Research in Pharmaceutical Sciences, August 2018; 13(4): 353-359
Received: January 2018
Accepted: June 2018

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Construction and characterization of human embryonic kidney-(HEK)-293T cell overexpressing truncated α4 integrin

Azam Fatahi[1], Ilnaz Rahimmanesh[2], Mina Mirian[3], Fattah Rohani[4], Maryam Boshtam[5], Azam Gheibi[6], Laleh Shariati[5,7], Hossein Khanahmad[2,*], and Shirin Kouhpayeh[8,*]

[1]Department of Biology and Biochemistry, Payame Noor University, Taft, Yazd, I.R. Iran.
[2]Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.
[3]Department of Pharmaceutical Biotechnology, Isfahan Pharmaceutical Science Research Center, School of Pharmacy and Pharmaceutical Science, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.
[4]Faculty of veterinary medicine, University of Shahrekord, Shahrekord, I.R. Iran.
[5]Isfahan Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.
[6]Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, I.R. Iran.
[7]Applied physiology research center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.
[8]Isfahan Neurosciences Research Center, Alzahra Research Institute, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

Abstract

Blockade of α4 integrin by antibodies could be an appropriate treatment strategy in multiple sclerosis and Crohn's disease. Considering disadvantages of antibodies, other elements (e.g. aptamers) have been proposed for antibodies replacement. Isolation of aptamers through cell-SELEX (systematic evolution of ligands by exponential enrichment) method requires positive and negative α4 integrin expressing cell lines. For a better isolation, we intended to construct a negative cell line lacking of specific ligand binding site of α4 integrin. *Escherichia coli* strain top 10 was used for truncated integrin subunit α4 *(TITGA-4)* expression. Human embryonic kidney (HEK)-293T cell was transfected with linearized *TITGA-4* plasmid and subsequently screened for stable *TITGA-4* expressing cells. Chromosomal DNA of *TITGA-4*-transfected cells was extracted and the presence of *TITGA-4* gene in HEK-293T genome was confirmed by polymerase chain reaction (PCR). The expression level of *TITGA-4* on HEK-293T cells was also analysed by real-time PCR and flow cytometry. Real-time PCR and flow cytometric analysis showed significant difference of *TITGA-4* expression between untransfected HEK-293T cells compared to transfected cells. The results suggest that we have successfully constructed the truncated integrin α4 expressing HEK-293T cell, which will facilitate further research into the production of antibody, nanobody, and aptamer against α4 integrin.

Keywords: HEK-293T; Integrin α4; *ITGA4;* Multiple sclerosis.

INTRODUCTION

The pathogenesis of multiple sclerosis (MS) involves several steps including immigration of autoreactive immune cells to the central nervous system (CNS), inflammation, blood-brain barrier (BBB) breakdown and demyelination, though the mechanism of MS progression is not clearly understood. Since then numerous animal model studies confirmed and extended the predominant involvement of α4 integrin and vascular cell adhesion molecule-1 (VCAM-1) interaction in inflammatory cell recruitment into the CNS and pathogenesis of MS (1). Integrins are heterodimeric glycoprotein receptors that function as the major metazoan receptors for cell adhesion and connection of intracellular and extracellular environments (2,3).

*Corresponding authors:
Sh. Kouhpayeh, Tel: +98-3137929144, Fax: +98-3136688597
Email: Shirin_ake@yahoo.com
H. Khanahmad, Tel: +98-3137929144, Fax: +98-3136688597
Email: H_khanahmad@med.mui.ac.ir

Access this article online

Website: http://rps.mui.ac.ir

DOI: 10.4103/1735-5362. 235162

Integrins exist as two non-covalently bound α and β subunits, which pair to form heterodimers (4,5). Integrins can bind to extracellular matrix (ECM) glycoproteins including collagens, fibronectins, laminins, and cellular receptors such as VCAM-1 and the intercellular cell adhesion molecule (ICAM) family (5,6). Integrin subunit alpha 4 (ITGA4) as a part of integrin α4β1 (CD49d/CD29) or very late antigen 4 (VLA-4) recognizes both type III connecting segment of fibronectin (3,7) and VCAM-1 expressed on activated endothelial cells and bone-marrow stromal cells (8-12). Previous studies indicated that the putative binding site for various ligands in ITGA4 structure is distinct but located in a small region of 107-268 residues. α4β1 is possibly involved in the interaction of leukocytes with activated endothelial cells which has a leading role in atherosclerosis, Crohn's disease, and MS (13-16).

Regarding the α4 integrin role in MS pathogenesis, natalizumab (Tysabri®), a monoclonal antibody (mAb), was introduced to block α4 integrin and inhibit α4-mediated migration of leukocytes through BBB (17), that is still prescribed for relapsing-remitting MS (RRMS) patients (18). There are many disadvantages for mAbs including high immunogenicity and molecular weight and low heat stability, which led to vast researches on other agents to overcome the above problems. One of the selected agents for mAb substitution is aptamer (19) which is short single-stranded DNA or RNA sequences with low molecular weight and immunogenicity and high heat stability (20). Aptamers could bind to specific surface markers and inhibit their function, thus applied as diagnostic and therapeutic tools (21).

Systematic evolution of ligands by exponential enrichment (SELEX) process is used to select sequence of specific aptamers. Cell SELEX is the most convenient SELEX methods, in which protein targets expressed on the cell surface. Thus, cell types applied for negative and positive selection is of great importance in cell SELEX (20).

In this research, a recombinant human embryonic kidney-293 (HEK-293T) cell overexpressing truncated human ITGA4 (HEK-293/TITGA4) lacking ligand binding site was constructed. HEK293/TITGA4 could further be utilized as negative cell in SELEX process to isolate a specific aptamer against ITGA4 ligand binding site. The generated aptamer might be employed in future studies for treatment of MS and Chrohn's disease.

MATERIALS AND METHODS

Transformation

Z2827-M67-(ITGA4) expression vector (laboratory stock) encoding the human ITGA4, pcDNA3.1/Hygro (+) vector and *Escherichia coli* (*E. coli*) strain TOP 10F′ (Pasteur Institute of Iran, Tehran, I.R. Iran) as the host were used in this study. *E.coli* was grown in Luria-Bertani, (Sigma, USA) broth medium supplemented with 50 µg/mL tetracycline under aerobic condition and shaking at 250 rpm and 37 °C. *E.coli* was then separately transformed with the mentioned expression vectors in order to amplify the plasmid using $CaCl_2$ chemical DNA transfer method. Subsequently, the transformed cells were spread on Luria-Bertani agar plates containing 100 µg/mL ampicillin incubated at 37 °C overnight. Appeared colonies were inoculated into Luria-Bertani broth containing ampicillin and incubated at 37 °C for 24 h with shaking at 250 rpm in order to extract the plasmid (Fermentas, Canada). The extracted Z2827-M67-(ITGA4) plasmids were digested with *Nhe*I enzyme (Thermo Scientific, USA) according to manufacturer's instruction to make a linear plasmid to apply for overlap polymerase chain reaction (PCR). The digested plasmids were then evaluated using agarose gel electrophoresis. Extracted pcDNA3.1/Hygro (+) was digested with *Bgl*II, treated with *Pfu* and finally digested with *Xho*I.

Polymerase chain reaction

Two 879 and 2312 bp segments of linear Z2827-M67-(ITGA4) were amplified using forward and reverse primers containing *Nhe*I and *Xho*I enzyme cutting sites, respectively. The PCR program for 879 bp segment amplification included a primary denaturation at 94 °C for 4 min followed by 35 cycles of 94 °C for 30 s, 58 °C for 30 s,

72 °C for 2 min, with final extension step at 72 °C for 10 min. The PCR program for 2312 bp fragment amplification was exactly the same with an extension step of 72 °C for 4.5 min. The PCR products then were visualized by 1.5% agarose gel electrophoresis. The 879 and 2312 bp fragments were extracted from the gel using gel extraction kit (Bioneer, Korea). Overlap extension PCR was performed using a pair of overlapping primers (Table 1). The PCR program was initiated with one cycle at 94 °C for 4 min, continued by 5 cycles at 94 °C for 30 s and 58 °C for 7 min followed by 30 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 7 min, and ended with a final extension cycle at 72 °C for 10 min. The overlap PCR product was then confirmed on 1% agarose gel. Overlap PCR product (3191 bp) was treated with *Xho*I (Fermentase, USA) restriction enzyme and finally subcloned in *Xho*I-treated Z2827-M67-(ITGA4) vector in a ligation reaction. The recombinant vector was named as pcDNA-TITGA4. Transformation of ligation product was then performed and positive clones were extracted and evaluated with *Xho*I and *Nhe*I. The extracted pcDNA-TITGA4 plasmids were linearized with *Nhe*I restriction enzyme.

Cell culture

The HEK-293T cell line (Pasteur Institute of Iran, Tehran, I.R. Iran) was grown in DMEM (Sigma, USA) supplemented with 10% fetal calf serum (Gibco, USA), 100 U/mL penicillin (Gibco, USA) and 100 µg streptomycin (Gibco, USA) at 37 °C in a 5% CO2 incubator.

Transfection of human embryonic kidney-293T cell

Human embryonic kidney -293T cells in exponential growth phase were seeded (5×10^6 cells) a day before transfection. The medium was then changed with fresh medium 2 h before transfection. Transfection was performed using TurboFect reagent (Thermo Fisher Scientific, USA) based on manufacturer's instruction. The transfected cells were grown in a non-selective medium for 48 h. Subsequently, the cells were exposed to 200 µg/mL hygromycin B (Gibco, USA) for 21 days. Hygromycin-resistant clones were randomly picked up, and cultured in separated flasks containing the medium supplemented with 200 µg/mL hygromycin B. The resistant clones are considered as stable transfected cell lines. The HEK-293T cell line which was left untreated, served as negative control.

DNA extraction and polymerase chain reaction on genomic DNA

Chromosomal DNA was extracted from *TITGA-4*-transfected cells using commercial extraction kit (BIONEER, Korea) according to manufacturer's instructions. The presence of *TITGA4* fragment in genome of transfected cells was assayed by PCR using specific primers designed for amplification of partial human *TITGA4*. The same program for amplification of fragment 879 bp was applied for verification of *TITGA4* internalization into the HEK-293T cell genome. Agarose gel electrophoresis was performed to visualize the PCR product.

Table 1. The primer designation. Forward primer (PF) *NheI* and reverse primer (PR) *XhoI* were used for amplification of 879 and 2312 bp fragments respectively. PF and PR over truncated α4 integrin (TITGA4) were used for overlap polymerase chain reaction (PCR). PF and PR TITGA4 were used for real-time PCR with the pair of β-actin primers as internal control.

Primer name	Primer sequence
PF *Nhe*I genecopoeia	5′ AAAGCTAGCTGCCACCTGACGTCTAAGA 3′
PR *Xho*I TITGA4	5′ CACTCGAGCTAATCATCATTGC 3′
PF Over TITGA4	5′ TGAGAGCGCGCTGGTCGGAGGAGCTCCCCAAC 3′
PR Over TITGA4	5′ TCCTCCGACCAGCGCGCTCTCAGTGTC 3′
PF β-Actin	5′ ACCAACTGGGACGATATGGAGAAGA 3′
PR β-Actin	5′ TACGACCAGAGGCATACAGGGACAA 3′
PF TITGA4	5′ ACGTGCGAACAGCTCCAG 3′
PR TITGA4	5′ ACTCCATAGCAACCACCAG 3′

Real-time polymerase chain reaction

Total RNA of about 10^6 transfected and control cells was extracted using RNX kit (Cinagen, I.R. Iran) and treated with DNase I enzyme (Thermo Scientific, USA). First strand cDNA was synthesized using 2 µg of the total RNA according to first strand cDNA synthesis kit instruction (Thermo Scientific). Relative quantification was performed by specific primers and β-Actin was used as internal control (Table 1). All real-time RT-PCR reactions were prepared in triplicate by mixing 10 µL of SYBR green master mix (Ampliqon kit, Denmark), 0.5 µL of each primer (10 µM), 2 µL of synthesized cDNA and 7µL RNase-free water. The reaction program was 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min.

Flow cytometric analysis

Human embryonic kidney (HEK)-293/TITGA4 recombinant cells were initially trypsinized, washed twice with phosphate-buffered saline (PBS) and then stained with anti-human CD49d antibody (Biolegend, USA) conjugated with phycoerythrin for 30 min at 4 °C. Cell suspension was then resuspended in 500 µL of PBS. Flow cytometric analysis was performed on FACS Calibur flow cytometer (BD Bioscience, USA) by accumulating up to 100,000 cells per tube. The untransfected cells were used as a negative control.

RESULTS

Truncated ITGA4 construction

Z2827-M67-(ITGA4) and pcDNA3.1Hygro (+) plasmids were applied in order to construct truncated *ITGA4* through transfection, which were further verified subsequent to positive clone selection (data not shown). Subsequently, agarose gel electrophoresis results showed the amplification of two 879 and 2312 bp fragments of *ITGA4* (Fig. 1a). Visualization of overlap PCR product also revealed a 3191 bp band (Fig. 1b).

Chromosomal DNA extraction and amplification

Genomic DNA of the transfected cells was extracted and PCR amplified. The PCR product illustrated amplification of an 879 bp DNA band on 1% agarose gel, which verified the integration of *TITGA4* in to the HEK-293T cells (Fig. 2).

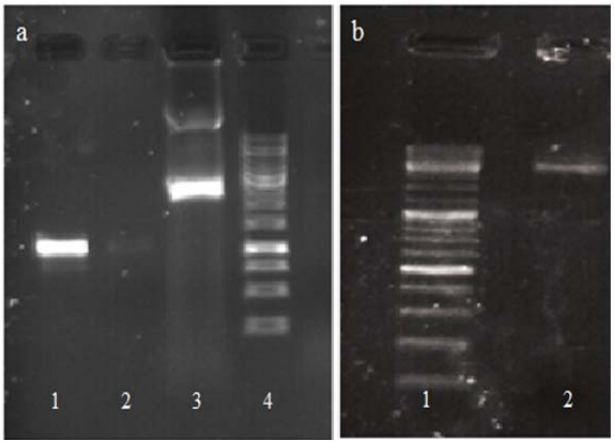

Fig. 1. Polymerase chain reaction (PCR) and overlap PCR results. (a) Amplification of two α4 integrin (*ITGA4)* gene fragments; lane 1, 879 bp fragment; lane 3, 2312 bp fragment; lane 4, marker 1kb. (b) Overlap PCR product; lane 1, marker mix, lane 2, 3191 bp fragment.

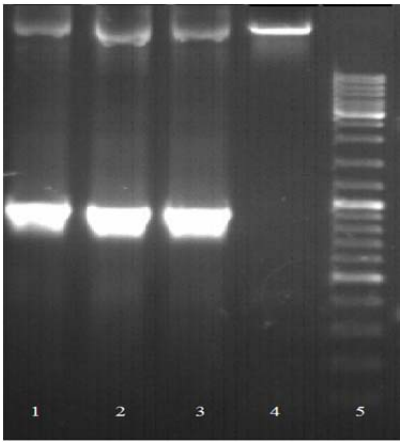

Fig. 2. Chromosomal DNA amplification. Agarose gel electrophoresis results illustrated the insertion of truncated α4 integrin (*TITGA4)* into the genome of transfected cells. Lane 1-3, amplified 879 bp fragment of transfected cells; lane 4, untransfected cell, and lane 5, marker 1 kb.

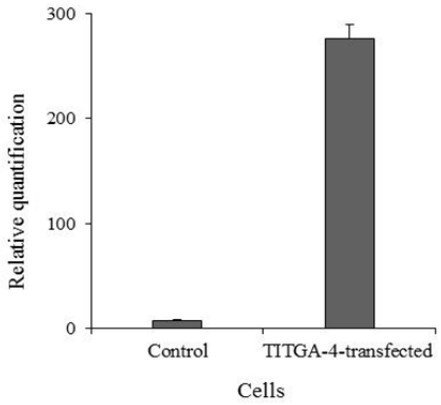

Fig. 3. Real-time polymerase chain reaction (PCR) results. Truncated α4 integrin (*TITGA4)* expression level was significantly increased in transfected cells in comparison with negative control ($P < 0.001$) as illustrated in the graph.

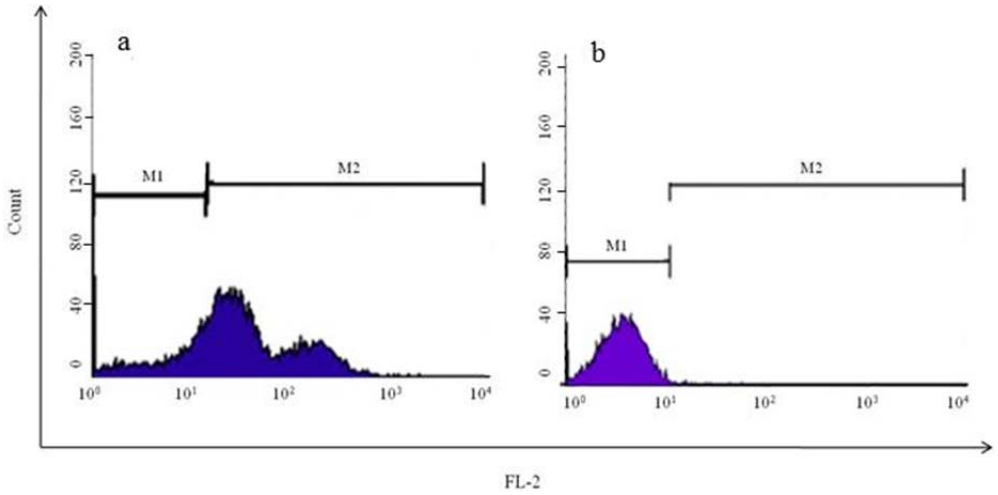

Fig. 4. Flow cytometric analysis. The results showed the overexpression of α4 integrin (ITGA4) in transfected cells (a) when compared to untransfected cells (b).

Real-time polymerase chain reaction and flow cytometric analysis

The mean value of *TITGA4* relative quantitation illustrated that expression level of *TITGA4* was significantly increased in transfected cells (276 ± 2.9) compared with control cells (8 ± 0.8, $P < 0.001$) as shown in Fig. 3.

As shown in Fig. 4, HEK-293T cells have no expression of α4 integrin on their surface while the expression level of α4 integrin in HEK-293/TITGA4 cells was 76%, which correlates well with genomic DNA PCR amplification and real-time PCR results.

DISCUSSION

The role of α4 integrin in pathogenesis of a few diseases (15) has been approved and thus blockade of this molecule has been considered as a promising therapeutic approach. Aptamers have been introduced as appropriate candidates to achieve this purpose. Aptamers were developed by application of a SELEX method, cell SELEX, as the most common technique of aptamer preparation amongst different available strategies. Cell-SELEX requires both positive and negative cell lines to facilitate the aptamers development against the target of interest (20). In the current study we constructed an artificial HEK-293T cell enable to overexpress truncated α4 integrin to serve as a negative cell for SELEX. The HEK-293T cell line was transfected with *TITGA4* lacking ligand binding site. The integration of *TITGA4* in HEK-293T cells was evaluated using PCR on genomic DNA, which was followed by assessment of *TITGA4* gene expression on

RNA and protein levels. Real-time PCR results revealed a great number of *TITGA4* copy that was in accordance with flow cytometry data showing 76% transfected cells overexpressing TITGA4. A recent study constructed HEK-293T cells overexpressing α4 integrin for further use as a positive cell in SELEX strategy (22). In this study, PCR and flow cytometry methods were used similarly in order to verify α4 integrin integration into genomic DNA and the protein expression, respectively (22). In three separate studies, exactly the same procedures were applied to conduct the generation of recombinant cells overexpressing HBsAg (23), MCP-1 (24) and TIM-1 (25) for diagnostic and therapeutic purposes.Greater similarity of the target with intact molecule results in a more specific aptamer isolation. Moreover, limiting of the target to one or a few specific sites in target structure leads to reduction of cross-reaction and side effects of aptamer in future.

Mammalian cells including HEK-293T are widely used in laboratory research (26). Due to post-translational modifications (PTM) of proteins in eukaryotic cells like HEK-293T and production of appropriately folded protein, mammalian cells are considered as a good choice for target gene expression (25). There are several lines of evidences which have shown the use of same cell due to necessity of PTM or precise assembly of target. For instance Aricescu *et al.* showed the importance of using mammalian cells like HEK-293 for expression of targets which have a high rate of PTM or need to be supra-assembled (26).

There are three general systems for transferring gene into the cells including biological, chemical and physical methods (27,28). In the present study, a chemichal method with polycationic reagent was applied to transfer *TITGA4* cDNA into the HEK-293T cells. Due to the possibility of viral DNA integration into the cell genome (29,30) biological method was not used in this study. Moreover, as physical methods need special instruments (29), unavailable in our laboratory, were not used in the current study. Transfection efficiency of 76% using chemical methods indicates high efficiency of the method for transferring genes into the cells.

Recombinant HEK-293T cell over-expressing *TITGA4* could further be applied in monoclonal and polyclonal antibody production, nanobody or aptamer selection against ITGA4 ligand binding site. This strategy leads to production of mentioned agents with high specificity and affinity against a particular site in target structure.

CONCLUSIONS

In the present study, we developed an artificial cell that overexpresses TITGA4. This cell provides a platform for selection of aptamers with highest affinity and specificity against ITGA4 ligand binding site. This cell could be further considered as the negative cell to avoid inclusion of aptamers against all parts of ITGA4 structure in SELEX process.

ACKNOWLEDGEMENT

The content of this paper is extracted from a M.Sc. thesis (Grant No. 292136) submitted by Hossein Khanahmad which was financially supported by the Pediatric Inherited Diseases Research Center, Research Institute for Primordial Prevention of Non-Communicable Disease, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

REFERENCES

1. Goverman J. Autoimmune T cell responses in the central nervous system. Nat Rev Immunol. 2009;9(6):393-407.
2. Burke RD. Invertebrate integrins: structure, function, and evolution. Int Rev Cytol. 1999;191:257-284.
3. Guan JL, Hynes RO. Lymphoid cells recognize an alternatively spliced segment of fibronectin via the integrin receptor alpha 4 beta 1. Cell. 1990;60:53-61.
4. Darzi L, Boshtam M, Shariati L, Kouhpayeh S, Gheibi A, Mirian M, *et al*. The silencing effect of miR-30a on ITGA4 gene expression in vitro: an approach for gene therapy. Res Pharm Sci. 2017;12(6):456-464.
5. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell. 2002;110(6):673-687.
6. Plow EF, Haas TA, Zhang L, Loftus J, Smith JW. Ligand binding to integrins. J Biol Chem. 2000;275(29):21785-21788.
7. Wayner EA, Garcia-Pardo A, Humphries MJ, McDonald JA, Carter WG. Identification and

characterization of the T lymphocyte adhesion receptor for an alternative cell attachment domain (CS-1) in plasma fibronectin. J Cell Biol. 1989;109(3):1321-1330.
8. Elices MJ, Osborn L, Takada Y, Crouse C, Luhowskyj S, Hemler ME, *et al*. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. Cell. 1990;60(4):577-584.
9. Rice GE, Bevilacqua MP. An inducible endothelial cell surface glycoprotein mediates melanoma adhesion. Science. 1989;246(4935):1303-1306.
10. Osborn L, Hession C, Tizard R, Vassallo C, Luhowskyj S, Chi-Rosso G, *et al*. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. Cell. 1989;59(6):1203-1211.
11. Miyake K, Medina K, Ishihara K, Kimoto M, Auerbach R, Kincade PW. A VCAM-like adhesion molecule on murine bone marrow stromal cells mediates binding of lymphocyte precursors in culture. J Cell Biol. 1991;114(3):557-565.
12. Kina T, Majumdar AS, Heimfeld S, Kaneshima H, Holzmann B, Katsura Y, *et al*. Identification of a 107-kD glycoprotein that mediates adhesion between stromal cells and hematolymphoid cells. J Exp Med. 1991;173:373-381.
13. Kamata T, Puzon W, Takada Y. Identification of putative ligand-binding sites of the integrin alpha 4 beta 1 (VLA-4, CD49d/CD29). Biochem J. 1995;305(Pt 3):945-951.
14. Galkina E, Ley K. Immune and inflammatory mechanisms of atherosclerosis (*). Annu Rev Immunol. 2009;27:165-197.
15. Mitroulis I, Alexaki VI, Kourtzelis I, Ziogas A, Hajishengallis G, Chavakis T. Leukocyte integrins: role in leukocyte recruitment and as therapeutic targets in inflammatory disease. Pharmacol Ther. 2015;147:123-135.
16. Cybulsky MI, Gimbrone MA Jr. Endothelial expression of a mononuclear leukocyte adhesion molecule during atherogenesis. Science. 1991;251(4995):788-791.
17. Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N. Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. Nature. 1992;356(6364):63-66.
18. Outteryck O. Natalizumab in relapsing-remitting multiple sclerosis. Expert Rev Neurother. 2016;16(5):471-481.
19. Song KM, Lee S, Ban C. Aptamers and their biological applications. Sensors (Basel). 2012;12:612-631.
20. Sefah K, Shangguan D, Xiong X, O'Donoghue MB, Tan W. Development of DNA aptamers using Cell-SELEX. Nat Protoc. 2010;5(6):1169-1185.
21. Thiviyanathan V, Gorenstein DG. Aptamers and the next generation of diagnostic reagents. Proteomics Clin Appl. 2012;6(11-12):563-573.
22. Naderi Beni S, Kouhpayeh S, Hejazi Z, Heidari Hafshejani N, Khanahmad H. Construction and characterization of recombinant HEK cell over expressing alpha4 integrin. Adv Pharm Bull. 2015;5(3):429-434.
23. Mirian M, Taghizadeh R, Khanahmad H, Salehi M, Jahanian-Najafabadi A, Sadeghi-Aliabadi H, *et al*. Exposition of hepatitis B surface antigen (HBsAg) on the surface of HEK293T cell and evaluation of its expression. Res Pharm Sci. 2016;11(5):366-373.
24. Rahimmanesh I, Khanahmad H, Boshtam M, Kouhpayeh S, Hejazi Z. Cell surface display of rabbit MCP1 on human embryonic kidney 293T cell line. Cell surface display of rabbit MCP1 on human embryonic kidney 293T cell line. 2017;16(3): 284-288.
25. Ebrahimi M, Kazemi T, Ganjalikhani-Hakemi M, Majidi J, Khanahmad H, Rahimmanesh I, *et al*. Development of a stable cell line, overexpressing human T-cell immunoglobulin mucin 1. Iran J Biotechnol. 2015;13(4):25-31.
26. Portolano N, Watson PJ, Fairall L, Millard CJ, Milano CP, Song Y, *et al*. Recombinant protein expressionfor structural biology in HEK 293F suspension cells: a novel and accessible approach. J Vis Exp. 2014;(92):e51897.
27. Zhu J. Mammalian cell protein expression for biopharmaceutical production. Biotechnol Adv. 2012;30(5):1158-1170.
28. Arcescu AR, Owens RJ. Expression of recombinant glycoproteins in mammalian cells: towards an integrative approach to structural biology. Curr Opin Struct Biol. 2013;23(3):345-356.
29. Kim TK, Eberwine JH. Mammalian cell transfection: the present and the future. Anal Bioanal Chem. 2010;397(8):3173-3178.
30. Kealy B, Liew A, McMahon JM, Ritter T, O'Doherty A, Hoare M, *et al*. Comparison of viral and nonviral vectors for gene transfer to human endothelial progenitor cells. Tissue Eng Part C Methods. 2009;15(2):223-231.