Biomaterials 34 (2013) 9728–9735

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

A polyvalent aptamer system for targeted drug delivery

Zhiqing Zhang [a, b, c], M. Monsur Ali [b, c], Mark A. Eckert [b, c], Dong-Ku Kang [b, c], Yih Yang Chen [b, c], Leonard S. Sender [d, e], David A. Fruman [f], Weian Zhao [b, c, *]

[a] State Key Laboratory of Heavy Oil Processing, College of Science, China University of Petroleum, Qingdao 266580, People's Republic of China
[b] Department of Pharmaceutical Sciences, Sue and Bill Gross Stem Cell Research Center and Chao Family Comprehensive Cancer Center, University of California, Irvine, 845 Health Sciences Road, Irvine, CA 92697, USA
[c] Department of Biomedical Engineering, and Edwards Lifesciences Center for Advanced Cardiovascular Technology, University of California, Irvine, 845 Health Sciences Road, Irvine, CA 92697, USA
[d] Department of Medicine, University of California, Irvine, Irvine, CA, USA
[e] Hyundai Cancer Institute, CHOC Children's Hospital, Orange, CA, USA
[f] Department of Molecular Biology & Biochemistry, University of California, Irvine, CA, USA

ARTICLE INFO

Article history:
Received 19 July 2013
Accepted 27 August 2013
Available online 14 September 2013

Keywords:
Aptamer
Rolling circle amplification
Multivalency
Drug delivery
Leukemia
Cancer

ABSTRACT

Poor efficacy and off-target systemic toxicity are major problems associated with current chemotherapeutic approaches to treat cancer. We developed a new form of polyvalent therapeutics that is composed of multiple aptamer units synthesized by rolling circle amplification and physically intercalated chemotherapy agents (termed as "Poly-Aptamer-Drug"). Using a leukemia cell-binding aptamer and doxorubicin as a model system, we have successfully constructed Poly-Aptamer-Drug systems and demonstrated that the Poly-Aptamer-Drug is significantly more effective than its monovalent counterpart in targeting and killing leukemia cells due to enhanced binding affinity (~40 fold greater) and cell internalization via multivalent effects. We anticipate that our Poly-Aptamer-Drug approach will yield new classes of tunable therapeutics that can be utilized to effectively target and treat cancers while minimizing the side effects of chemotherapy.

1. Introduction

Targeted cancer therapy is still a major unmet need. Current chemotherapeutic drugs lack selectivity and are associated with major side-effects and lack of efficacy in many patients. Recent effort has therefore focused on the development of targeted approaches (e.g., antibody–drug conjugates, targeted drug delivery (TDD) systems with nanoparticles) to deliver drugs selectively to cancerous cells, thus enhancing drug efficacy and reducing non-specific toxicity [1–6]. However, current targeted cancer therapy systems, which typically utilize monovalent molecular recognition [7–11] between targeting molecules (e.g., antibody and aptamers) and receptors on cancer cells, still suffer from poor targeting and cellular internalization efficiency, selectivity, and overall killing efficacy.

In nature, biological systems often use multivalent, cooperative interactions where multiple ligands on one biological entity simultaneously bind to receptors on another to achieve high binding affinity and selectivity [12]. Inspired by nature, engineered multivalency has become an emerging and powerful strategy to improve targeting efficacy and selectivity in drug delivery [12–17]. For example, cooperative, multivalent binding between targeting molecules immobilized on a polymer scaffold or nanoparticle and receptors at target sites can improve not only the affinity but also the specificity of molecular interactions involved in drug delivery [2,12–14]. Intriguingly, multivalent ligand–receptor binding at the cell membrane can promote cellular internalization, likely through energy-dependent endocytic pathways [2,12–14]. While these examples demonstrate the advantages of multivalency in drug targeting and delivery, current methods to prepare multivalent TDD systems are complex and often involve chemistries that are not easily modified [2,12–14].

We have recently exploited a simple, powerful isothermal enzymatic reaction called rolling circle amplification (RCA) to synthesize multivalent scaffolds [18–20]. In RCA, DNA polymerase (e.g., phi29 DNA polymerase) extends DNA from a primer by replicating a circular DNA template many times to yield a single-stranded (ss) DNA product that is typically tens of thousands of nucleotides long (Fig.1a) [21–28]. Note also that RCA products

* Corresponding author. Department of Pharmaceutical Sciences, Sue and Bill Gross Stem Cell Research Center and Chao Family Comprehensive Cancer Center, University of California, Irvine, 845 Health Sciences Road, Irvine, CA 92697, USA.
E-mail address: weianz@uci.edu (W. Zhao).

0142-9612/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biomaterials.2013.08.079

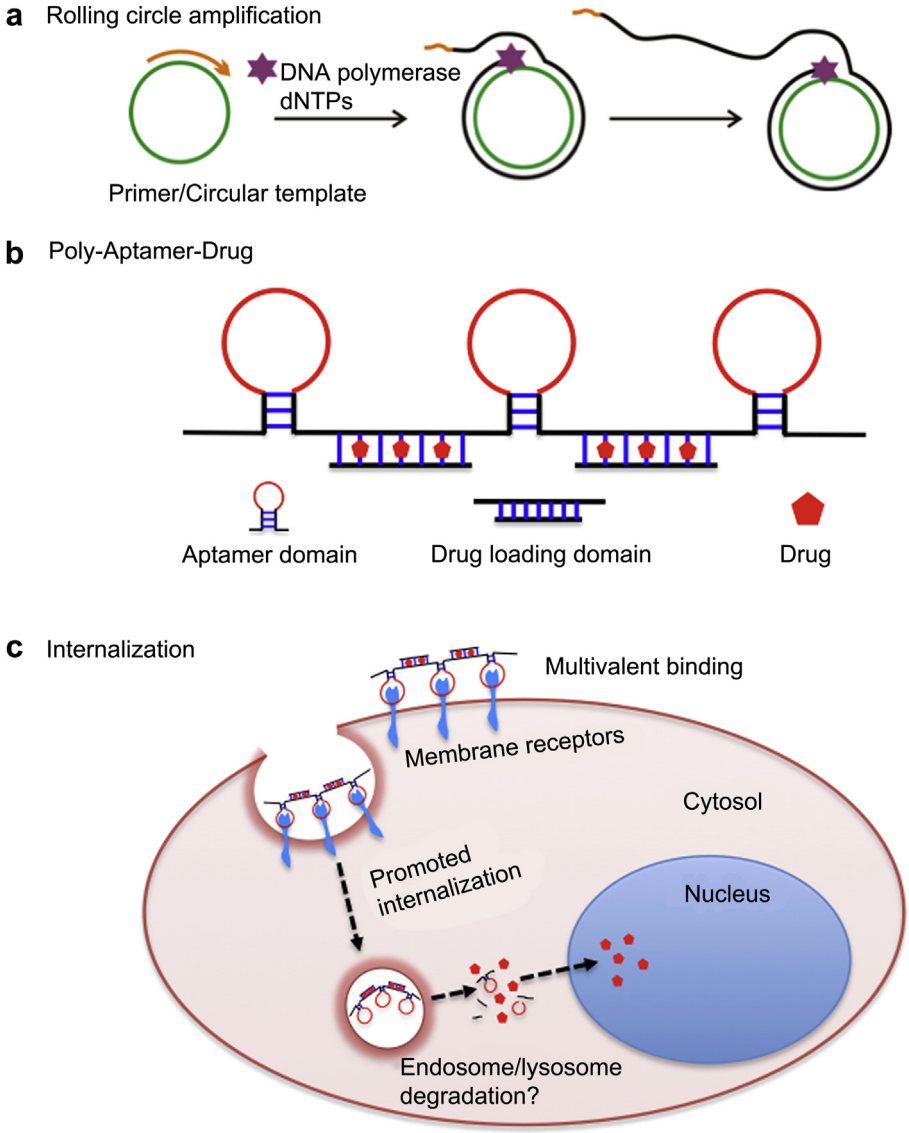

Fig. 1. Concept of using Poly-Aptamer-Drug to target and kill cancer cells. a) Generation of long single-stranded DNA using rolling circle amplification (RCA). A short DNA primer is annealed to a circular DNA template. Amplification initiates upon addition of Phi29 DNA polymerase (purple star) in the presence of dNTP mix. b) Poly-Aptamer-Drug composition: Poly-Aptamer (red loops) complex with a short complementary sequence (shown as duplex) and drug. c) Poly-Aptamer-Drug specifically binds to target receptors on cancer cells followed by enhanced cellular internalization due to multivalency effects. Poly-Aptamer-Drug might be degraded by intracellular nucleases to facilitate drug release. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

contain repetitive sequence units that are complementary to the circular DNA template and therefore can be easily modified. This versatility allows us to incorporate aptamers (single-stranded nucleic acid sequences that specifically bind to non-nucleic acid targets) [7] into the RCA product that selectively bind to cancer but not normal cells. We have recently demonstrated that multivalent aptamers produced by RCA on a microfluidic-device can selectively capture flowing cancer cells with a significantly higher efficiency than monovalent aptamers or antibodies due to their high binding avidity and unique structural and mechanical properties [18].

We reasoned that the aptamer-containing DNA molecules produced by RCA (Poly-Aptamer) could act as a simple molecular scaffold to construct multivalent TDD systems. The multivalent aptamer composition may induce cooperative binding that increases the strength and frequency of interactions with target cells. Additionally, the DNA scaffold can be tailor-designed to incorporate a spacer domain between aptamers; these spacers can hybridize with complementary strands to form duplex, drug-loading domains [8,29] on which DNA-intercalating chemotherapeutic agents (e.g., doxorubicin, abbreviated as Dox) can be readily incorporated via physical association without the need for chemical modification of the drug or the scaffold (i.e. "Poly-Aptamer-Drug") (Fig. 1b). We hypothesized that our Poly-Aptamer-Drug system will exhibit high cancer cell targeting efficiency and enhanced cellular internalization due to multivalent effects (Fig. 1c). To test our hypothesis, we used a previously identified aptamer sequence [30] that specifically binds to protein tyrosine kinase 7 (PTK7), a protein marker overexpressed in up to 70% of some subtypes of leukemia including T-cell acute lymphoblastic leukemia (ALL) [31]. Leukemia is a particularly appealing target as it is one of the most common cancers especially in children for which chemotherapy is the main treatment but unfortunately associated with severe side-effects [1,2].