Supplementary Materials

Aptamer Internalization via Endocytosis Inducing S-Phase Arrest and Priming Maver-1 Lymphoma Cells for Cytarabine Chemotherapy

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1. Identification of ssDNA aptamer sequences specific for Maver-1 lymphoma cells

In this study, a hybrid SELEX approach was employed, including both cancer cell- and biomarker-based selection steps as shown in Fig. S1. The ssDNA library used for aptamer selection consisted of a central, continuous stretch of 40 randomized bases flanked by arm sequences at both ends. For PCR annealing, the primer sequences (5'- ATC CAG AGT GAC GCA GCA - 40 random-base sequences - TG GAC ACG GTG GCT TAG T-3') were used. The Cy3-labeled 5′ primer sequence (5′-Cy3- ATC CAG AGT GAC GCA GCA-3′), and the biotinylated 3′ primer sequence (5′-biotin- ACT AAG CCA CCG TGT CCA 3′) were used for the initial PCR amplification. The ssDNA library and primers were synthesized, and purified by HPLC (Integrated DNA Technologies, Coralville, IA). The enriched aptamer pools were amplified by PCR with Taq polymerase (Takara Bio, Mountain View, CA, USA).

In each round of cell-based SELEX, the ssDNA library underwent enrichment with Maver-1 lymphoma cells and subsequent counter-selection with Jeko-1 cells that are negative for lambda light chain. After a total of 7 cell-based SELEX rounds, the obtained ssDNA pools underwent protein-based SELEX by using an affinity column containing purified recombinant proteins of Ig lambda light chain (Abnova, Walnut, CA). The protein-based SELEX was repeated twice and the final aptamer pools were amplified for sequencing analysis. All SELEX experiments were conducted at room temperature.

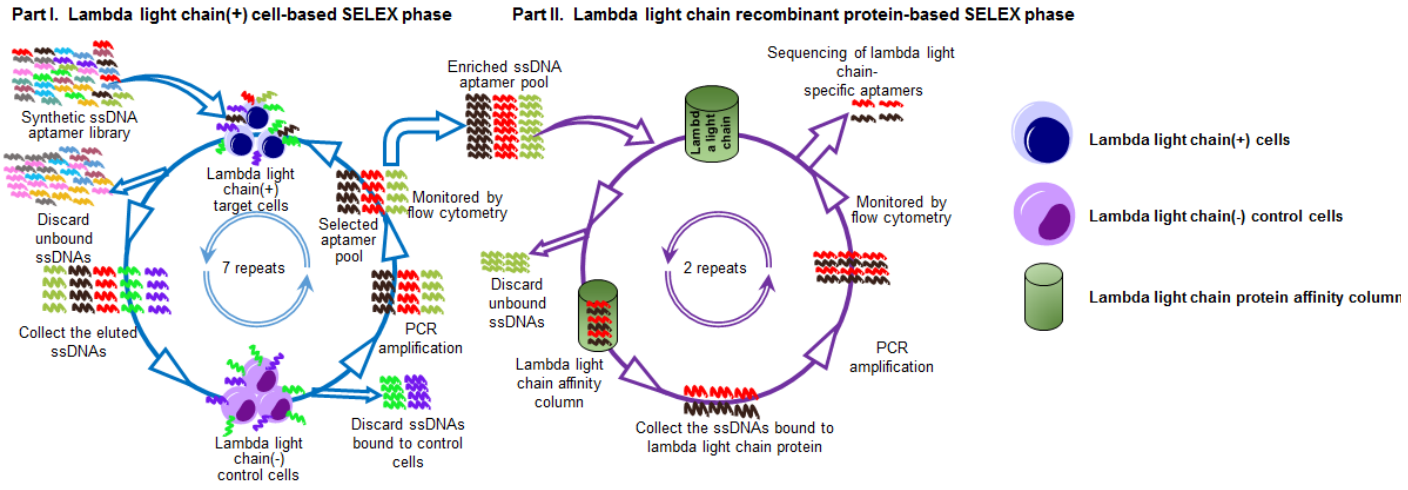

Figure S1. Scheme of hybrid SELEX

2. Characterization of aptamer sequence evolution

To monitor evolution of cell binding capacity, aptamer pools from the 4th, 7th, and 9th rounds of SELEX were amplified and labeled with Cy3 fluorochrome reporter. Flow cytometry analysis showed that the obtained aptamer pools gradually gained binding capacity to Maver-1 lymphoma cells as the SELEX process progressed, but did not react with control Jeko-1 cells (Fig. S2A).

To obtain sequence information, the aptamer pools derived from the 4th, 7th and 9th rounds of SELEX were purified with a ChargeSwitch PCR Clean-Up Kit (Life Technologies, Carlsbad, CA), and submitted for next-generation sequencing (LC Sciences LLC, Houston, TX, USA). The 10 dominant sequences from the 9th pool are listed in Fig. S2B. The three top aptamer sequences, named HL-1, HL-2, and HL-3, accounted for 36.62, 3.80%, and 3.47% out of a total of over 5 million reads, respectively. Notably,

the sequences of aptamers HL-1 and HL-2 are almost identical with single nucleotide mismatches in the 40 nt central core region (Fig. S2C).

In addition, the evolution of these 10 dominant aptamers in the 9th pool was calculated (%) and compared to that identified in the 4th and 7th pools (Fig. S2C). Interestingly, as the SELEX rounds progressed, the top three aptamer sequences showed dramatically different changes: the percentage of aptamer HL-1 sharply increased only at the 7th round and reached a maximum at the 9th round; in contrast, aptamer HL-2 reached about 10% of total sequences at the 4th and 7th rounds of SELEX and decreased to 3% at the 9th round, while aptamer HL-3 slowly increased at the 7th round, and finally reached about 4% at the 9th round.

Moreover, for the functional assays, aptamers HL-1, HL-2 and HL-3 were synthesized and labeled with Cy3 fluorescence reporter and incubated with cells at final concentration of 50 nmol/L. Flow cytometry revealed that aptamers HL-1 and HL-2 bound Maver-1 lymphoma cells with high affinities that were comparable, whereas HL-3 had lower binding affinity for Maver-1 cells, although all aptamer probes showed negligible reaction to control Jeko-1 cells under the same treatment conditions (Fig. S2D). These findings indicate that the developed aptamers HL-1 and HL-2 are specific for Maver-1 lymphoma cells, and were thus selected for further analysis.

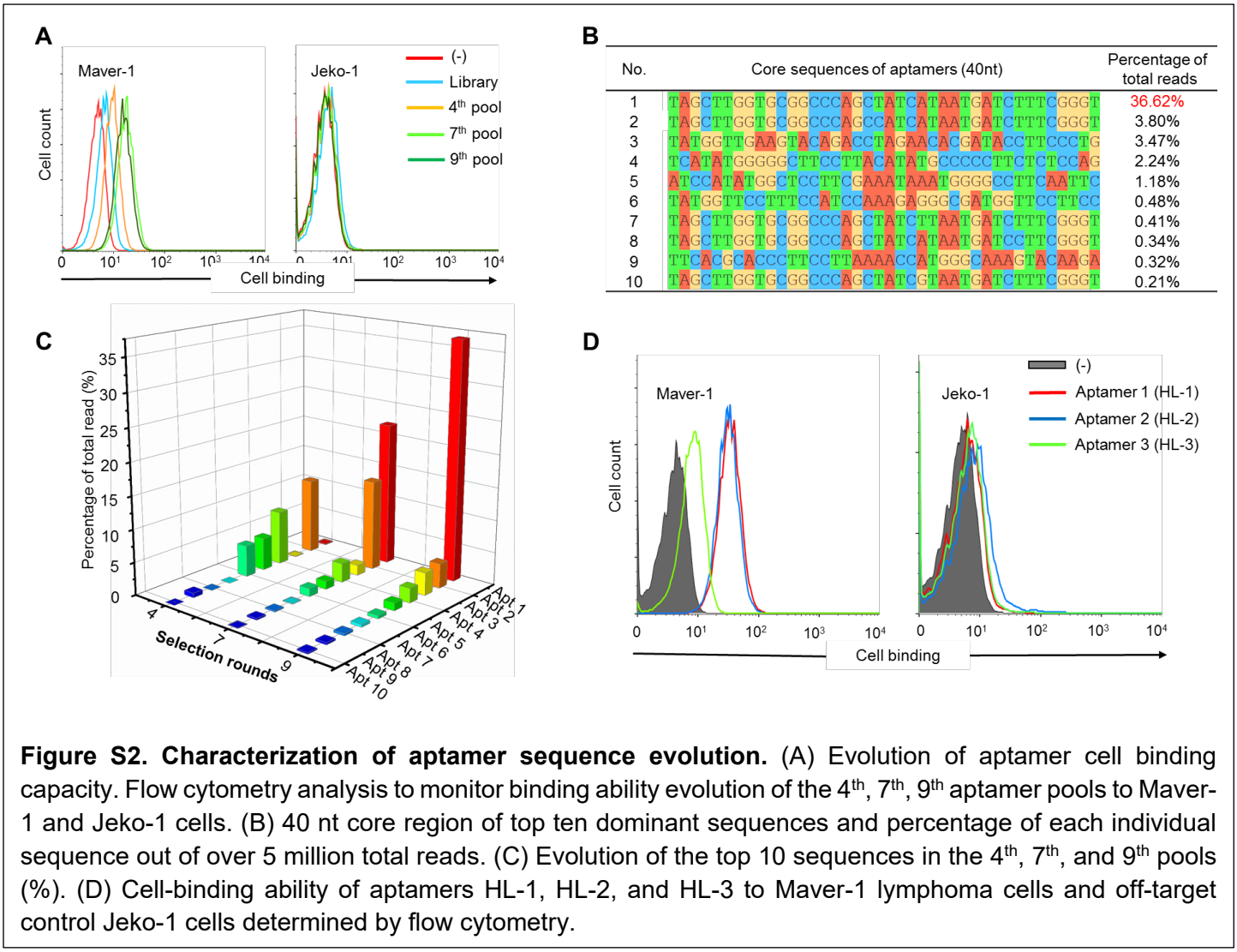

Figure S2. Characterization of aptamer sequence evolution. (A) Evolution of aptamer cell binding capacity. Flow cytometry analysis to monitor binding ability evolution of the 4th, 7th, 9th aptamer pools to Maver-1 and Jeko-1 cells. (B) 40 nt core region of top ten dominant sequences and percentage of each individual sequence out of over 5 million total reads. (C) Evolution of the top 10 sequences in the 4th, 7th, and 9th pools (%). (D) Cell-binding ability of aptamers HL-1, HL-2, and HL-3 to Maver-1 lymphoma cells and off-target control Jeko-1 cells determined by flow cytometry.

3. The presence of G-quadraplex structure in aptamer HL-1

To detect G-quadraplex structure, Thioflavin T (ThT) staining assay of aptamers was performed as the previously reported [1]. Briefly, synthetic HL-1 aptamers at 1 umol/L final concentrations were incubated with equal molar amount of Thioflavin T dye (Sigma Aldrich, St. Louis, MO) for 30 min at room temperature. The aptamer AS1411 that contains G-quadraplex structure was used as positive control [2,3], and the aptamer CD30 [4] that doesn't have G-quadraplex structure was used as negative control. Fluorescence of each reaction was detected (excitation=425nm and emission=490nm) and quantified using a microplate reader (Biotek, Winooski, VT). Results confirm the presence of G-quadraplex structure in the aptamer HL-1.

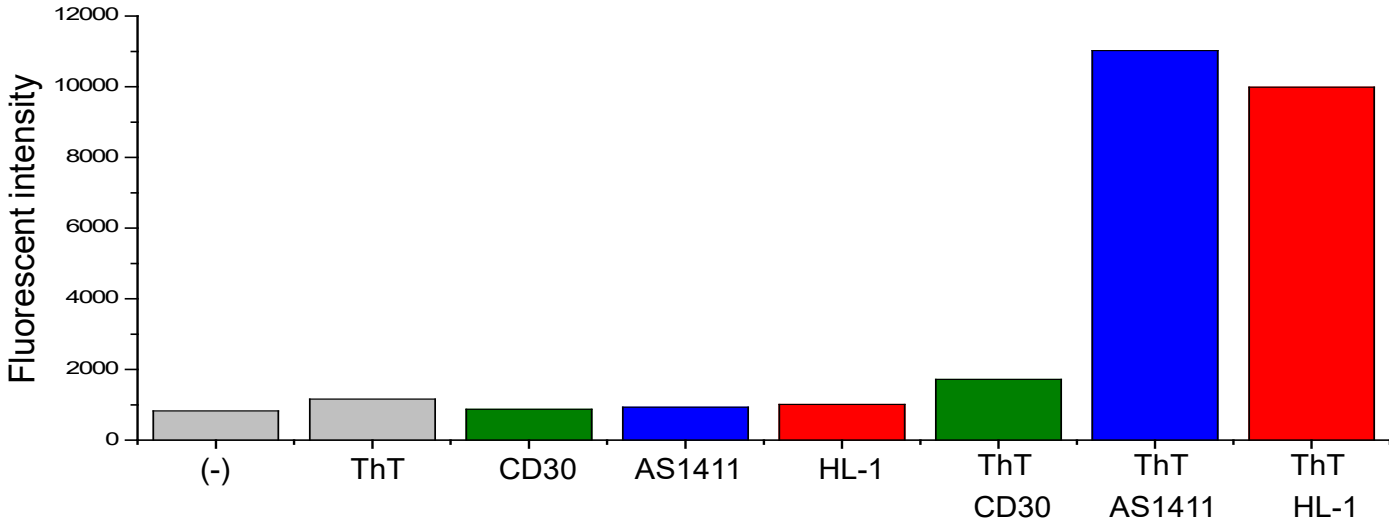

Figure S3. The presence of G-quadraplex structure in aptamer HL-1. Synthetic aptamers HL-1, CD30, and AS1411 were treated with Thioflavin T (ThT) and changes in reaction fluorescent intensity were quantified (excitation=425nm and emission=490nm).

4. Aptamer HL-1 did not react to cellular nucleolin protein

To investigate potential target, interaction of aptamer HL-1 and cellular nucleolin protein were tested. For this purpose, freshly cultured Maver-1 cells (1.0×10^7) were lysed with 1 mL cell lysis buffer (Thermo Fisher Scientific, Rockford, IL) for 30 min on ice, and centrifuged at 14,000×g for 10 min. The supernatants were collected and incubated with biotinized aptamer HL-1 (100 nmol/L) overnight at 4°C and then with 100 µL of streptavidin beads (Thermo Fisher scientific) for 1 hour. After washed twice with Phosphate-Buffered Saline buffer, the streptavidin beads were suspended in 60 µL Laemmli Sample buffer (Bio-rad, Hercules, CA) and then boiled for 5 min. The precipitated cellular proteins were separated on 4-20% SDS Mini Protein Gels (Life Technologies) and transferred to membrane. Western blot assay was performed using anti-nucleolin antibody and precipitated cellular nucleolin protein was visualized by color development system (Thermo Fisher scientific). As a positive control, aptamer AS1411 was used for immunoprecipitated under the same conditions. Results revealed that aptamer HL-1 did not bind to cellular nucleolin although aptamer AS1411 did as previously reported [2].

Figure S4. Nucleolin is not a target for aptamer HL-1. Immunoprecipitation using aptamers as indicated and Western blotting with anti-Nucleolin antibody demonstrated that aptamer HL-1 did not react to nucleolin in Maver-1 cell lysates. Aptamer AS1411 was used as a positive control.

References

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