Medicine in Drug Discovery 3 (2019) 100011

Contents lists available at ScienceDirect

Medicine in Drug Discovery

journal homepage: https://www.journals.elsevier.com/medicine-in-drug-discovery

# RNA binding protein as monodisperse carriers for siRNA delivery

Wen Wang, Wanyi Tai *

*Key Laboratory of Combinatorial Biosynthesis and Drug Discovery (Ministry of Education), School of Pharmaceutical Sciences, Wuhan University, Wuhan, Hubei 430071, China*

ARTICLE INFO

*Article history:*
Received 27 September 2019
Received in revised form 24 November 2019
Accepted 12 December 2019
Available online 24 December 2019

*Keywords:*
dsRBD
siRNA delivery
Protein carrier
RNA binding protein

ABSTRACT

siRNA is a powerful gene silencing tool that suppresses the expression of the disease-causing protein by degrading its mRNA directly. It exhibits the profound potential in drug development with applications in both target validation and disease intervention. The most established methods for nonviral delivery of siRNA employ the use of nanoparticles, in particular, the cationic nanocarriers. Despite the popularity and feasibility, nanoparticle-based delivery carriers possess some undesired features such as polydisperse sizes, heterogeneous contents, nonspecific binding and technical challenges on large-scale fabrication, which limit their efficacy and potential in clinic utility. Protein-based siRNA carriers show the potentiality to solve some of these problems faced by nanoparticle carriers. In this review, we surveyed the current studies of using RNA binding proteins for siRNA packing and targeted delivery. RNA binding protein recognizes siRNA independent of charges, thus assembles into monodisperse carrier that has a discrete structure and drug ratio. This review discusses the most widely used RNA binding protein, including dsRBD, p19 and U1A RBD. Chemical and genetic modification to modulate the proteins' functionalities is also covered.


## 1. Introduction

Small interfering RNA (siRNA) mediated the gene silencing by the degradation of a particular mRNA in a highly sequence-specific manner [1]. Given the powerfulness in gene regulation, siRNAs are gaining great interest in therapeutics with the application of both target validation and disease intervention [2,3]. Despite the significant promise, siRNA delivery in vivo, however, remains the biggest barrier in the path to its therapeutic application [4]. To deliver siRNA to diseased sites, various carriers such as cell-penetrating peptides, cationic polymers and nanoparticles have been developed and evaluated [5]. However, the majority of current siRNA delivery systems failed in the clinic either due to the low efficiency or systemic toxicity [6]. The current siRNA delivery systems share common problems. Regardless of the chemical composition (e.g., peptides, polymers, and inorganic materials), most siRNA delivery carriers are positive charged [7]. The positive charges are important to condense the negatively charged RNA molecules, promote cell uptake and sometimes involve in endosome escape; on the other hand, the prosperity of positively charged surface induces some adverse effects including cationic toxicity, nonspecific binding, complex formulation that make them recalcitrant to meet the rigorous demands of clinic utility [4,8,9]. More importantly, siRNA condensation based on electrostatic interactions is an aggregation process that defies the rigorous standard of pharmaceutical formulations [10]. The final siRNA particles have heterogeneous sizes, scattered surface charge, and poor batch consistency, making it extremely difficult for scale-up and quality management [11].

One solution is to deliver siRNA with a neutral-charged carrier. However, eliminating the cationic charges requires a new mechanism for carrier-siRNA association. In this context, RNA binding proteins (RBPs) provides a valuable tool for siRNA packing via a distinct structure recognition. RBPs are ribonucleoprotein that binds to single or double-stranded RNAs and participate in many biological events of cells [12,13]. The secondary and tertiary structure of RBPs provides the basis for the binding with RNAs [14]. In contrast to the cationic carriers, the RNA-protein complexes would have several advantages including higher stability, lower toxicity, and better specificity. There has been emerging interest to develop the new class of siRNA carriers that harnesses the new packing mechanism [15]. Here, we discuss the structural features of these ribonucleoproteins, its recognition to siRNAs and the consequence of the siRNA delivery carriers that contain the ribonucleoproteins.

## 2. dsRBD from PKR

The double-strand RNA binding domain (dsRBD) is a truncated protein kinase R (PKR) that lacks the region of C-terminal kinase but retains the binding affinity to double-stranded RNA (dsRNA) [16]. dsRBD is consisted of two dsRNA binding motifs (dsRBM1 and dsRBM2) plus a highly flexible, unstructured linker that allows two dsRBMs to position on the opposite faces of siRNA duplex in a close-packed fashion

* Corresponding author.
*E-mail address:* wanyi-tai@whu.edu.cn (W. Tai).

https://doi.org/10.1016/j.medidd.2019.100011

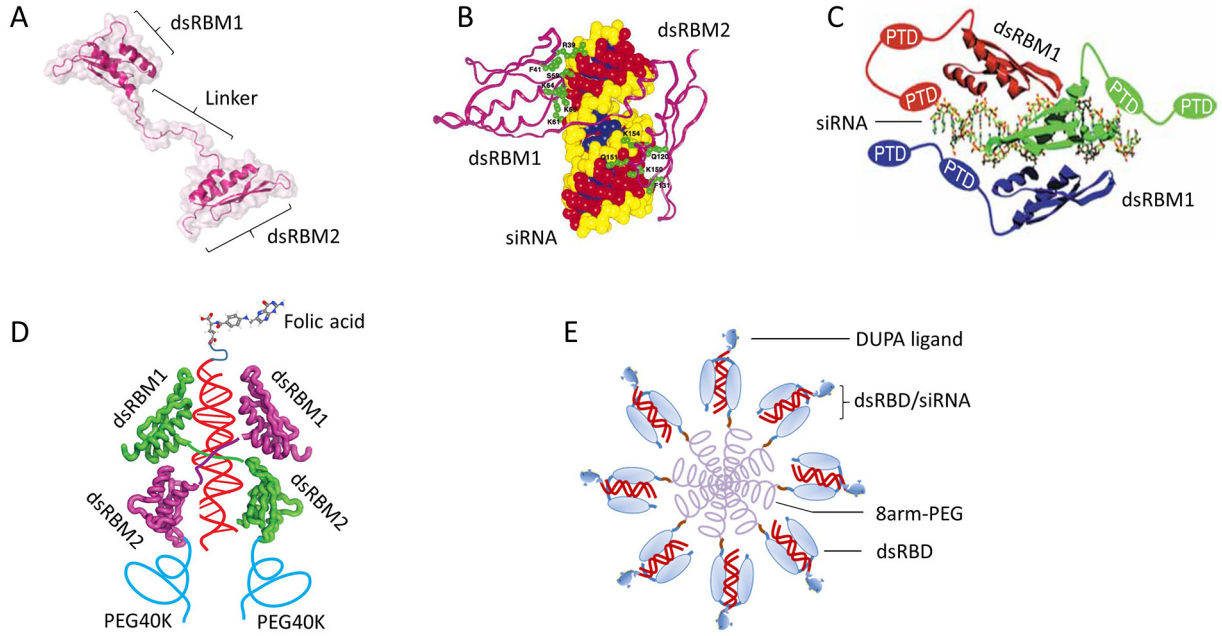


**Fig. 1.** (A) Ribbon representation of dsRBD containing dsRBM1 and dsRBM2 linked by a flexible loop. The figure was drawn using the PyMOL Molecular Graphics System based on crystal structure information from PDB ID 1QU6. (B). Simulation of the interaction between the dsRBD and a siRNA helix. Adapted with permission from EMBO J. 1998, 17(18), 5458–5465. (C) The cartoon schematic of dsRBM1-PTD fusion protein bound to siRNA. Adapted with permission from Nat. Biotechnol. 2009, 27(6), 567–571. (D) Schematic representation of ribonucleoprotein carriers assembled by PEGylated dsRBD (full-length) and siRNA (conjugated with folic acid for tumor-cell targeting). (E) Schematic representation of dsRBD octamer for targeted siRNA delivery. Adapted with permission from Nat. Biomed. Eng. 2018, 2(5), 326–337.

(Fig. 1a) [17]. The binding between dsRBD and siRNA is structure-dependent rather than sequence-dependent since dsRBDs recognize the A-form helical axis of dsRNA but not the specific RNA sequence (Fig. 1b) [18,19]. When extra dsRBD is available, the second dsRBD wraps around siRNA helix at the symmetrical position of the first dsRBD for cooperative binding, resulting in two dsRBD encompassing a single siRNA (Fig. 2a) [20].

dsRBD protein is the first ribonucleoprotein carrier explored for siRNA delivery. In 2009, Dowdy group reported a dsRBD fusion protein that can bind siRNA and mediate cellular uptake with the aid of the

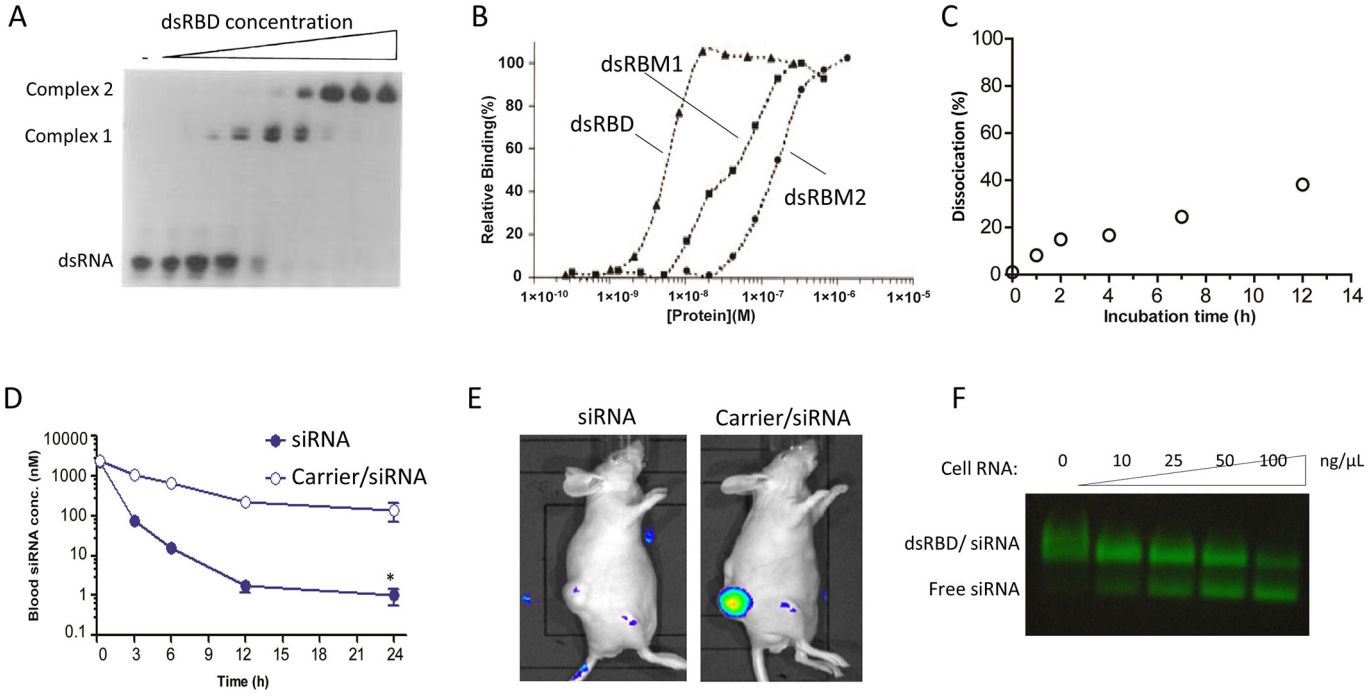


**Fig. 2.** (A) Gel mobility-shift experiment reveals that dsRNA (21 bp) can associate with one dsRBD (complex 1) and two copies of dsRBD (complex 2), respectively. Adapted with permission from Biochemistry, 1996, 35, 9983–9994. (B) Saturation Binding Curves of dsRBM1, dsRBM2 and dsRBD to dsRNA. The graph depicts the $K_D$ values for binding at $5 \times 10^{-9}$ M, $5 \times 10^{-8}$ M and $2 \times 10^{-7}$ M, respectively. Adapted with permission from J. Biol. Chem. 2001, 276(13), 9936–9944. (C) The dsRBD-PEG/siRNA complex shows slow dissociation in the mouse serum at 37 °C. (D) The dsRBD-PEG carrier extended the blood half-life of siRNA from 10 min to 3 h in vivo. (E) dsRBD-PEG carrier promotes the deposition of siRNA in the tumor site in the xenograft mouse model. The figure C, D and E were adapted with permission from Adv. Funct. Mater. 2019, 29, 1902221. (F) The displacement of siRNA (Fam labeled) from dsRBD/siRNA complex by total cellular RNA. Adapted with permission from Nat. Biomed. Eng. 2019, 2, 326–337.

peptide transduction domain (PTD) (Fig. 1c) [21]. The association of dsRBD-PTD to siRNA masks the siRNA's negative charge and induces rapid RNAi response in primary cells. It is of note that the dsRBD in the study was just the domain dsRBM1, which has a weaker binding affinity to siRNA (around 150 nM, roughly 10 times weaker than the full-sequence dsRBD) (Fig. 2b) [22]. It was a concern that excessively strong siRNA binding might prevent the discharge of siRNA from the ribonucleoprotein and result in a decreased RNAi response. James et al. compared the two types of dsRBD (dsRBM1 vs full-length dsRBD) and found that the dsRBM1 alone is insufficient to stably complex siRNA [23]. The full-length dsRBD binds siRNA and yields a specific and stable ribonucleoprotein complex that can deliver siRNA into the endosomal compartment. However, the endosomal escape of the siRNA complex is impeded with this full-length dsRBD. The endosomolytic agent has to be added to facilitate the endosomal release of siRNA complex. Wittrup group optimized the dsRBD-mediated siRNA delivery approach by co-delivery of a protein perfringolysin O (PFO) that disrupts endosomal compartments to allow the trapped siRNA to access cytoplasm [24]. It worth noting that both dsRBD and PFO were grafted with the same targeting ligand (E6N2), which allows the two components to simultaneously transport into the same endosomal vesicle for a cooperative effect on siRNA delivery.

Our lab has combined the above two components into one construct and developed a biomimetic platform for targeted siRNA delivery [25]. This ribonucleoprotein carrier is consisted of three building blocks: a dsRBD (full-length) for siRNA packing, an endosomal destabilization peptide and a polyethylene glycol (PEG) chain (40 kDa) for better biocompatibility (Fig. 1d). The endosomal destabilization peptide is a histidine-rich peptide and genetically fused to the C-terminus of dsRBD. Histidine, more specifically the imidazole ring, is one of the neutral-charged components that are in favor of the escape of siRNA from endosome [26]. The imidazole group of histidine has a pKa of 6.0 and can absorb protons in the acidic environment of the endosome (pH 5–6.5), leading to osmotic swelling, membrane disruption and eventually siRNA escape [27]. This proof of concept has been proved by numbers of histidine-containing biomaterials that dramatically enhance gene delivery efficiency by orders of magnitude [28–30]. To improve the blood retention profile, the 40 kDa PEG is site-specifically conjugated to the terminal end of the dsRBD fusion protein by Sortase A ligation. Once assembled with siRNA (folic acid is conjugated for tumor-cell targeting), the resulting complex presents a single macromolecule of ~150 kDa, that is capable of tumor cell targeting, endosomal siRNA escape, and long-term blood circulation, with a benefit of good biocompatibility (Fig. 1d) [25]. The dsRBD carrier forms a stable complex with siRNA and exhibits a half-life of around 18 h in mouse serum (Fig. 2c). Moreover, pharmacokinetic analysis reveals that the dsRBD carrier extends the blood half-life of siRNA from 10 min to 3 h in mice (Fig. 2d). The long blood retention of the carrier/siRNA complex also results in a high deposition of siRNA drug in tumor site and a selective knockdown of the targeted gene (Fig. 2e). To further improve the delivery efficacy, we constructed a dsRBD octamer by covalently conjugated the dsRBD with an 8-arm PEG via click chemistry (Fig. 1e) [31]. The new protein carrier has a sphere-shaped scaffold, which can sterically mask the endocytic peptide. Despite the outstanding binding stability of dsRBD and siRNA, our studies indicated that the octamer/siRNA complex can dissociate in the cytosol by the mRNA displacement [31]. The bioactive mRNA exhibits the complicate 3D dimension. Around one third of the mRNA bases was self-annealed into short duplex, which is structurally similar to the siRNA. Once octamer/siRNA enters into cytosol, the abundant mRNA would compete the siRNA binding sites of dsRBD and results in fast release of free siRNA. Our studies revealed that around 60% dsRBD/siRNA was disassembled in 1 h in the presence of cellular RNAs (Fig. 2f) [31]. The dsRBD octamer provides a discrete number of siRNA loading sites (8 siRNA per particle) and has a high siRNA payload (>30%). Moreover, the multivalent effect of the protein carrier dramatically enhances the binding affinity of siRNA ligand to tumor cells by over 100 times, which leads to more efficient tumor deposition and specific gene silencing in vivo.

## 3. p19 protein from carnation Italian ringspot virus

p19 is a natural siRNA inhibitor expressed by many tombusviruses (for example the carnation Italian ringspot virus) [32]. The eukaryotes, particularly plants, use the evolutionarily conserved RNA interference pathway to deal with the invaded virus. As a result, many plant viruses fight back by producing a 19 kDa protein (p19) and utilizing it as a blocker of the anti-viral siRNA generated by the infected plants [33]. The p19 protein binds tightly to the minor groove of siRNA duplex with affinity ranging from nM to pM ($K_D$) (different measurement method) [34]. Similar to dsRBD, p19 binds siRNA in a sequence-independent manner and its specificity is based on the length of the siRNA (Fig. 3a).

In the process of discovering therapeutic targets, RNAi has been an important technology [35]. Although it has attracted increased interest for potential therapeutics, the poor cellular uptake and rapid enzymatic degradation are the main barriers that impede the therapeutic applications [3]. Some reports demonstrate that p19 is an extraordinarily strong binder of siRNA ($K_D$ ~ 0.2 nM) and able to enhance the siRNA stability in serum conditions [24,34,36]. Choi et al. report that when p19 fuse to a peptide, ephrin mimetic peptide (called YSA peptide), the p19-YSA fusion protein could efficiently delivery the siRNA into the EphA2 receptor overexpressed cancer cells [36]. The strong 'caliper-like' association buried siRNA inside the cavity of p19 dimer and protected it from the degradation of external RNase. The results showed that the expression of the red fluorescent protein (RFP) gene was suppressed when the SKOV3 cells treated with p19-YSA/siRNA complexes. As a homodimer, p19-YSA interacts with siRNA through hydrogen bonding, hydrophobic and salt bridge interaction, which can tolerate the interruption of polyanionic biomaterials found in extracellular matrix [36].

In designing the p19 protein as a siRNA delivery carrier, Danielson et al. fused a Tat peptide to enhance the cytosolic delivery of siRNA (Fig. 3b) [34]. Tat peptide is a well-known cell-penetrating peptide (CPP) derived from amino acid 49–57 (RKKRRQRRR) of the HIV-1 TAT protein [34]. It is a cationic short peptide that has been widely used for mediating cell entry of fusion protein through the transient pore on the plasma membrane [37,38]. Recombinant p19 proteins fused with a Tat peptide has been constructed to deliver siRNA into the cytoplasm of hepatoma cells by harnessing this cell entry mechanism. The luciferase reporter assay reveals that p19-Tat/siRNA complex elicits potent and sustained gene knockdown in human cells without cytotoxic effects [34]. Similarly, Yang et al. engineered a series of p19 derivatives that show ultra-high affinity to siRNA duplex [24]. By fusing with a peptide ligand E18, p19 carrier can selectively carry siRNA into A431 cell that overexpresses the epidermal growth factor receptor (EGFR). Surprisingly, it was found that the stronger siRNA-carrier affinity correlated with more potent silencing (Fig. 3d). The best siRNA binder $p19^{N15K,G16R}$ showed the most potent silencing activity, with an EC50 of 0.23 nM for knockdown of GFP, in comparison to the EC50 of 5.9 nM by canonical p19 protein. This reinforced p19 carrier, with the aid of endosome-disrupting protein PFO, successfully carries the anti-GFP siRNA into cytoplasm of A431-EGFP cells and give a silencing effect of ~82% (GFP is knockdown to ~18% at the siRNA concentration of 5 nM), which is slightly better than lipofectamine (~80% at the siRNA concentration of 10 nM) (Fig. 3e). It is worth noting that the combination of p19 and PFO is essential for the siRNA delivery. The p19 carrier plays the major role to carry siRNA into endosomal compartment of the target cells, but lack of the endosome escaping functionality. The majority of siRNA is degraded in endosome/lysosome vesicles and give a silencing effect as low as around 10% when the siRNA is delivered by p19 carrier alone (Fig. 3d).

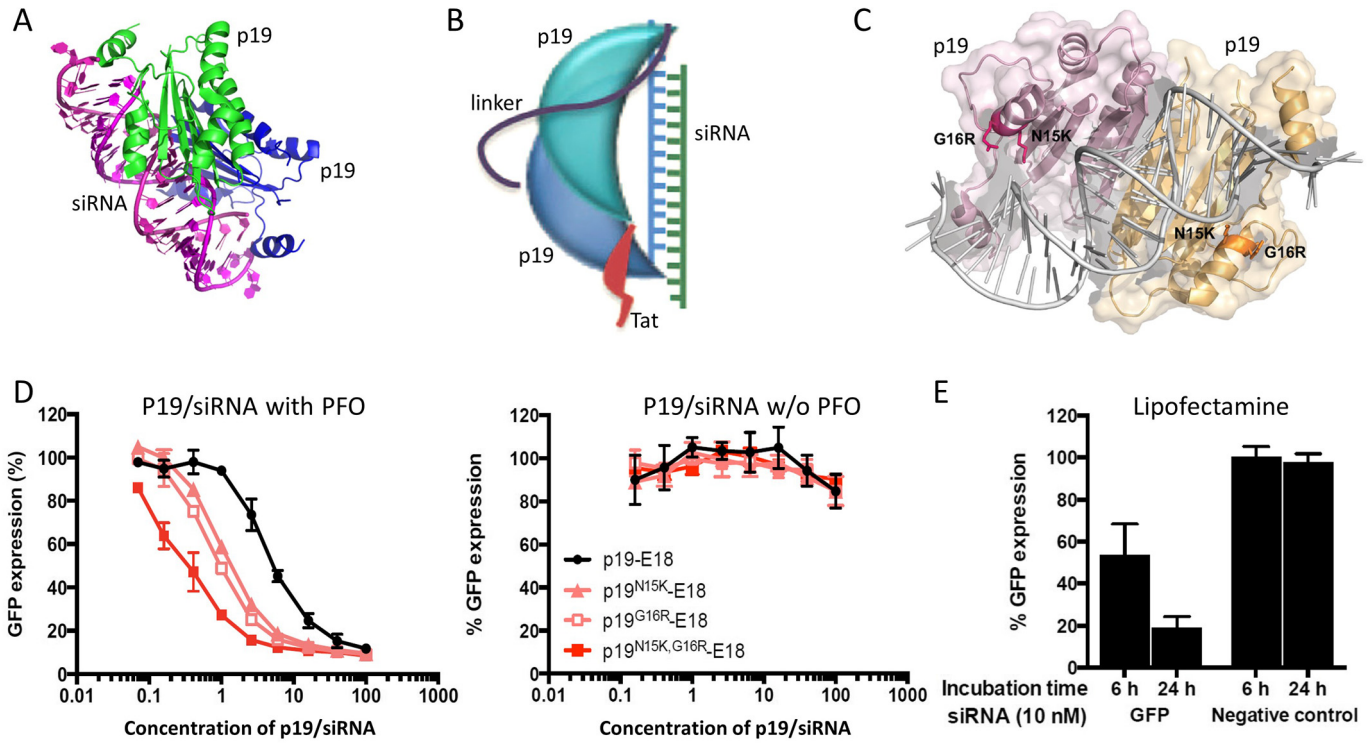


**Fig. 3.** (A) Cartoon representation of siRNA bound with two copies of p19 proteins. The figure was drawn using the PyMOL Molecular Graphics System based on crystal structure information from PDB ID 1RPU. (B) Tandem p19 proteins conjugated with Tat peptide and binds with siRNA. Adapted with permission from Mol. Ther. Nucleic Acids 2016, 5, e303 (C) The p19 mutant (G16R and N15K) binds siRNA with extremely high affinity. (D) Affinity-dependent silencing mediated by the p19 carriers. Pore forming protein PFO plays an essential role in siRNA delivery. The mutated p19 shows different binding affinity and stability to siRNA. An EGFR binder E18 engineered on the Fn3 scaffold was fused to the C terminus of p19. (E) Gene silencing effect of lipofectamine/siRNA complex at 6 h and 24 h post transfection. The figure C, D and E were adapted with permission from Nucleic Acids Res. 2017, 45, 7602–7614.

## 4. U1A RBD

The U1 small nuclear ribonucleoprotein A (U1A) is a component of the spliceosome that recognizes pre-mRNA by binding stem-loop II of U1 snRNA [39]. Protein U1A has a relatively small RBD of 98 amino acid, but it can recognize a short RNA loop with high affinity and sequence specificity [40]. Saito group utilized the loop-sequence specificity of U1A to design a protein device that can be responsive to a short hairpin RNA (shRNA) [41]. To further explore the potential of U1A as a siRNA carrier, Endoh et al. prepared a special siRNA bearing a short loop sequence at the 3′ end of the sense strand, which assigned to be recognized by U1A RBD (Fig. 4a) [42]. Gel mobility shift assay confirmed the special interaction between the U1A carrier and the loop siRNA. However, the intracellular trafficking experiment reveals that the U1A/siRNA complex could be uptake by CHO cells but the majority exhibited the punctuate cytoplasmic distribution, a sign of trapping the cargo in the endosome. Even with the aid of the conjugated Tat peptide, the U1A mediated siRNA delivery can only achieve a moderate level (~30%) of EGFP gene knockdown at a concentration of 1 μM. Finally, the trapped siRNA was rescued from endocytic compartments by photostimulation of TatU1A (Alexa Fluor 546 labeled) for 60 s (Fig. 4b).

## 5. Other RBPs

RBP family contains a large number of ribonucleoproteins that take part in many biological events from mRNA splicing, signal transduction to gene regulation. The diversity of RBP functions would suggest a correspondingly large number of structural variants that are responsible for RNA recognition [14]. In the approximately 20 known structures of RBP/RNA complexes, however, only a few RBPs have been utilized for siRNA packing and delivery. Besides the aforementioned RBPs, it is worth noting that another dsRBD analogue, human trans activation response element RNA Binding Protein (TARBP2), has been developed to deliver siRNA to the brain of AβPP-PS1 mouse model of Alzheimer's

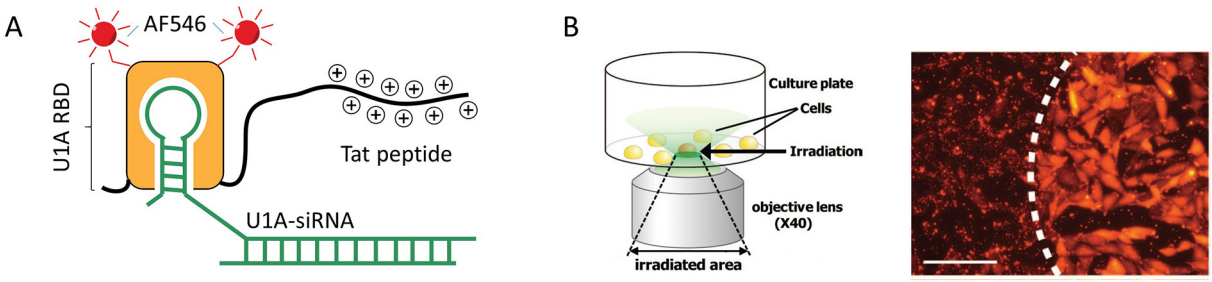


**Fig. 4.** (A) U1A RBD binds with hairpin siRNA by recognizing the stem loop. The Tat peptide tail facilitates the internalization of the complex to endocytic compartments. (B) Region-specific photostimulation of cells triggers the release of U1A/siRNA from endocytic compartments. Scale bar, 100 μm. Adapted with permission from Bioconjugate Chem. 2008, 19, 1017–1024.

disease [43,44]. Importantly, the siRNA recognition nature of TARBP2 resulted in a monodisperse serum-stable RBP/siRNA complex and showed a potential to cross the blood–brain barrier (BBB). By fusing with a brain targeting peptide that binds to monosialoganglioside GM1, TARBP2 led to distinctive localization of the siRNA in the cerebral hemisphere and a significant knockdown of the targeted gene in the brain of both AβPP-PS1 mice and wild type C57BL/6 [43]. This study expands the path of RBPs to deliver siRNA into the brain tissue.

## 6. Conclusion

Safety and clearance of the siRNA carrier are of major concern, because repeated injection, probably long-term treatment, are often required. The conventional cationic carriers condense siRNA and assemble into the polydisperse nanoparticles that possess excessive positive charges. There is an ongoing appreciation that charge-neutral carriers can avoid the possibility of the undesirable electrostatic interaction between the cationic complex and surrounding components (negative-charged cell membrane, serum proteins, and others), thus enhancing the specificity and safety during siRNA delivery. RBPs interact with siRNA by a structural recognition of RNA duplex in a charge-independent manner. It is emerging as a popular building block to develop the charge-free and monodisperse carriers for siRNA delivery. Because of the monodisperse nature, RBP carriers act more like macromolecules in vivo, and thus both pharmacokinetics and bioactivity are more predictable than polydisperse nanoparticles.

To achieve a targeted delivery to the disease sites, it is essential to avoid the premature release of siRNA from the RBP/siRNA complex. However, most of the RBPs only shows a moderate affinity to siRNAs, and exhibits a half-life of around 10 h or less in mouse serum at 37 °C. p19 is the only RBP that binds siRNA with $K_D$ in the picomolar rang. Its siRNA complex is very stable in serum, but it is a virus-derived protein and may have a limited therapeutic window due to immunogenicity. Besides the siRNA affinity, RBP carriers may encounter the problem of endosomal entrapment, a rate-determining step of siRNA delivery. Many endocytic agents have been investigated to facilitate the endosomal escape of the carrier/siRNA complex. Combined with the pore-forming protein PFO, RBP p19 carrier has achieved the potent in-vitro silencing effect even higher than commercial lipofectamine. Collectively, the current studies of RBP carriers have led to multiple improvements in siRNA delivery. The monodisperse nature is the unique feature [1] of this siRNA delivery system, which increases the feasibility of delivery in vivo and is expected to enhance the clinical performance in the future.

## Author statement

**Wen Wang**: Writing-Original draft preparation.

**Wanyi Tai**: Conceptualization, Writing - Original Draft, Review & Editing, Supervision.

## Conflict of Interest

The authors declare no conflict of interest.

## Acknowledgements

This work was supported by the start-up package of Wuhan University.

## References

[1] Tolia NH, Joshua-Tor L. Slicer and the argonautes. Nat Chem Biol 2007;3(1):36–43.
[2] Liu B, et al. Effects of gold nanoprism-assisted human PD-L1 siRNA on both gene down-regulation and photothermal therapy on lung cancer. Acta Biomater 2019;99:307–19.
[3] Singh A, Trivedi P, Jain NK. Advances in siRNA delivery in cancer therapy. Artif Cells Nanomed Biotechnol 2018;46(2):274–83.
[4] Whitehead KA, Langer R, Anderson DG. Knocking down barriers: advances in siRNA delivery. Nat Rev Drug Discov 2009;8(2):129–38.
[5] Oh YK, Park TG. siRNA delivery systems for cancer treatment. Adv Drug Deliv Rev 2009;61(10):850–62.
[6] Bobbin ML, Rossi JJ. RNA interference (RNAi)-based therapeutics: delivering on the promise? Annu Rev Pharmacol Toxicol 2016;56:103–22.
[7] Kanasty R, et al. Delivery materials for siRNA therapeutics. Nat Mater 2013;12(11):967–77.
[8] Kedmi R, Ben-Arie N, Peer D. The systemic toxicity of positively charged lipid nanoparticles and the role of Toll-like receptor 4 in immune activation. Biomaterials 2010;31(26):6867–75.
[9] Tao W, et al. Mechanistically probing lipid-siRNA nanoparticle-associated toxicities identifies Jak inhibitors effective in mitigating multifaceted toxic responses. Mol Ther 2011;19(3):567–75.
[10] Kim HK, et al. Enhanced siRNA delivery using cationic liposomes with new polyarginine-conjugated PEG-lipid. Int J Pharm 2010;392(1–2):141–7.
[11] Anilmis NM, et al. Designing siRNA-conjugated plant oil-based nanoparticles for gene silencing and cancer therapy. J Microencapsul 2019:1–14.
[12] Turner M, Diaz-Munoz MD. RNA-binding proteins control gene expression and cell fate in the immune system. Nat Immunol 2018;19(2):120–9.
[13] Velasco MX, et al. The diverse roles of RNA-binding proteins in glioma development. Adv Exp Med Biol 2019;1157:29–39.
[14] Lunde BM, Moore C, Varani G. RNA-binding proteins: modular design for efficient function. Nat Rev Mol Cell Biol 2007;8(6):479–90.
[15] Peng L, Wagner E. Polymeric carriers for nucleic acid delivery: current designs and future directions. Biomacromolecules 2019;20:3613–26.
[16] Toroney R, et al. Mechanistic characterization of the 5′-triphosphate-dependent activation of PKR: lack of 5′-end nucleobase specificity, evidence for a distinct triphosphate binding site, and a critical role for the dsRBD. RNA 2012;18(10):1862–74.
[17] Spanggord RJ, Vuyisich M, Beal PA. Identification of binding sites for both dsRBMs of PKR on kinase-activating and kinase-inhibiting RNA ligands. Biochemistry 2002;41(14):4511–20.
[18] Ryter JM, Schultz SC. Molecular basis of double-stranded RNA-protein interactions: structure of a dsRNA-binding domain complexed with dsRNA. EMBO J 1998;17(24):7505–13.
[19] Manche L, et al. Interactions between double-stranded RNA regulators and the protein kinase DAI. Mol Cell Biol 1992;12(11):5238–48.
[20] Husain B, Mukerji I, Cole JL. Analysis of high-affinity binding of protein kinase R to double-stranded RNA. Biochemistry 2012;51(44):8764–70.
[21] Eguchi A, et al. Efficient siRNA delivery into primary cells by a peptide transduction domain-dsRNA binding domain fusion protein. Nat Biotechnol 2009;27(6):567–71.
[22] Tian B, Mathews MB. Functional characterization of and cooperation between the double-stranded RNA-binding motifs of the protein kinase PKR. J Biol Chem 2001;276(13):9936–44.
[23] Geoghegan JC, Gilmore BL, Davidson BL. Gene silencing mediated by siRNA-binding fusion proteins is attenuated by double-stranded RNA-binding domain structure. Mol Ther Nucleic Acids 2012;1:e53.
[24] Yang NJ, et al. Cytosolic delivery of siRNA by ultra-high affinity dsRNA binding proteins. Nucleic Acids Res 2017;45(13):7602–14.
[25] Tai W, Gao X. Ribonucleoprotein: a biomimetic platform for targeted siRNA delivery. Adv Funct Mater 2019;29(35):1902221.
[26] Sun P, et al. siRNA-loaded poly(histidine-arginine)6-modified chitosan nanoparticle with enhanced cell-penetrating and endosomal escape capacities for suppressing breast tumor metastasis. Int J Nanomedicine 2017;12:3221–34.
[27] Lo SL, Wang S. An endosomolytic Tat peptide produced by incorporation of histidine and cysteine residues as a nonviral vector for DNA transfection. Biomaterials 2008;29(15):2408–14.
[28] Qin B, et al. Development of cholesteryl peptide micelles for siRNA delivery. J Control Release 2013;172(1):159–68.
[29] Asayama S, Kumagai T, Kawakami H. Synthesis and characterization of methylated poly(L-histidine) to control the stability of its siRNA polyion complexes for RNAi. Bioconjug Chem 2012;23(7):1437–42.
[30] Nakagawa O, et al. Conjugation with receptor-targeted histidine-rich peptides enhances the pharmacological effectiveness of antisense oligonucleotides. Bioconjug Chem 2014;25(1):165–70.
[31] Tai W, et al. A ribonucleoprotein octamer for targeted siRNA delivery. Nature Biomedical Engineering 2018;2(5):326–37.
[32] Lakatos L, et al. Molecular mechanism of RNA silencing suppression mediated by p19 protein of tombusviruses. EMBO J 2004;23(4):876–84.
[33] Kontra L, et al. Distinct effects of p19 RNA silencing suppressor on small RNA mediated pathways in plants. PLOS Pathogens 2016;12(10):e1005935.
[34] Danielson DC, et al. A novel p19 fusion protein as a delivery agent for short-interfering RNAs. Mol Ther Nucleic Acids 2016;5:e303.
[35] Wittrup A, Lieberman J. Knocking down disease: a progress report on siRNA therapeutics. Nat Rev Genet 2015;16(9):543–52.
[36] Choi KM, et al. Efficient siRNA delivery into tumor cells by p19-YSA fusion protein. Mol Pharm 2013;10(2):763–73.
[37] Milletti F. Cell-penetrating peptides: classes, origin, and current landscape. Drug Discov Today 2012;17(15–16):850–60.
[38] Jiang T, et al. Dual-functional liposomes based on pH-responsive cell-penetrating peptide and hyaluronic acid for tumor-targeted anticancer drug delivery. Biomaterials 2012;33(36):9246–58.

[39] Oubridge C, et al. Crystal structure at 1.92 A resolution of the RNA-binding domain of the U1A spliceosomal protein complexed with an RNA hairpin. Nature 1994;372(6505):432–8.
[40] Hall KB, Stump WT. Interaction of N-terminal domain of U1A protein with an RNA stem/loop. Nucleic Acids Res 1992;20(16):4283–90.
[41] Kashida S, Inoue T, Saito H. Three-dimensionally designed protein-responsive RNA devices for cell signaling regulation. Nucleic Acids Res 2012;40(18):9369–78.
[42] Endoh T, Sisido M, Ohtsuki T. Cellular siRNA delivery mediated by a cell-permeant RNA-binding protein and photoinduced RNA interference. Bioconjug Chem 2008;19(5):1017–24.
[43] Haroon MM, et al. A designed recombinant fusion protein for targeted delivery of siRNA to the mouse brain. J Control Release 2016;228:120–31.
[44] Haroon MM, et al. Delivery of BACE1 siRNA mediated by TARBP-BTP fusion protein reduces beta-amyloid deposits in a transgenic mouse model of Alzheimer's disease. J Biosci 2019;44(1).