*RNA & DISEASE* 2019; 6: e1534. doi: 10.14800/rd.1534; © 2019 by Hong Yan Liu
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REVIEW

# Bivalent aptamer-dual siRNA chimera is emerging as a new combination therapy

Hong Yan Liu

*Center for Biotechnology and Genomic Medicine, Department of Biochemistry and Molecular Biology, Georgia Cancer Center Medical College of Georgia, Augusta University, Augusta, GA, 30912, USA*

Correspondence: Hong Yan Liu
E-mail: HOLIU@augusta.edu
Received: March 06, 2018
Published: April 10, 2019

**The selective delivery of siRNAs in a cell type-specific manner represents the major challenge for the application of RNA interference for disease treatment. Aptamers have great potentials as carriers for tumor specific siRNA delivery. With the nature of nucleic acid, aptamers can be ease of modification and editing. Novel bivalent aptamer-dual siRNA chimera (PSMA aptamer- survivin siRNA -EGFR siRNA -PSMA aptamer, PSEP) was developed by fusing two siRNAs (specific to EGFR and survivin) between two PSMA aptamers. Bivalent aptamer offers increased siRNA internalization compared with monovalent counterpart. PSEP chimera is able to inhibit EGFR and survivin simultaneously in a cell type-specific manner. In PSMA expressing tumor xenografts, PSEP significantly inhibits tumor growth and angiogenesis. Our results highlight that co-delivery of multiple siRNAs with bivalent aptamer represents a novel approach for targeting combination therapy.**

***Keywords:*** bivalent aptamer; siRNA delivery; combination therapy; oncogene silencing

**To cite this article:** Hong Yan Liu. Bivalent aptamer-dual siRNA chimera is emerging as a new combination therapy. RNA Dis 2019; 6: e1534. doi: 10.14800/rd.1534.



Aptamers are synthetic single stranded DNA or RNA that can bind to a wide range of targets, including small organic molecules, peptides, proteins, nucleic acids, and whole cells with high affinity and specificity. Aptamers are generated from an iterative process called systematic evolution of ligands by exponential enrichment (SELEX), first established in 1990s by Ellington and Gold [1, 2]. Ellington reported that a selected RNA molecule can specifically bind to organic dyes, and Gold demonstrated that an RNA could interact with T4 DNA polymerase. Since then, aptamers have become a notable class of targeting ligand for both diagnostics and therapeutics.

As nucleic acid-based molecules, aptamers possess significant advantages over antibodies including ease of modification, ease of production, low cost, low immunogenicity, low toxicity and small size, all of which make aptamers ideal candidates for targeted cancer therapy.

Cancer is highly heterogeneous and complex, particular at advanced stages. Single-agent treatments exhibit limited activity in clinical setting because cancer cells are able to switch to alternative survival pathways. Combination therapy to target several oncogenic pathways simultaneously, therefore, may have better efficacy in eradicating tumors. Current chemical drug combination is associated with

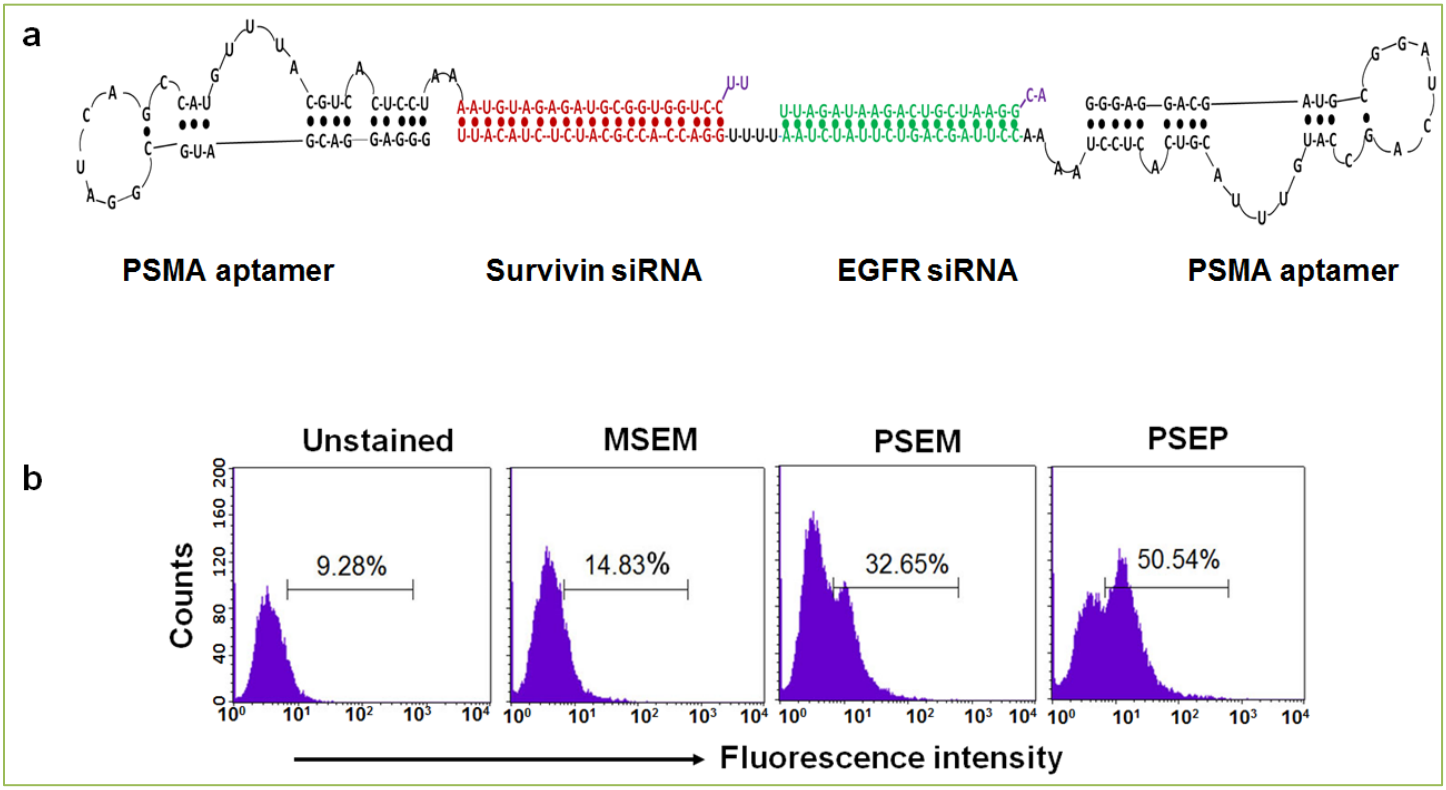


**Figure 1. Design and characterization of bivalent aptamer-dual siRNA chimera.** (**a**) Structure of PSEP: PSMA aptamer-survivin siRNA-EGFR siRNA-PSMA aptamer. PSEP chimera consists of a bivalent PSMA aptamer and two siRNAs specific to survivin and EGFR, respectively. Each antisense strand of siRNAs has a 2-nt overhang at the 3′ end. (**g**) Detection of internalization. C4-2 cells were treated with Cy3-labeled PSEP (bivalent PSEP aptamer), PSEM ( monovalent PSMA aptamer), or MSEM ( bivalent non-targeting MG aptamer ) for 2 h at 37 °C. Cells were washed with DPBS plus 0.5 M NaCl to remove surface bound RNAs. The amount of fluorescently labeled chimeras that internalized into cells was measured using flow cytometry. The fluorescent intensity of PSEP has about 1-fold increase compared with monovalent counterpart PSEM.

overlapping toxicity to normal organs and quickly developed drug resistance. Although kinase inhibitor combinations show the efficacy and certain molecule targeting, most kinase inhibitors are able to target on multiple kinases (low specificity), and combination of different kinases may easily cause increased toxicity like other chemical drugs. Combination of monoclonal antibodies are usually more specific, however, antibodies cannot block intracellular targets and have high immunogenicity due to their membrane impermeability and recognition by host as foreign.

siRNA has great potential for sequence-specific silencing of any genes and has emerged as a promising new therapeutic paradigm for "undruggable" targets [3-5]. However, cell type -specific delivery of siRNA in vivo still represents a major technical hurdle [6]. Carriers using small molecules, lipids, peptides and polymers have been linked with siRNA for delivery of siRNA to diseases cells [7]. Considering the complex physical and chemical structures of various formulations, current siRNA delivery vehicles face difficulties in large-scale production and regulatory approval of clinical uses.

Aptamer-siRNA chimera (AsiC), employing only RNA molecules, is emerging as a highly promising approach for cell type-specific RNA interference (RNAi). Cell-based SELEX allows the selection of internalized aptamer, which can introduce the intracellular delivery of cargo through receptor-mediated endocytosis [8, 9]. Aptamers have specific 3-dimentional structures for target binding, and specific binding capability can be kept in vivo. Aptamer-siRNA chimera has shown the advantages of low off-target effect and low immunogenicity. On the contrary, small chemical drugs suffer severe off-target effect that can target normal organs, and antibody -based therapeutic can incur strong immunogenicity. As a single-component entity, AsiC also has advantages in ease of synthesis and high tissue penetrability. Importantly, AsiC-based drugs can utilize endogenous enzymes (e.g. dicer, argonaute) and trigger gene silencing in cell type- and mRNA sequence-specific manner. Therefore, AsiC can provide selective and effective inhibition of any protein targets that can locate in cell membrane, cytoplasm and nucleus. CD4 aptamer-tat/rev siRNA chimera can inhibit HIV transmission [10]. PSMA aptamer-PLK1siRNA enables the regression of prostate cancer [11]. CTLA4 aptamer-STAT3 siRNA inhibits

tumor-associated Tregs and reduces tumor burden in multiple mouse tumor models [12]. EpCAM aptamer-survivin siRNA can reverse doxorubicin resistance and increase survival time in mice bearing chemoresistant tumors [13].

Most current chimeras are designed as the fusion of one aptamer with one siRNA [11, 12, 14-16]. However, it is important to simultaneous delivery of multiple siRNAs in one chimera. In recent studies, we have designed and developed a bivalent PSMA aptamer for co-delivery of two siRNAs (specific to EGFR and survivin) as shown in Figure 1a. Bivalent aptamer is expected to have antibody-like properties in terms of inducing cell activation by cross-linking two receptors. Many studies have shown that bivalent ligands can induce receptor clustering which will trigger signaling transduction and cell activation [17, 18]. For example, bivalent antibody can form non-covalently crosslinked complex with the BCR (B cell antigen receptor), but not monovalent single chain antibody. BCR clustering can change membrane organization and elicit robust calcium signals [19]. Ligand induced receptor dimerization represents a powerful regulatory mechanism that can activate intracellular signal pathways [20]. Bivalent ligands enable easily induction of receptor dimerization. In our studies, to increase the specificity, binding avidity and internalization of aptamer-siRNA chimera, we designed a bivalent aptamer to facilitate the engagement of cell surface protein and trigger cell activation [21]. Activated cells will allow increased cargo internalization [22]. PSMA aptamer against prostate-specific membrane antigen (PSMA) has been proven to enable efficient delivery of siRNAs into PSMA-expressing prostate cancer (PCa) cells [23, 24]. In our studies, two important oncogenes including EGFR and survivin were targeted and fused with two PSMA aptamers. By fusing of two aptamers and two siRNAs, we have developed a novel bivalent aptamer-dual siRNA chimera: PSMA aptamer-survivin siRNA-EGFR siRNA-PSMA aptamer (PSEP) [25]. We proved that PSEP chimera can be cut by dicer. Two tandem siRNAs spaced with four "U"s warrant the cleavage with dicer enzyme but not inactivating two genes, since dicer is able to measure and cut 21-25-nt RNA duplex [26-28]. We have designed survivin anti-sense and EGFR anti-sense strands with 2-nt overhang at the 3′ end of siRNAs, which will aid the siRNA-RISC (RNA-induced silencing complex) formation (Figure 1a). It is worth emphasizing, in the design, it is important to exam whether there are significant complementarities among aptamer and siRNAs. No significant complementarity will ensure the correct folding by annealing.

We have demonstrated that bivalent aptamer offers increased siRNA internalization compared with monovalent aptamer. C4-2 cells were treated with Cy3-labeled chimeras followed by washing with 0.5M NaCl containing DPBS to remove surface bound chimeras. The amount of internalized chimeras was quantitated using flow cytometry. As shown in Figure 1b, the fluorescence intensity in PSEP treated C4-2 cells have increased about 1-fold compared with that in monovalent PSEM-treated cells, the results confirm the advantage of bivalent aptamer over the monovalent counterpart in promoting siRNA internalization.

Unmodified RNA aptamers are susceptible to nuclease-mediated degradation. RNA aptamers can be modified by replacing ribose 2'-OH group with fluoro (F), amino (NH2), or O-methyl (OCH3). Because ribonucleases select 2′-OH group for cleavage of phosphodiester bonds. The ribose 2'-OH modified aptamers have significantly increased nuclease resistance while also enhancing binding affinity. In the process of synthesis of PSEP, 2′ F-pyrimidines are incorporated into entire chimera via *in vitro* transcription. We have characterized the serum stability of 2' F-modified PSEP in denaturing gel electrophoresis. We incubated PSEP chimera in PBS containing 50% fresh human serum for 1, 2, 3 and 4 h. Electrophoresis revealed that 2′ F-modified PSEP did not show detectable degradation within 4 h, and over 60% of modified RNA does not have degradation for 24h. On the contrary, degraded RNA pattern was observed for unmodified PSEP as early as at 1 h incubation.

Notably, bivalent aptamer and dual siRNA chimera will have longer circulation time than aptamer and siRNA alone. Most aptamers and siRNAs are 5-20 Kd and are therefore susceptible to renal filtration since renal glomerulus has 30-50 Kd of molecular mass cutoff. To overcome short circulation half-life, PEG has been conjugated with aptamer -siRNA chimera to increase circulation time in vivo [11]. In our new chimera, the size of PSEP is bout 59Kd, which is much larger than current PSMA aptamer-siRNA chimeras [11, 29] (around 29Kd). We envision that chimera with a bivalent aptamer and dual siRNAs should possess increased circulating half-life and reduced renal excretion. Owing to being nucleic acids, PSEP will perform better than PEG in ease of clearance. We demonstrated that PSEP chimera specifically and effectively suppresses the endogenous expression of EGFR and survivin in PSMA expressing PCa cells, and clearly inhibits tumor growth in mouse models. Notably, co-delivery of two siRNAs (EGFR and survivin) significantly inhibit tumor angiogenesis. Our work is the first time report that two different siRNAs can be simultaneously delivered by a bivalent aptamer. Co-delivery of two siRNAs in one RNA chimera provides a new and efficient approach for combination therapy. Since the system is highly modular, our work can be used to design many co-delivery systems by using siRNAs and aptamers.

## Conclusions

Aptamer and siRNAs are highly compatible with combinational therapy, and may address the limitations of current combination treatments with antibodies and small chemical drugs in terms of immunogenicity and systemic toxicity. Aptamers opened a new horizon for targeting cancer treatment. Concomitant delivery of multiple siRNAs with bivalent aptamer will be potential to provide synergistic efficacy for cancer therapy.

## Conflicting interests

The authors have declared that no conflict of interests exist.

## Acknowledgements

This work is supported by start-up funding of Augusta University and Department of Defense W81XWH-15-1-0333 (to H.Y.L.).

### References

1. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. Science 1990; 249: 505-510
2. Ellington AD, Szostak JW. In vitro selection of RNA molecules that bind specific ligands. Nature 1990; 346: 818-822.
3. Hannon GJ. RNA interference. Nature 2002; 418: 244-251.
4. Jensen SA, Day ES, Ko CH, Hurley LA, Luciano JP, Kouri FM, *et al*. Spherical nucleic acid nanoparticle conjugates as an RNAi-based therapy for glioblastoma. Sci Transl Med 2013; 5: 209ra152.
5. Wu SY, Lopez-Berestein G, Calin GA, Sood AK. RNAi therapies: drugging the undruggable. Sci Transl Med 2014; 6: 240ps7.
6. Whitehead KA, Langer R, Anderson DG. Knocking down barriers: advances in siRNA delivery. Nat Rev Drug Discov 2009; 8: 129-138.
7. Jeong JH, Mok H, Oh YK. & Park TG. siRNA conjugate delivery systems. Bioconjug Chem 2009; 20: 5-14.
8. Dassie JP, Giangrande PH. Current progress on aptamer-targeted oligonucleotide therapeutics. Ther Deliv 2013; 4: 1527-1546.
9. Zhou J, Li H, Zhang J, Piotr S, Rossi J. Development of cell-type specific anti-HIV gp120 aptamers for siRNA delivery. J Vis Exp 2011; 52: pii: 2954. doi: 10.3791/2954.
10. Neff CP, Zhou J, Remling L, Kuruvilla J, Zhang J, Li H, *et al*. An aptamer-siRNA chimera suppresses HIV-1 viral loads and protects from helper CD4(+) T cell decline in humanized mice. Sci Transl Med 2011; 3: 66ra6. doi: 10.1126/scitranslmed.3001581.
11. Dassie JP, Liu XY, Thomas GS, Whitaker RM, Thiel KW, Stockdale KR, *et al*. Systemic administration of optimized aptamer-siRNA chimeras promotes regression of PSMA-expressing tumors. Nat Biotechnol 2009; 27: 839-849.
12. Herrmann A. Priceman SJ, Swiderski P, Kujawski M, Xin H, Cherryholmes GA, *et al*. CTLA4 aptamer delivers STAT3 siRNA to tumor-associated and malignant T cells. J Clin Invest 2014; 124: 2977-2987.
13. Wang T, Gantier MP, Xiang D, Bean AG , Bruce M, Zhou SF, *et al*. EpCAM Aptamer-mediated Survivin Silencing Sensitized Cancer Stem Cells to Doxorubicin in a Breast Cancer Model. Theranostics 2015; 5: 1456-1472.
14. Zhou J, Li H, Li S, Zaia J, Rossi JJ. Novel dual inhibitory function aptamer-siRNA delivery system for HIV-1 therapy. Mol Ther 2008; 16: 1481-1489.
15. Hussain AF, Tur MK, Barth S. An aptamer-siRNA chimera silences the eukaryotic elongation factor 2 gene and induces apoptosis in cancers expressing alphavbeta3 integrin. Nucleic Acid Ther 2013; 23: 203-212.
16. Wheeler LA, Trifonova R, Vrbanac V, Basar E, McKernan S, Xu Z, *et al*. Inhibition of HIV transmission in human cervicovaginal explants and humanized mice using CD4 aptamer-siRNA chimeras. J Clin Invest 2011; 121: 2401-2412.
17. Gestwicki JE, Cairo CW, Strong LE, Oetjen KA, Kiessling LL. Influencing receptor-ligand binding mechanisms with multivalent ligand architecture. J Am Chem Soc 2002; 124: 14922-14933.
18. Mammen M, Choi S K, Whitesides GM. Polyvalent interactions in biological systems: Implications for design and use of multivalent ligands and inhibitors. Angewandte Chemie-International Edition 1998; 37: 2755-2794.
19. Puffer EB, Pontrello JK, Hollenbeck JJ, Kink JA, Kiessling LL. Activating B cell signaling with defined multivalent ligands. ACS Chem Biol 2007; 2: 252-262.
20. Klemm JD, Schreiber SL. Crabtree GR. Dimerization as a regulatory mechanism in signal transduction. Annual Review of Immunology 1998; 16: 569-592.
21. Cochran JR, Aivazian D, Cameron TO, Stern LJ. Receptor clustering and transmembrane signaling in T cells. Trends Biochem Sci 2001; 26: 304-310.
22. Qureshi OS, Kaur S, Hou TZ, Jeffery LE, Poulter NS, Briggs Z, *et al*. Constitutive clathrin-mediated endocytosis of CTLA-4 persists during T cell activation. J Biol Chem 2012; 287:9429-9440.
23. McNamara JO 2nd, Andrechek ER, Wang Y, Viles KD, Rempel RE, Gilboa E, *et al*. Cell type-specific delivery of siRNAs with aptamer-siRNA chimeras. Nat Biotechnol 2006; 24: 1005-1015.
24. Pastor F, Kolonias D, Giangrande PH, Gilboa E. Induction of tumour immunity by targeted inhibition of nonsense-mediated mRNA decay. Nature 2010; 465: 227-230.
25. Liu H Y, Yu X, Liu H, Wu D, She JX. Co-targeting EGFR and survivin with a bivalent aptamer-dual siRNA chimera effectively suppresses prostate cancer. Sci Rep.2016; 6: 30346; doi: 10.1038/srep30346.
26. Siomi H, Siomi MC. On the road to reading the RNA-interference code. Nature 2009; 457: 396-404.
27. Jinek M, Doudna JA. A three-dimensional view of the molecular machinery of RNA interference. Nature 2009; 457: 405-412.
28. Bernstein E, Caudy AA, Hammond SM, Hannon GJ. Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 2001; 409: 363-366.

29. Wullner U, Neef I, Eller A, Kleines M, Tur MK, Barth S. Cell-specific induction of apoptosis by rationally designed bivalent aptamer-siRNA transcripts silencing eukaryotic elongation factor 2. Curr Cancer Drug Targets 2008; 8: 554-565.