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Oligonucleotide aptamer-drug conjugates for targeted therapy of acute myeloid leukemia

Nianxi Zhao [a, 1], Sung-Nan Pei [a, 1, 2], Jianjun Qi [a], Zihua Zeng [a], Swaminathan P. Iyer [b], Pei Lin [c], Ching-Hsuan Tung [d], Youli Zu [a, *]

[a] Department of Pathology and Genomic Medicine, Houston Methodist Hospital, and Cancer Pathology Laboratory, Houston Methodist Research Institute, Houston, TX, 77030, USA
[b] Houston Methodist Cancer Center, Houston, TX, 77030, USA
[c] Department of Hematopathology, The University of Texas MD Anderson Cancer Center, Houston, TX, 77030, USA
[d] Molecular Imaging Innovations Institute, Department of Radiology, Weill Cornell Medical College, New York, NY, 10021, USA

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ABSTRACT

Oligonucleotide aptamers can specifically bind biomarkers on cancer cells and can be readily chemically modified with different functional molecules for personalized medicine. To target acute myeloid leukemia (AML) cells, we developed a single-strand DNA aptamer specific for the biomarker CD117, which is highly expressed on AML cells. Sequence alignment revealed that the aptamer contained a G-rich core region with a well-conserved functional G-quadruplex structure. Functional assays demonstrated that this synthetic aptamer was able to specifically precipitate CD117 proteins from cell lysates, selectively bound cultured and patient primary AML cells with high affinity (Kd < 5 nM), and was specifically internalized into CD117-expressing cells. For targeted AML treatment, aptamer-drug conjugates were fabricated by chemical synthesis of aptamer (Apt) with methotrexate (MTX), a central drug used in AML chemotherapy regimens. The formed Apt-MTX conjugates specifically inhibited AML cell growth, triggered cell apoptosis, and induced cell cycle arrest in G1 phase. Importantly, Apt-MTX had little effect on CD117-negative cells under the same treatment conditions. Moreover, exposure of patient marrow specimens to Apt-MTX resulted in selective growth inhibition of primary AML cells and had no toxicity to off-target background normal marrow cells within the same specimens. These findings indicate the potential clinical value of Apt-MTX for targeted AML therapy with minimal to no side effects in patients, and also open an avenue to chemical synthesis of new, targeted biotherapeutics.

1. Introduction

Aptamers are a class of molecular ligands composed of synthetic single-stranded oligonucleotides (DNA or RNA) that are able to specifically bind their targets with high affinity [1]. As “chemical antibodies”, oligonucleotide aptamers can be chemically synthesized and easily conjugated with different functional molecules [2–6]. Due to these unique chemical and biological properties, synthetic aptamers have been widely used in biomedical applications to specifically target biomarkers [6–11] for cancer cell detection and targeted therapy [5,11,12]. Personalized medicine employing targeted therapeutic approaches not only enhances therapy efficacy, but also reduces adverse side effects in cancer patients. Recent studies have shown that antibody-drug conjugates are a promising technology for targeted cancer therapy [13–16]. However, production of humanized monoclonal antibodies and subsequent drug conjugation are costly and time and labor consuming. In contrast, aptamers can be easily chemically synthesized and, due to their smaller size, exhibit more efficient tissue penetration, faster binding capacity to tumor cells, and no immunogenicity *in vivo* [17,18]. These advanced chemical and biological features indicate a potential use for synthetic aptamers in clinical applications.

* Corresponding author. Department of Pathology and Genomic Medicine, Houston Methodist Hospital, 6565 Fannin Street, Houston, TX, 77030, USA.
E-mail address: yzu@houstonmethodist.org (Y. Zu).
[1] Equal contributions.
[2] Current institution: Division of Hema-Oncology, Kaohsiung Chang Gung Memorial Hospital, and Chang Gung University College of Medicine, Kaohsiung, Taiwan.

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The current treatment regimen for acute myeloid leukemia (AML) is remission-induction chemotherapy, followed by either consolidation chemotherapy or allogeneic stem cell transplantation [19–22]. As most patients diagnosed with AML are in their sixth or seventh decade of life, many are not candidates for standard induction chemotherapy because of the severe adverse side effects, such as profound myelosuppression, life-threatening infections, and cardiotoxicity [23–26]. Therefore, a personalized medicine specific for AML with fewer side effects is urgently needed [27–31]. CD117 is a transmembrane receptor that is highly expressed on leukemia cells in 95% of patients with relapsed AML [32]. In addition, CD117-expressing AML patients have a worse survival prognosis than CD117-negative patients, and high levels of CD117 expression correlate with low rates of complete remission [33–37]. These data indicate that the CD117 receptor may be a potential therapeutic biomarker for the treatment of AML.

To develop new therapeutics targeting AML, we identified a CD117-specific ssDNA aptamer sequence through a hybrid selection approach. Subsequently, an aptamer-drug conjugate was formulated by synthesizing the aptamer sequence with methotrexate (MTX), a drug used to treat AML. Functional analysis with clinical specimens demonstrated that the aptamer-methotrexate (Apt-MTX) conjugates specifically killed patient primary AML cells and had no toxicity to the off-target background marrow cells of the same specimens.

2. Materials and methods

2.1. Reagents and cell lines

CD117-expressing HEL cells (a leukemia cell line from ATCC, Manassas, VA) were used to select single stranded (ss) DNA aptamers. CD117 recombinant protein with a polyhistidine (his) tag at the C-terminus (Sino Biological Inc. Beijing, China) was used to further enrich ssDNA sequences specific for the CD117. The CD117-negative cell lines included: the B lymphoma cell line CA46 (ATCC, Manassas, VA); the breast cancer cell line 468 (kindly provided by Dr. Haifa Shen, Houston Methodist Hospital); and the prostate cancer cell line LNCaP (ATCC, Manassas, VA). All suspension cells were cultured with RPMI 1640 medium (Fisher Scientific, Pittsburgh, PA) with 10% FBS (Atlanta Biologicals, Lawrenceville, GA). All adhesion cell lines were cultured in DMEM (Atlanta Biologicals, Lawrenceville, GA) with 10% FBS.

2.2. Hybrid systematic evolution of ligands by exponential enrichment (SELEX)

For hybrid SELEX (Fig 1A), the ssDNA library consisted of a central, continuous stretch of 35 randomized sequences flanked by PCR primer sequences (5′-GAGGCATACCAGCTTATTCAA-35N-ATAGTAAGTGCAATCTGCGAA-3′). Cy3-labeled 5′ primer (5′-Cy3-GAGGCATACCAGCTTATTCAA-3′) and biotinylated 3′ primer (5′-

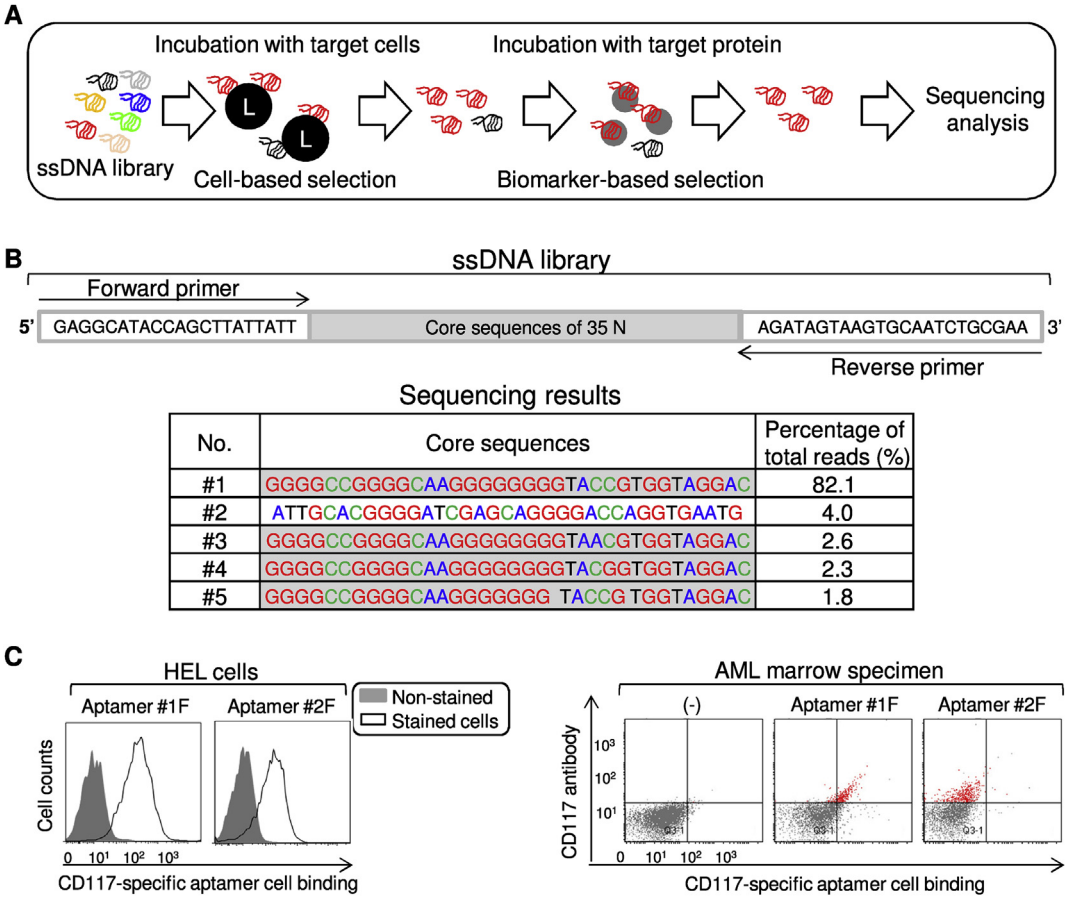

Fig. 1. Development of CD117-specific ssDNA aptamers. (A) The process of aptamer selection using a hybrid cell- and protein-based enrichment approach. (B) Sequencing results of selected ssDNA aptamers, including forward primer region (23 nt), 35 nt random core region, and reverse primer region (23 nt). The top five dominant aptamer sequences with percentage of total sequencing reads. (C) Binding ability of aptamers #1 and #2 to cultured CD117-positive HEL cells (left panel) and patient primary AML cells (right panel) assessed by flow cytometry.