Selection of DNA Aptamers for Differentiation of Human Adipose-Derived Mesenchymal Stem Cells from Fibroblasts

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Abstract
In recent years, stem cell therapy has shown promise in regenerative medicine. The lack of standardized protocols for cell isolation and differentiation generates conflicting results in this field. Mesenchymal stem cells derived from adipose tissue (ASC) and fibroblasts (FIB) share very similar cell membrane markers. In this context, the distinction of mesenchymal stem cells from fibroblasts has been crucial for safe clinical application of these cells. In the present study, we developed aptamers capable of specifically recognize ASC using the Cell-SELEX technique. We tested the affinity of ASC aptamers compared to dermal FIB. Quantitative PCR was advantageous for the in vitro validation of four candidate aptamers. The binding capabilities of Apta 2 and Apta 42 could not distinguish both cell types. At the same time, Apta 21 and Apta 99 showed a better binding capacity to ASC with dissociation constants (Kd) of 50.46 ± 2.28 nM and 72.71 ± 10.3 nM, respectively. However, Apta 21 showed a Kd of 86.78 ± 9.14 nM when incubated with FIB. Therefore, only Apta 99 showed specificity to detect ASC by total internal reflection microscopy (TIRF). This aptamer is a promising tool for the in vitro identification of ASC. These results will help understand the differences between these two cell types for more specific and precise cell therapies.

Keywords DNA aptamers • Cell-SELEX • Human adipose-derived stem cells • Human dermal fibroblasts • Quantitative PCR assay

Extended author information available on the last page of the article
Introduction

Nucleic acid aptamers can be defined as single-stranded DNA or RNA oligonucleotides, generally smaller than 100-mer, capable of binding to a wide variety of target molecules with high affinity and specificity [1]. During aptamers selection, a set of random sequences are enriched by generating target-specific sequences by an in vitro and an iterative process called systematic evolution of ligands by exponential enrichment (SELEX) [2, 3]. Once the target molecules are anchored to the cell surface, the bound aptamers can be easily separated from unbound oligonucleotides by washing and centrifugation during the Cell-SELEX process. This method supports the development of probes and the discovery of cell biomarkers [1, 4] since Cell-SELEX can be applied without prior knowledge of the target on the cell membrane.

The mesenchymal stem cells can differentiate into distinct cell types of cells and have been isolated from the stromal fraction of practically all body tissues. Adipose tissue is an accessible source that provides higher yields of stem cells than bone marrow [5]. These human adipose-derived stem cells (ASC) are promising self-renewing and multipotent mesenchymal stem cells. They have shown strong immunosuppressive effects and regenerative actions in vivo, features needed to develop novel cell-based therapy and tissue engineering strategies [6]. However, in the therapeutic application of stem cells, there is a high risk of contamination with human dermal fibroblasts (FIB), which can lead to a series of losses for the therapy, such as the decrease in the stem cell differentiation capacity and the induction of senescence [7–9].

Aiming the identification of ASC as mesenchymal stem/stromal cells, the International Society for Cell Therapy (ISCT) provided a set of criteria, such as plastic adherence, colony formation capacity, tri-lineage differentiation potential, the presence of mesenchymal, and the absence of hematopoietic cell surface markers [10]. Like ASC, FIB are plastic-adherent spindle-shaped cells. These cells can be found in the stromal portion of several tissues, such as skin, adipose, and cardiac tissue, and muscle, showing an essential role in tissue development and wound healing. Both cells have an elongated, spindle-like morphology, making them indistinguishable [11, 12]. Moreover, the cell surface proteins of FIB are very similar to the profile observed in ASC [8, 12–14]. In the context of cell therapy, for better preparation of ASC without fibroblasts, it is necessary to find markers that differentiate ASC from FIB without impairing its application condition.

Studies to identify mesenchymal stem cells (MSCs) derived from various tissues using aptamers were conducted in recent years. SELEX was used to generate aptamers against human jaw periosteal cells and the aptamer 74 showed binding capacities to MSCs derived from placental tissue and bone marrow [15]. Ueki and colleagues [16] developed a DNA aptamer named TD0 capable of supporting self-renewal and pluripotency of induced pluripotent stem cells (iPSCs). A study led by Hou and collaborators [17] discovered an aptamer called Apt19S that demonstrated a high affinity for induced iPSCs, mouse embryonic stem cells (mESCs), Rhesus monkey ESCs, and human embryonic stem cells (hESCs). Wang and colleagues [18] used whole-cell SELEX to generate a novel MSCs aptamer termed HM69. Another group developed a cartilage tissue-engineering scaffold functionalized with an aptamer (Apt19s) that specifically recognizes pluripotent stem cells [19].

Although recent studies have developed aptamers capable of identifying many categories of stem cells, selecting specific aptamers to identify ASC using FIB as control cells is still unprecedented. Thus, considering the high specificity and affinity of aptamers for their targets, we aimed to select novel aptamers after ASC (positive) and FIB (negative) selection pressures using Cell-SELEX and quantitative PCR techniques.
Materials and Methods

Isolation and Cell Culture

Human skin and adipose tissue were freely provided by healthy donors that underwent liposuction and abdominoplasty, according to the guidelines of Universidade Federal de Minas Gerais Research Ethics Committee (Number: 02508018.1.0000.5149). Human ASC and FIB were isolated using mechanical and enzymatic digestion as previously described by our group [20]. Cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM, Thermo Fisher Scientific, MA, USA) supplemented with 5 mmol/L of sodium bicarbonate (Sigma-Aldrich, MO, USA), 1% of PSA solution (penicillin/streptomycin/amphotericin B; Sigma-Aldrich), and 10% of fetal bovine serum (FBS). The cells were kept in a humidified incubator at 37°C and 5% CO2. The medium was changed three times a week, and cells at 4th and 5th passages were used in the experiments.

Design of Oligonucleotides

The forward (5’-ATGGTCTCCTGCGTTCA-3’) and the reverse (5’- AGATTCGG TAGCACAGGA-3’) primers were used in the Cell-SELEX process. The reverse primer was labeled at the 5’ end with biotin to allow single strand separation during Cell-SELEX. The ssDNA library comprised a 60 nucleotide (60N) randomized uniform region flanked by two constant primer annealing sequences: 5’-ATGGTCTCCTGCGTTCA-60N-TCCTGTGTACCGAATCT-3’ totaling 96 bases of DNA. The ssDNA library was purchased with the following specifications: 4 nmol Ultramer® DNA Oligo with Tm = 72.5°C, GC content = 50%, and molecular weight = 29,490.1, without base modifications. The scrambled sequence has the same primer hybridization region as the candidate aptamers: 5’-ATGGTCTCCTAGGGCCCTTAAGGCCTGCTCTGCTACCGAATCT-3’.

Based on the qPCR reaction normalization method developed by Graziani and colleagues [21], an exogenous nucleotide sequence extracted from the genome of Arabidopsis thaliana (AT1) was synthesized (5’-GCATGTTTGAACAGACACTGTATCTGTATTGTTACATGGATATTGTGTGGTTGACAGATCCACCAGAAGCAGGCGAAACAGGCA-3’) and the primers AT1 forward 5’-GCATGTTTGAACAGACACT-3’ and AT1 reverse 5’-TGCCTTTGCTCTGCTACCGA-3’ were used for PCR amplification. All oligonucleotides were synthesized by Integrated DNA Technologies (IDT, IA, USA) and purified using standard desalting method.

Cell-SELEX protocol

Aptamers selection was carried out based on the Cell-SELEX protocol [1], with modifications. Previously, 2 nmol of ssDNA were diluted in binding buffer [25 mM of glucose, 0.05 mM of MgCl₂, 15 mM of BSA, 4 μM of yeast tRNA in 100 mL of phosphate-buffered saline solution (PBS pH 7.4; Sigma-Aldrich)], heated at 95°C for 5 min and snap cooled on ice before binding. Then, the ssDNA library was incubated with 3.5×10⁶ in one dish (100 mm × 20 mm) for 1.5 h at room temperature. After incubation, the supernatant was discarded, and cells were washed with washing buffer (25 mM of glucose, 0.05mM of MgCl₂ in 1L of PBS; Sigma-Aldrich). Subsequently, cells with bound aptamers (ligands) were removed using a cell scraper and nuclease-free water. To separate cell-ligands complex, the mixture was heated to 95°C
and, subsequently, centrifuged at 13,100× g for 5 min. The optimal number of PCR cycles for each selection round was determined according to the literature. The protocol recommends selecting a cycle number that produces a shiny band without nonspecific amplicons by visual selection [1] (Supplementary Information Fig. S1 and Fig. S2). After the first round of positive selection, recovered sequences were submitted to six cycles of amplification. The pool of ssDNA was amplified using 5× colorless buffer GoTaq® flexi buffer (Promega, WI, USA), 2.5 mM of each dNTP (dATP, dCTP, dGTP, dTPP), 50 mM of MgCl$_2$, 0.5 μM of primers (R labeled with biotin), and 5 U/μL of GoTaq® DNA polymerase (Promega). The amplification conditions were initial stage at 95°C for 3 min, followed by 10 cycles of 95°C for 30 s, 53.3°C for 30 s, and 72°C for 30 s. The final extension step was performed at 72°C for 5 min. The double-strand DNA (dsDNA) was separated using Magnesphere® Paramagnetic Particles kit (Promega) (Item 2.4). In the second round of incubation with ASC, the recovered ssDNA were submitted to 12 amplification cycles. In the third round of selection, the negative selection was introduced: 3×10$^6$ FIB cells were seeded in one cell dish. The ssDNA ligands obtained from the second round were incubated with fibroblasts for 1 h. After this period, the supernatant containing the aptamers that did not bind to the fibroblasts was extracted, heated, and centrifuged for PCR cycle test. The optimal number of 20 PCR cycles was chosen, repeated in the subsequent negative selection cycles. After amplification and separation of the double-stranded DNA, the ligands were incubated with the target cell for 1 h. The number of 16 PCR cycles was defined as the optimal cycle number, which was also used in subsequent positive selection rounds. From the 4th round on, modifications such as cell density and incubation time were introduced in the selection (Table 1). These alterations aimed to raise the stringency of the Cell-SELEX procedure until the final 8th round. The enriched aptamers of the 7th and 8th round after incubation with ASC and the ligands extracted after the incubation with FIB in the 8th round were sequenced using Illumina MiSeq platform (Illumina Inc., CA, USA). Sequencing and bioinformatics analysis were performed by GenXPro GmbH (Frankfurt am Main, Germany).

### Single-Strand DNA Preparation

The Streptavidin Magnesphere® Paramagnetic Particles kit (Promega) was used to separate the dsDNA after every PCR amplification cycle. After the PCR cycle, one strand of the dsDNA is labeled with biotin, since the reverse primer was labeled at the 5’ end with biotin to allow single strand separation (Item 2.2). Briefly, magnetic particles were washed three times with 300 μL of 0.05X SSC buffer (solution containing NaCl, trisodium citrate dehydrate, and

| Rounds | Positive selection: ASC | Dish number | Time of incubation | PCR cycles | Negative selection: FIB | Time of Incubation | Dish number | PCR cycles |
|--------|------------------------|-------------|--------------------|------------|-------------------------|--------------------|-------------|------------|
| 1      | 3.5×10$^6$             | 1           | 1 h 30 min         | 6          | -                       | -                  | -           | -          |
| 2      | 3.5×10$^6$             | 1           | 1 h 30 min         | 12         | -                       | -                  | -           | -          |
| 3      | 3×10$^6$               | 1           | 1 h               | 16         | 3×10$^6$                | 30 min             | 1           | 20         |
| 4      | 3×10$^6$               | 1           | 45 min            | 16         | 3×10$^6$                | 30 min             | 1           | 20         |
| 5      | 5×10$^5$               | 1           | 30 min            | 16         | 3×10$^6$                | 30 min             | 1           | 20         |
| 6      | 5×10$^5$               | 1           | 30 min            | 16         | 3×10$^6$                | 30 min             | 1           | 20         |
| 7      | 5×10$^5$               | 1           | 30 min            | 16         | 3×10$^6$                | 30 min             | 1           | 20         |
| 8      | 5×10$^5$               | 1           | 20 min            | 16         | 3×10$^6$                | 20 min             | 2           | 20         |
DEPC-treated water). After each wash, the particles were captured using the magnetic stand (Promega) and the wash solution was carefully removed. The particles were resuspended in 100 μl of 0.05X SSC buffer and the tube was ready to be used in ssDNA preparation. The dsDNA was previously heated at 65°C for 10 min, mixed with 20X SSC buffer, cooled at room temperature, and then added to the tube. The dsDNA was incubated with the magnetic particles for 10 min at room temperature and mixed by inversion every 1–2 min. Then, the tube was positioned in the magnetic stand and the supernatant was removed. The particles were washed with 0.1X SSC buffer and captured using a magnetic stand. The supernatant was discarded and this step was repeated four times. After the final wash, the supernatant was removed without disturbing the particles and the ssDNA was eluted in 250 μL of nuclease-free water.

**Melting Curve Analysis**

The progress of aptamers selection was monitored by analyzing the dissociation curve (DNA melt) of ligands after each round as previously described [22, 23]. For this purpose, 5 μL of SYBR® Green PCR Master Mix 2x reagent (Applied Biosystems, CA, USA) was added to 5 μL of the PCR products containing the ligands. The samples were subjected to gradual denaturation according to the protocol: 95°C for 15 min, 95°C for 15 s, denaturation ramp from 40 to 95°C and 25°C for 15 s. Reactions were performed on the 7500 Fast Real-Time PCR System (Applied Biosystems). The graphs of fluorescence intensity versus temperature were generated on the 7.500 Software v. 2.0.6 (Applied Biosystems).

**Selection and Characterization of Candidate Aptamers**

Post-sequencing analyzes were performed using the Galaxy server available at https://usegalaxy.org/ [24]. The following criteria were established for the selection of candidate aptamers: (a) the more prevalent sequence in ASC compared to FIB and enriched from the 7th to the 8th round; (b) sequences with specific affinity to ASC and enriched from the 7th to the 8th round; and (c) sequences with the lowest value of ΔG at 25°C. The secondary structures and Gibbs free energy (ΔG) of aptamers were determined using the mfold web server available at http://unafold.rna.albany.edu28, under 25°C condition. The phylogenetic tree of candidate aptamers was created using the neighbor joining method [25], using the MEGA-X 10.0.5 software [26] available at https://www.megasoftware.net/. The four aptamers were synthesized by IDT for in vitro validation assays.

**In Vitro Binding Assays of Aptamers and Scramble**

The four candidate aptamers and the scrambled sequence were tested for the binding capacity to human cells. ASC and FIB were plated in 6-well plates at a density of 5×10^5 cells/well and maintained in a CO2 incubator for 24 h. The next day, 250 nM aptamers or scrambled sequences were heated to 95°C and cooled on ice for the three-dimensional structure formation. Then, they were incubated with cells for 20 min at room temperature under gentle agitation. Next, the cells were washed three times with cold PBS to remove unbound sequences. To recover the cell-aptamer complex, cells were removed using a cell scraper, and sequences were eluted in nuclease-free water under heating at 95°C for 10 min. The AT1 sequence (100 fM) was added and the samples were centrifuged for 5 min at 13,200×g. The
supernatants containing aptamers were recovered and amplified using the Go Taq® qPCR Master Mix Kit (Promega), in a CFX Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories, CA, USA). The following PCR conditions were used in all reactions: 95°C for 2 min, followed by 40 cycles of 95°C for 15 s and a single stage of hybridization and extension at 60°C for 1 min. After 40 cycles of amplification, all samples were subjected to gradual denaturation. Reactions were normalized by the AT1 amplification as an internal reference control. Two different reaction mixes for each sequence were prepared: one mix in which the sequences were amplified with the original pair of primers and another mix with a pair of primers for AT1 sequence. The efficiency of each reaction was calculated based on the method described by Liu and Saint [27] and Cikos and colleagues [28]. The relative quantification was calculated using the method developed by Pfaffl [29], with pair wise fixed reallocation randomization test and FIB as the reference condition.

**Determination of Aptamers Dissociation Constant**

The Kd value of each candidate aptamers was estimated using the non-linear regression curve as previously described by our group [30]. ASC and FIB were plated at a density of 2.5×10^5 cells per well in 12-well plates and maintained in a CO₂ incubator for 24 h. The next day, cells were incubated with different aptamer concentrations for 20 min at room temperature under gentle agitation. After incubation, the cell-aptamer complex was extracted with nuclease-free water and a cell scraper. Bound aptamers were recovered by heating the complex at 95°C and subsequent centrifugation for 5 min at 13,200× g. The supernatant was collected and amplified by qPCR using GoTaq® qPCR Master Mix Kit (Promega). The reaction mixture contained 5 μL of GoTaq® qPCR Master Mix (2X), 0.2 μM of each primer, 4 μL of aptamers samples, and 0.6 μL of nuclease-free water. The amplification steps were initial heating of 95°C for 2 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. After amplification, samples were subjected to gradual denaturation to determine the melting curve using CFX Touch™ Real-Time PCR Detection System (Bio-Rad Laboratories). Standard curves for each pure aptamer were generated and the correspondent equations were used in the conversion of Ct values from bound aptamers to nanograms. After obtaining these values, a saturation curve was plotted (cell bounded aptamers in nanograms vs the aptamer concentration used in the incubation). The Kd was determined using non-linear regression in GraphPad Prism 7 software (Graph-Pad Software Inc., La Jolla, CA, USA).

**Statistical Analysis**

Data were expressed graphically as mean ± standard deviation (SD). Normal distribution was verified using the D’Agostino & Pearson normality test. Unpaired t-test with Welch’s correction was used to compare pairs of means. The non-linear regression equation (specific binding with Hill Slope) was used to determine Kd values. Statistical significance was considered when p<0.05. All statistical analyzes were performed using GraphPad Prism 7 software.

**Results and Discussion**

Cell-SELEX was performed to select aptamers that specifically bind to ASC, with negative selection steps using human FIB. Due to the similarity of the surface markers (CD) between
ASC and FIB, the search for one biomarker to selectively differentiate these two cell types becomes of great interest. Our group recently identified several genes that are differentially expressed between ASC and FIB. Among them, several genes encoding membrane proteins were found [20]. To find new cell membrane specific targets to identify ASC, aptamers were selected for this work.

We monitored the progression of the aptamers selection by DNA melt assay, an informative method for rapid qualitative evaluation of the aptamers pool’s diversity [22, 23]. Aptamers melting profiles in all selection rounds are shown in Supplementary Information (Fig. S3). The aptamers, corresponding to the 7th round of selection in ASC and the 8th round of selection in ASC and FIB, were sequenced and analyzed by bioinformatics to select candidate aptamers for in vitro validation. Based on the criteria established, four candidate nucleotide sequences were selected and named based on their position in the 8th round of positive selection (Table 2).

Data obtained from the four selected aptamers regarding the number of reads, enrichment (fold), and GC content (%) are shown in Table 3. The number of reads corresponds to the number of times that the oligonucleotide sequence was identified in the bioinformatics analysis after sequencing. The enrichment (fold) was calculated by dividing the number of reads from the 8th round by the number of reads from the 7th round in ASC. Apta 21 showed the highest enrichment value between rounds (3.8) and GC content (cytosine and guanine bases) of 57.3% compared to the other aptamers.

The secondary structure of candidate aptamers and scrambled aptamer was simulated using the mfold web server at 25°C (Fig. 1). Apta 99 showed the lowest value of Gibbs free energy (−12.71 kcal/mol), followed by Apta 21 (−11.49 kcal/mol), Apta 2 (−10.49 kcal/mol), and Apta 42 (−9.00 kcal/mol). Scrambled showed Gibbs free energy value of −24.92 kcal/mol. We based the binding characteristic on a low Gibbs free energy values and the aptamer’s high thermodynamic stability, optimizing contact with the cell membrane target. Therefore, Apta 99 is the candidate aptamer with the greatest thermodynamic stability. Furthermore, among the Cell-SELEX studies present in the literature, it was observed that the total ΔG values of DNA and RNA aptamers are variable within a group of negative values [31, 32], which is similar to the calculated values for candidate aptamers. These observations indicate that the selection of thermodynamically stable aptamers is important for in vitro validation assays. The selected aptamers were clustered using the neighbor joining tree provided by MEGA-X 10.0.5 software (Fig. 2). Two clusters were observed: Apta 2 and Apta 99 showed sequence homology, while Apta 21 and Apta 42 showed sequence similarity and were classified in another branch.

One of the suggested and most commonly used methods to assess aptamers’ binding to cultured cells is flow cytometry [1, 33]. Although the flow cytometry assay is commonly used

| Aptamer       | Nucleotide sequence                                                                 |
|---------------|--------------------------------------------------------------------------------------|
| Apta 2        | 5’ATGGTCTCACTGCGTTCCAGCAACTCCCGTGTAGCCGTATCTCCCTCCA |
| (Apta 2)      | TGGCGGCTCCAGTGTGGCTACTGCTTCAATCCGCCATGGAATGCTTCAGAAC                      |
| Apta 21       | 5’ATGGTCTCACTGCGTTCCAGAAAGCGGTTCACAGATGTGTGCCCGCAAATCCCCC |
| (Apta 21)     | GAACCTCCGGCCGCTATGACGTATCTCCCTCTGCTGCTGCTCCGAATCT-3’                        |
| Apta 42       | 5’ATGGTCTCACTGCGTTCCAGACGTGCTACTAGTTGCTTACCATCAGTGTGCTCTTACGAAGA             |
| (Apta 42)     | ACTCCCGCTACCTCCGCCCTACACGGTGCTCTCTGCTACGGCGCAATCT-3’                       |
| Apta 99       | 5’ATGGTCTCACTGCGTTCCAAGCAGACATGATTTGGCGGACGATTTGTCTCGTACTT |
| (Apta 99)     | CTGCAGCCTTTGATCATTTCTCCCTTCGCTTGCTACCGAATCT-3’                             |

Random regions of sequences are highlighted in bold

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to assess the binding of aptamers to cultured cells, quantitative PCR validation stands out due to the reduction amount of the number of cells required for these validation assays. Besides that, aptamer binding by flow cytometry requires dissociation of cells, which is not ideal for adherent cells. Dissociation of ASC from the plastic surface can modify protein conformation and other molecules in the cell membrane and could introduce bias for the selection and validation of candidate aptamers.

Table 3  Number of reads, enrichment and GC content of candidate aptamers

| Aptamer | ASC Number of reads—7th round | ASC Number of reads—8th round | Enrichment (fold)* | FIB Number of reads—8th round | GC content (%)** |
|---------|-------------------------------|-------------------------------|-------------------|-------------------------------|------------------|
| Apta 2  | 100                           | 269                           | 2.6               | 57                            | 54.2%            |
| Apta 21 | 25                            | 95                            | 3.8               | 26                            | 57.3%            |
| Apta 42 | 30                            | 78                            | 2.6               | 0                             | 53.1%            |
| Apta 99 | 22                            | 49                            | 2.2               | 0                             | 53.1%            |

*Ratio between the number of reads in the 8th and 7th rounds in ASC; **GC content = percentage equivalent to the amount of cytosine and guanine bases of the oligonucleotides

Fig. 1  Predicted secondary structures of four aptamers selected for in vitro analyses. The structural predictions and ΔG values of a Apta 2, b Apta 21, c Apta 42, d Apta 99, and e scrambled were obtained using the mfold web server, including the primer regions under 25°C
Aptamers can be recovered by elution after incubation with the target cells, amplified by PCR and evaluated quantitatively compared to the control cells. To avoid errors in this quantification, the presence of a normalizing sequence is necessary. Thus, the AT1 sequence from the vegetable *Arabidopsis thaliana* was inserted in binding assays according to the method proposed by Graziani and colleagues [21]. We and other groups have also demonstrated the binding of DNA aptamers and the internalization of RNA aptamers in various types of cells using qPCR [22, 30, 34, 35], opening a possibility of future clinical application of this technique as an efficient alternative to the use of flow cytometry. The results of the binding assays are shown in Figure 3. Box-plot graphs showed no difference in the binding capacity of aptamer 42 (Fig. 3c) and scrambled (Fig. 3e) when comparing ASC to FIB. We also observed that the interaction of aptamers with the molecular targets present on cells’ surface occurs with more affinity than the scrambled sequence, whose interaction is nonspecific. Aptamers 2 (Fig. 3a), 21 (Fig. 3b), and 99 (Fig. 3d) showed better binding capacity to ASC compared to FIB (**p < 0.001).

The Kd of these three potential candidates are shown in Figure 4. The Kd for Apta 2 incubated with ASC was 82.44 ± 4.44 nM (Fig. 4a), and when incubated with FIB was 44.39 ± 2.14 nM (Fig. 4d). The Kd for Apta 21 incubated with ASC was 50.46 ± 2.28 nM (Fig. 4b) and 86.78 ± 9.14 nM with FIB (Fig. 4e). For Apta 99 incubated with ASC, the Kd was 72.71 ± 10.3 nM (Fig. 4c), and when incubated with FIB was undetermined (ambiguous) (Fig. 4f).

Candidate aptamers 21 and 99 showed more affinity for ASC and less affinity for FIB, unlike aptamer 2 whose affinity was greater for fibroblasts. Considering aptamers 21 and 99, we hypothesize that both aptamers have important binding motifs to detect molecular targets present in the ASC membrane. Still, only Apta 99 showed specificity for the target cells since the Kd for FIB was undetermined. Although Apta 21 showed greater enrichment between rounds and higher percentages of GC content (Table 3), Apta 99 showed the lowest value of Gibbs free energy (Fig. 1), indicating better structural stability under room temperature conditions.

Total internal reflection microscopy (TIRF) confirmed that Apta 99 was more capable of binding to ASC than FIB (Fig. S4 a and b). The TIRF fluorescence of Apta 21 was similar between ASC and FIB (Fig. S4 c and d). TIRF is a powerful technique for observing
fluorescent molecules in living cells attached to a coverslip (solid surface with a high index of refraction). Depending on the objective numerical aperture (N.A.) and excitation wavelength, the thickness of the excitation depth, which is defined as the evanescent field, can be less than 100 nm from the coverslip [36]. The advantages of such a small volume of illumination are as follows: (1) the cells are exposed to significantly less light; (2) the background is less than 2,000 times when compared to normal epifluorescence microscopes; and (3) there is less out of focus fluorescence collected when compared to a confocal microscope [37].
Researchers have conducted several studies to identify MSCs derived from diverse human tissues [15–19]. However, the application of aptamers in studying normal human stem cells is still little compared to cancer stem cells [38]. Due to the scientific value of studies involving human stem cells, our data appear as pioneers in the application of Cell-SELEX to identify ASC using dermal fibroblasts as a negative selection control. This tool can be applied to isolation protocols to ensure the preparation of ASC free from other cellular contaminants, especially fibroblasts. Also, Apta 99 can bind to an extracellular marker, which allows the identification of ASC in matrix/scaffold culture systems. Future research will use the selected aptamers as bait followed by mass spectrometry analysis to determine the specific biomarkers they bind, as described by aptamer-facilitated biomarker discovery technology (AptaBiD) [39].

Conclusions

In conclusion, the Cell-SELEX method has been successfully adapted to select aptamers capable of binding with more affinity and efficiency to mesenchymal stem cells derived from human adipose tissue. It was possible to identify thermodynamically stable candidate sequences and determine the dissociation constant (Kd) of these aptamers by quantitative PCR. This technique is highly sensitive and effective, but it is not commonly used in aptamer validation tests. According to our results, Apta 99 was the most promising aptamer for accurate identification of ASC in future cell purification assays and cell therapy protocols.
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12010-021-03618-5.

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Author Contribution All authors contributed to the study’s conception and design. Material preparation, data collection, and analysis were performed by Mariane Izabella Abreu de Melo, Pricila da Silva Cunha, Marcelo Coutinho de Miranda, Joana Lobato Barbosa, Jerusa Araújo Quintão Arantes Faria, and Davidson Assis Gomes. Funding acquisition, resources, and supervision were provided by Michele Angela Rodrigues, Priscila da Silva Cunha, Alfredo Miranda de Goes, and Davidson Assis Gomes. Mariane Izabella Abreu de Melo wrote the first draft of the manuscript, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The data that supports the findings of this study are included in this article and available in the supplementary information.

Declarations

Ethics Approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Research Ethics Committee of the Universidade Federal de Minas Gerais (No. 02508018.1.0000.5149).

Consent to Participate Informed consent was obtained from all individual participants included in the study.

Competing Interests The authors declare no competing interests.

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