Nucleic Acid Aptamer- Drug Delivery for Targeted Cancer Therapy

Introduction

Today, cancer therapy is based primarily on chemotherapy and radiation, both of which have well-established side effects. Chemotherapy drugs typically kill not only cancer cells but also normal cells, and administration of bolus doses of these strong drugs can result in unpredictable side effects(1) . One way of generating effective molecular probes to differentiate diseased cells is to use aptamers, that are capable of identifying molecular level differences between a healthy cell and a diseased cell(2).Aptamers are single-stranded oligonucleotides that can specifically recognize their targets, including live cancer cells, and bind to the targets with high affinity(3). DNA aptamers targeting cancer cells can be selected from a random library of 1013–1016 ssDNA or ssRNA molecules through an in vitro technology known as cell-SELEX (systematic evolution of ligands by exponential enrichment) technology.(4). They are generated through cell-SELEX can be acquired without having prior knowledge of the molecular signatures of proteins found on the cell surface, making aptamers ideal tools for applications in cancer diagnosis and treatment. The three major steps of cell-SELEX, including incubation, partitioning

and amplification, are shown in Figure 1. Using this method, DNA aptamers against several kinds of cancer cells, such as lung cancer, liver cancer,[7] ovarian cancer,[8] ALL (T-cell leukemia),[ 9] colorectal cancer,[10] B-cell lymphoma,[11] breast cancer,[12] and acute myeloid leukemia (AML),[13] have been developed.

Figure 1. Schematic illustration of the cell-SELEX (systematic evolution of ligands by exponential enrichment) process. DNA sequence specifically recognizing target cells is enriched and cloned. Positive clones are sequenced to identify individual aptamers. Copyright \_ 2006 National Academy of Sciences. Reproduced with permission from Reference [9].

Aptamer−Drug Conjugates for Targeted Drug Delivery

1. Phosphoramidite D

Aptamer−drug conjugates (ApDCs) have been developed to exploit aptamer-based targeted drug delivery.14a,b,c current ApDC technologies largely rely on the noncovalent association of drug and specific DNA sequences,14d or complicated and less efficient organic synthesis.14a These technologies suffer from complicated preparation, low controllability of site-specific drug conjugation on vehicles, low synthesis yield in some cases, low drug loading capacity and the accompanying high cost and low spatiotemporal controllability in drug release.

Solid-phase synthesis technology15 technology is able to generate DNA from individual phosphoramidite building blocks (A, T, C, and G) with high efficiency, providing a platform for automated and sequencepredesigned DNA synthesis. Inspired by this technology, we are interested in the development of therapeutic modules that can be integrated into ApDCs, as well as the downstream automated and modular ApDC preparation by solid-phase synthesis technology. Here we report the design and synthesis of the next-generation of ApDCs for targeted drug delivery, by first developing a drug-incorporated module and then using the solid-phase synthesis technology to automatically integrate aptamers and drug modules. As shown in Figure 1A, the aptamer moiety on one end serves as the locomotive of the molecular trains MT, and the tandem drug modules serve as the “boxcars”. Each boxcar contains a drug (D) molecule, which was first integrated into phosphoramidite as a therapeutic module phosphoramidite D (Figure 1B). In addition, we incorporated spatiotemporal controllability of drug release into the therapeutic module through a photocleavable chemical linkage of drugs and vehicles. The ApDCs can be automatically prepared from modules D, A, T, C, and G on an automated DNA synthesizer in a tailor-designed manner (Figure 1A).16

Figure 1. (A) Automated and modular synthesis of ApDCs from phosphoramidites A, T, C, G, and D. (B) Structural features of phosphoramidite D.16

In this way, multiple drug moieties can be conjugated onto one aptamer at predesigned positions and drug loading capacity. The automated conjugation of aptamers with drugs is operationally simple and highly efficient. It also improves vehicle economy and achieves spatiotemporally controllable drug release. To realize the automated and modular synthesis of ApDCs, the key is the design and synthesis of the therapeutic module: phosphoramidite D. As shown in Figure 1B, phosphoramidite D is composed of three parts: solid phase synthesis functionalities, drug moiety, and a linker between. For synthesis simplicity, we chose glycol as the backbone instead of ribose.17 Various drug molecules can be covalently incorporated into the phosphoramidite as prodrugs, but subtle protecting strategy will be required for those with nucleophilic functionalities such as hydroxyl and amino groups. We chose Fluorouracil (5-FU), a simple and widely used anticancer drug for treatment of many types of cancers, including colorectal cancer and pancreatic cancer,18as a model in this study to test our idea. Between the backbone and drug, a functional linker is necessary for efficient and controllable drug release (as the results of ApDC sgc8-5FU demonstrated). In this design a nitrobenzene derivative was selected as the photocleavable linker (PC-linker) between drug and the backbone of MT to achieve photocontrollable drug release.19

2)Doxorubicin conjugates

Have been demonstrated that sgc8c can internalize into the target cells after the binding to its target protein.[20] For these reasons, sgc8c is considered a good candidate for proof-of-concept. (Dox) is the most utilized anticancer drug against a range of neoplasms, including acute lymphoblastic and myeloblastic leukemias, as well as malignant lymphomas.[21] Have molecularly assembled Dox into aptamer probe through a simple conjugation method in order to demonstrate the feasibility of this target-specific approach in intracellular drug delivery 22



Scheme 1. Conjugation of the drug doxorubicin (Dox) to aptamer sgc8c for targeted delivery to cancer cells.

The synthesis procedure for producing the sgc8c–Dox conjugate is shown in Scheme 1. In order to release the chemotherapeutic agent from the conjugate after internalization, we chose a hydrazone linker to conjugate sgc8c with Dox. Several studies have already demonstrated that Dox C-13 hydrazone derivatives possess a cytotoxic effect comparable to unconjugated Dox[23] and allow the release of Dox at pH 4.5–5.5.[24]

1. L. Brannon-Peppas, J. O. Blanchette, Adv. Drug Delivery Rev. 2004, 56, 1649 –1659.
2. D. Shangguan, Y. Li, Z. Tang, Z. C. Cao, H. W. Chen, P. Mallikaratchy, K. Sefah, C. J. Yang, W. Tan, Proc. Natl. Acad. Sci. USA 2006, 103, 11838– 11843.
3. (a) Ellington, A. D.; Szostak, J. W. Nature 1990, 346, 818. (b) Tuerk, C.; Gold, L. Science 1990, 249, 505.
4. (a) Shangguan, D.; Li, Y.; Tang, Z.; Cao, Z.; Chen, H. W.; Mallikaratchy, P.; Sefah, K.; Yang, C. J.; Tan, W. Proc. Natl. Acad. Sci. U. S. A. 2006, 103, 11838. (b) Sefah, K.; Shangguan, D.; Xiong, X.; O’Donoghue, M. B.; Tan, W. Nat. Protoc. 2009, 5, 1169.
5. C. P. Rusconi, J. D. Roberts, G. A. Pitoc, S. M. Nimjee, R. R. White, G. Quick, E. Scardino, W. P. Fay, B. A. Sullenger, Nat. Biotechnol. 2004, 22, 1423 –1428; b) S. Oney, R. T. Lam, K. M. Bompiani, C. M. Blake, G. Quick, J. D. Heidel, J. Y.-C. Liu, B. C. Mack, M. E. Davis, K. W. Leong, Nat. Med. 2009, 15, 1224 –1228.
6. Eyetech Study Group, Retina 2002, 22, 143 –152.
7. D. Shangguan, L. Meng, Z. C. Cao, Z. Xiao, X. Fang, Y. Li, D. Cardona, R. P. Witek, C. Liu, W. Tan, Anal. Chem. 2008, 80, 721– 728.
8. D. Van Simaeys, D. L\_pez-Col\_n, K. Sefah, R. Sutphen, E. Jimenez, W. Tan, PLoS One 2010, 5, e13770.
9. D. Shangguan, Y. Li, Z. Tang, Z. C. Cao, H. W. Chen, P. Mallikaratchy, K. Sefah, C. J. Yang, W. Tan, Proc. Natl. Acad. Sci. USA 2006, 103, 11838 – 11843.
10. K. Sefah, L. Meng, D. Lopez-Colon, E. Jimenez, C. Liu, W. Tan, PLoS One 2010, 5, e14269.
11. P. Mallikaratchy, Z. Tang, S. Kwame, L. Meng, D. Shangguan, W. Tan, Mol. Cell. Proteomics 2007, 6, 2230 –2238.
12. K. Zhang, K. Sefah, L. Tang, Z. Zhao, G. Zhu, M. Ye, W. Sun, S. Goodison, W. Tan, ChemMedChem 2012, 7, 79–84.
13. K. Sefah, Z. Tang, D. Shangguan, H. Chen, D. Lopez-Colon, Y. Li, P. Parekh, J. Martin, L. Meng, J. Phillips, Leukemia 2009, 23, 235– 244.
14. (a) Huang, Y.-F.; Shangguan, D.; Liu, H.; Phillips, J. A.; Zhang, X.; Chen, Y.; Tan, W. ChemBioChem 2009, 10, 862. (b) Meng, L.; Yang, L.; Zhao, X.; Zhang, L.; Zhu, H.; Liu, C.; Tan, W. PLoS One 2012, 7, e33434. (c) Dassie, J. P.; Liu, X.-Y.; Thomas, G. S.; Whitaker, R. M.; Thiel, K. W.; Stockdale, K. R.; Meyerholz, D. K.; McCaffrey, A. P.; McNamara, J. O.; Giangrande, P. H. Nat. Biotechnol. 2009, 27, 839. (d) Bagalkot, V.; Farokhzad, O. C.; Langer, R.; Jon, S. Angew. Chem., Int. Ed. 2006, 45, 8149.
15. (a)Letsinger, R. L.; Finnan, J. L.; Heavner, G. A.; Lunsford, W. B. J. Am. Chem. Soc. 1975, 97, 3278. (b) Letsinger, R. L.; Lunsford, W. B. J. Am. Chem. Soc. 1976, 98, 3655. (c) Beaucage, S. L.; Iyer, R. P. Tetrahedron 1992, 48, 2223.
16. J. Am. Chem. Soc. 2014, 136, 2731−2734
17. Zhang, L.; Peritz, A.; Meggers, E. J. Am. Chem. Soc. 2005, 127, 4174.
18. (a) Longley, D. B.; Harkin, D. P.; Johnston, P. G. Nat. Rev. Cancer 2003, 3, 330. (b) Heidelberger, C.; Chaudhuri, N. K.; Danneberg, P.; Mooren, D.; Griesbach, L.; Duschinsky, R.; Schnitzer, R. J.; Pleven, E.; Scheiner, J. Nature 1957, 179, 663.
19. (a) Hu, X.; Tian, J.; Liu, T.; Zhang, G.; Liu, S. Macromolecules 2013, 46, 6243. (b) Agasti, S. S.; Chompoosor, A.; You, C.-C.; Ghosh, P.; Kim, C. K.; Rotello, V. M. J. Am. Chem. Soc. 2009, 131, 5728.
20. Z. Y. Xiao, D. H. Shangguan, Z. H. Cao, X. H. Fang, W. H. Tan, Chem. Eur. J.2008, 14, 1769–1775.
21. R. B. Weiss, G. Sarosy, K. Clagett-Carr, M. Russo, B. Leyland-Jones, Cancer Chemother. Pharmacol. 1986, 18, 185–197.
22. ChemBioChem 2009, 10, 862 – 868
23. A. Lau, G. B\_rub\_, C. H. J. Ford, M. Gallant, Bioorg. Med. Chem. 1995, 3, 1305–1312.
24. D. Willner, P. A. Trail, S. J. Hofstead, H. D. King, S. J. Lasch, G. R. Braslawsky, R. S. Greenfield, T. Kaneko, R. A. Firestone, Bioconjugate Chem. 1993, 4, 521–527.