diseases that are refractory to traditional therapies. Recently, the programmable nuclease Cas9 and other genome-editing proteins have been shown to mediate editing of disease-associated alleles in the human genome, facilitating new treatments for many genetic diseases [2-6].

CRISPR-Cas Systems

The CRISPR/Cas systems are classified into three major types that differ by the repertoires of cas genes, the organization of cas operons and the structure of repeats in the CRISPR arrays contain cas3, cas9 and cas10 being the signature genes for the type I, type II and type III systems, respectively [7]. The simplest among the CRISPR-Cas systems is type II which consists of two components: a molecular scalpel (Cas9) that cuts DNA and an RNA guide complex is found Clustered with short DNA repeats of viral origin found in the bacterial genome (CRISPR) [8]. The endonuclease CRISPR-associated protein Cas9 cleaves DNA according to the sequence within an RNA duplex and creates site-specific double-strand break. [3,9].

Compared with other gene editing tools such as ZFNs and TALENs, target design simplicity with only three required components (Cas9 along with the crRNA and trRNA), high efficiency and multiplexed gene deletion or insertion makes this system top candidate for a new generation of powerful tools for new treatment of such genetic diseases as cystic fibrosis, muscular dystrophy and hemophilia. Efficient intracellular protein delivery system in vitro and especially in vivo has been a persistent challenge in biomedical research and protein therapeutics [10-18]. The CRISPR-Cas technology in combination with better delivery systems dramatically improved the specificity and efficacy and may find more applications in gene therapy. But to work well, the new gene-clipping tool must be delivered safely across the cell membrane and into its nucleus, a difficult process that can trigger the cell's defenses and trap CRISPR/Cas9, greatly reducing its treatment potential [19-21].

From Gene Editing to Gene Delivery

Recently, CRISPR/Cas9 system has attracted tremendous attention from both scientists and clinicians came out in different fields, especially with those diseases that remained untreatable for a long time such as AIDS, cancers and degenerative diseases. The following are just few examples to demonstrate the increasing popularity of this technology among clinical and scientific researchers [20]. CRISPR/Cas9 was thought to be a useful tool for in-depth understanding of carcinogenesis processes; facilitate systematic analysis of gene functions and

cells reprogramming [3,22-29]. Scientists demonstrated that CRISPR/ Cas9 technologies had the capability for a promising and sustained genetic therapy for HIV, neurodegenerative diseases and retinal degenerative disease [30-34].

CRISPR/Cas9 has also been applied in large-scaled genetic screening for drug targeting [3]; producing genetically engineered animal models, developing new antiviral treatments and drug discovery [28, 35]. All these researches made CRISPR/ Cas9 a "magic" technology in the scientific world and brought this new technology to an unprecedented level. The therapeutic potential of protein-based genome editing is dependent on the delivery of proteins to appropriate intracellular targets [36]. However, delivering protein and RNA remains a central challenge in drug delivery. Most protein therapeutics, such as enzymes [38], antibodies [39] or transcription factors [40] suffer from low stability and poor cell membrane permeability as a result of their fragile tertiary structures and large molecular sizes [37]. The strong negative charges of RNA therapeutics, including siRNA or miRNA, blocks them from diffusing across cell membrane and their susceptibility to endonuclease often requires chemical modification to prevent degradation [41].

A challenge to efficient delivery of genome-editing proteins is their proteolytic instability and poor membrane permeability. Thus, there remains a great demand for the development of novel platforms that efficiently assemble protein into nanoparticles for intracellular delivery while maintaining biological activity of the protein [16].

A Variety of non-viral vectors, including liposomes, cationic polymer, and nanoparticles have been employed in both gene therapy related experiments and gene therapy clinical trials. Unlike conventional delivery systems that primarily focus on direct, newer delivery modalities such as nanocarriers have been focused on more specificity and efficacy performance [20]. Thus non-viral gene delivery systems have been extensively studied in the past decades. Non-viral gene delivery techniques by nanotechnology tools such as application of polysaccharide nanoparticles, cationic liposome nanoparticles , calcium phosphate nanoparticles and nanoneedlesin parallel with efficient and safe genome editing tools such as ZFN, TALEN, and CRISPR/Cas9 nuclease systems, are new tools for developing clinically approved cell-based therapeutics [42-44].

These systems are more reliable since they avoid the high risks associated with using viral vectors such as insertional mutagenesis and undesired immune rejection, provide life-long therapy by more policed insertion of the therapeutic gene into the desired site, target a broader range of disorders due to their capability to accommodate genes of different sizes, and finally, provides higher activity owing to their ability to target hard- to-transfect human cells [20]. Additionally, nano drug has strong ability to cross biological membrane barriers which greatly improve the bioavailability of poorly soluble drugs.

Advances in Gene Editing Delivery Tools by Nanotechnology

RNA-guided endonucleases (RGENs) derived from the CRISPR/Cas system represent an efficient tool for genome editing. RGENs consist of two components: Cas9 protein and guide RNA. Recently, in vivo wound modification new delivery modalities include conjugated nanoparticles, multi-electrode arrays, microfabricated needles and nanowires are more efficiently managing the specific performance requirements of cutaneous modification [46]. In the case of conjugated nanoparticles, recently, Ramakrishna et al. [45] have demonstrated in human embryonic stem cells, dermal fibroblasts, human embryonic kidney cells (HEK), HeLa and embryonic carcinoma cells that cell-penetrating peptide conjugated with both the Cas9 protein and guide RNA sequences could achieve modest modification rates, a key milestone in terms of combining this delivery nanotechnology with CRISPR-Cas9.

Additionally, microfabricated needle and nanowire array concepts for delivery of genetic and drug constructs has been demonstrated with varying degrees of success in a wide range of human cell types which mostly rely on transient or semi-transient physical compromise of target cell membranes via micron-sized needle structures to pass transgenes to cells [47]. Hybridization of microneedle physical delivery design in concert with localized electroporation in a pseudo-multi-electrode array fashion is a technology called nanoinjection. More specifically, first-generation nanoinjection consisted of a micro-electromechanical system (MEMS) that uses electrostatic attraction of DNA onto a silicon micro-sized solid lance that is then inserted into mouse embryos before electrically releasing exogenous DNA into the host [46,48]. This same technology has been modified in a second generation to consist of a microfabricated silicon wafer that has a grid of four million 10-micron-length lances allow for effective delivery of genetic loads to hundreds of thousands of cells simultaneously [49].

As an emerging method, nanoinjection shows promise in regard to in vitro CRISPR-Cas9 chronic wound applications, given the high efficiency of transfection and high cell survival rates as well as the lack of cytotoxic effects. Utilizing this approach, researchers have recently modified a non-viral transfection technology, named lance array nanoinjection (LAN), utilizes a microfabricated silicon chip to physically and electrically deliver genetic material to large numbers of target cells by using the CRISPR-Cas9 system to edit the genome of isogenic cells. These findings have shown the LAN's ability to deliver genetic material to cells and indicate that successful alteration of the genome is influenced by a serial injection method as well as the electrical current settings [50]. Furthermore, researchers found that genome editing using LAN follows a non-linear injection-dose response, meaning samples injected three times had modification rates as high as nearly 12 times analogously treated single injected samples [46].

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In addition, researchers have successfully designeda promising delivery technology for CRISPR-Cas9 based on biologically inspired yarn-like DNA nanoclew (NC) genome editing [51]. The DNA NCs are synthesized by rolling circle amplification (RCA) with palindromic sequences encoded to drive the self-assembly of nanoparticles [52-57]. It has been shown previously that the DNA NC could encapsulate the chemotherapeutic agent doxorubicin and drive its release based on environmental conditions [58]. The biologically inspired vehicles were efficiently loaded with Cas9/sgRNA complexes and delivered the complexes to the nuclei of human cells, thus enabling targeted gene disruption while maintaining cell viability [59]. The DNA NC can load and deliver the Cas9 protein together with a sgRNA for genome editing. Inspired by the ability of single stranded DNA (ssDNA) to base pair with the guide portion of the Cas9-bound sgRNA [60].

Following loading of the DNA NC with the Cas9/sgRNA complex, a coating of the cationic polymer polyethylenimine (PEI) has applied to help induce endosomal escape [61]. The Cas9/sgRNA complex delivered to the cytoplasm could then be transported into the nucleus via nuclear-localization-signal peptides fused to Cas9 [51]. It has been approved that Editing was most efficient when the DNA nanoclew sequence and the sgRNA guide sequence were partially complementary, offering a design rule for enhancing delivery. Overall, this strategy provides a versatile method that could be adapted for delivering other DNA-binding proteins or functional nucleic acids [59].

One of the most advanced methodologies for delivering Cas9 in vivo is Gene therapy with adeno-associated viruses (AAVs) [62, 63]. However, there are some challenging issues aboutAAV delivery such as human population immunity towards AAV, significant off-target genomic damage due to the sustained expression of Cas9 [64-65] and using multiple viruses to Cas9 ribo nucleo protein (RNP) and donor DNA in vivo delivery regards to the AAV small packing size [21,66,67] which could decreases the efficiency of AAV-based Cas9-delivery methods [68]. To achieve targeted gene editing, more recently, researchers have explored a delivery vehicle composed of gold nanoparticles conjugated to DNA and complexed with cationic endosomal disruptive polymers (N-(N-(2-aminoethyl)-2-aminoethyl) aspartamide) (PAsp (DET)) which celled CRISPR–Gold. CRISPR–Gold can directly deliver Cas9 RNP and donor DNA in vivo via local administration and efficiently correct the DNA mutation that causes Duchenne muscular dystrophy in mice via local injection, with minimal off-target DNA damage [69].

After endocytosis, the PAsp (DET) polymer on CRISPR–Gold triggers endosomal disruption and causes the release of CRISPR–Gold into the cytoplasm. Importantly, once in the cytoplasm, glutathione releases the DNA from the gold core of CRISPR–Gold, which causes the rapid release of Cas9 RNP and donor DNA [70]. The challenge of using non-viral vectors for CRISPR-mediated genere pair is to deliver Cas9, sg RNA and is pair template simultaneously in vivo. Especially the non viral delivery of DNA into the nucleus in vivo with high efficiency and low to xicity remains difficult.

Recently researchers by combination of clinical suitable non-viral and viral delivery systems have succeeded to repair genes in vivo efficiently to minimize the side effects. They have combined lipid nanoparticle–mediated delivery of Cas9 mRNA with a deno-associated viruses encoding as gRNA and are pair template to induce repair of a disease gene in adult animals [71]. This allowed for short-term expression of the Cas9 nuclease, which provided efficient on-target geno meediting, while potentially reducing off-target editing. Genome editing through the delivery of CRISPR/Cas9-ribonucleoprotein (Cas9-RNP) reduces unwanted gene targeting and avoids integrational mutagenesis that can occur through gene delivery strategies. Direct and efficient delivery of Cas9-RNP into the cytosol followed by translocation to the nucleus remains a challenge. This approach uses gold nanoparticles to coassemble with engineered Cas9 protein and sgRNA into nanoassemblies.

These vectors deliver protein and nucleic acid efficiently to the cytoplasm, with concomitant transport to the nucleus. Using this approach, we achieved up to ~90% delivery efficiency in a range of cell types, with subsequent gene editing efficiency up to 30%. This approach can provides a direct platform for multiple in vitro applications and will greatly facilitate research in many other areas of rapidly growing genome engineering, including spatiotemporal control of gene transcription and imaging chromatin dynamics [72].

However, a central challenge to the development of protein-based therapeutics delivery methods is the inefficiency of delivery of protein cargo across the mammalian cell membrane, including escape from endosomes and limited in vivo efficacy [5,36]. Nucleic acid delivery has benefited greatly from the development of liposomal reagents over the past two decades. Scientists have previously shown that lipids designed in a combinatorial fashion have low immunogenicity and toxicity [73-74]. Cationic lipid formulations have enabled DNA and RNA transfection to become a routine technique in basic research and have even been used in clinical trials [75]. The lipid bilayer of the vehicle protects complexed nucleic acids from degradation and can prevent neutralization by antibodies [76]. Notably, fusion of liposomes with the endosomal membrane during endosome maturation can enable the efficient endosomal escape of cationic, lipid delivered cargo [77]. Researchers demonstrated that common cationic lipid nucleic acid transfection reagents can potently deliver proteins that are fused to negatively supercharged proteins, that contain natural anionic domains or that natively bind to anionic nucleic acids [69].

This method have used to deliver diverse protein classes, including the Cre tyrosine recombines, TALE transcription activators, and Cas9 nucleases, nickases and transcription activators to cultured cell lines, stem cell colonies and

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therapeutically relevant in vivo sites within the mouse inner ear [5]. This approach is highly efficient, producing modification rates similar to or exceeding those of established nucleic acid transfection methods in cell culture, and enabling Cre recombinase- and Cas9-mediated genome modification rates of up to 90% and 20%, respectively, within the inner ear hair cell population of live mice.

In addition, a similar clinical trial researchers have developed a lipid-like nanoparticles delivery system of supercharged Cre protein and Cas9: single-guide (sg) RNA complexed with bio reducible lipids into cells enables gene recombination and genome editing with efficiencies greater than 70% that mediate potent protein delivery and genome editing. They have reported the synthesis and utilization of a bio reducible lipid nanoparticle with negatively supercharged proteins or anionic Cas9: sgRNA complexes for genome editing in mammalian cells and in the rodent brain.

The integration of a bio reducible disulfide bond into lipids facilitates endosomal escape of nanoparticles containing protein cargo, enabling delivery into the nucleus for protein-based genome editing. The efficient and localized delivery of genome-editing proteins to the mouse brain demonstrated here may eventually lead to a protein-based approach for correcting genetic diseases and neurological disorders. For example, the single injection of nanoparticles containing a Cas9: sgRNA complex into brain regions rich in dopaminergic neurons could enhance dopamine signaling and potentially alleviate some symptoms of Parkinson's disease. One current treatment for Parkinson's disease is deep brain stimulation [36].

Conclusion and Future Perspectives

Genome editing is a group of technologies that give scientists the ability to insertion, deletion or replacement of DNA at a specific site in the genome of an organism or cell [5]. CRISPR-Cas9 is a genome-editing tool is used to addition or changing of a sequence of specific genes that are associated with human diseases and facilitate the development of therapies to correct the mutated gene [78-79]. Due to the unparalleled genetic specificity, speed and efficiency, CRISPR-Cas has become a very hot research field from bench to bedside [8,15]. Thus, devising an appropriate carrier to shield the protein and RNA from detrimental physiological environment and escort them simultaneously to cell nucleus is highly desirable. Advances in nanotechnology for synthesis of more cell-friendly structures, and in-depth knowledge of gene modification systems at the molecular level have encouraged scientists to develop novel non-viral gene delivery systems for gene editing [20].

In this review, we provided an overview of recent studies on CRISPR-Cas non-viral delivery systems by nanotechnology in genome engineering, and in particular, stress key toolsets and methodologies that will have particularly broad impact not only on basic researches such as creating animal models and generating cell lines but also on translational research like developing therapeutic methods for diseases in the future. These studies have established that non-viral protein delivery is a viable approach. However, more experiments must be done to characterize and optimize the pharmacokinetics, efficacy, and safety of these strategies in animal models.

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