Research in Pharmaceutical Sciences, October 2018; 13(5): 430-439
Received:January 2018
Accepted:May 2018

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Display of human and rabbit monocyte chemoattractant protein-1 on human embryonic kidney 293T cell surface

Maryam Boshtam[1], Seddigheh Asgary[1,*], Ilnaz Rahimmanesh[2], Shirin Kouhpayeh[3], Jamal Naderi[1], Zahra Hejazi[2], Hoda Mohammad-Dezashibi[4], Ina Laura Pieper[5], and Hossein Khanahmad[2,*]

[1]Isfahan Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, 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 Immunology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.
[4]Department of Genetics and Molecular Biology, School of Medicine, Semnan University of Medical Sciences, Semnan, I.R. Iran.
[5]Institute of Life Science, Swansea University Medical School, Swansea, United Kingdom.

Abstract

Monocyte chemoattractant protein-1 (MCP-1/CCL2) is a protein that is secreted immediately upon endothelial injury, and thereby it plays a key role in inflammation via recruitment of leucocytes to the site of inflammation at the beginning and throughout the inflammatory processes. Aim of this study was to develop two separate cell lines displaying either human MCP-1 (HMCP-1) or rabbit MCP-1 (RMCP-1) on their surface. A DNA fragment containing HMCP-1- or RMCP-1-encoding sequence was inserted into a pcDNA plasmid. *Escherichia coli* cells strain TOP 10F' was separately transformed with the pcDNA*/RMCP-1* or /*HMCP-1* ligation mixture. Following the cloning and construct verification, human embryonic kidney cell line (HEK 293T) was transfected with either of the linearized plasmids. Plasmid integration into the genomic DNA of HEK 293T cells was verified by polymerase chain reaction (PCR). HMCP-1 and RMCP-1 expression was evaluated at RNA and protein levels by real-time PCR and flow cytometry, respectively. PCR products of the expected sizes were amplified from the chromosomal DNA of transfected HEK 293T cells, i.e. 644 bp for H-MCP1 and 737 bp for RMCP-1 constructs. Real-time PCR revealed that the copy numbers of *RMCP1* and *HMCP1* mRNA per cell were 294 and 500, respectively. Flow cytometry analysis indicated 85% for RMCP-1 and 87% for HMCP-1 expression levels on the surface of transfected cells, when compared with an isotype control. The experiments thus confirmed that the *MCP-1* genes were integrated into the HEK 293T genomic DNA and the encoded proteins were stably expressed on the cell surface.

Keywords: Cell surface display; Chemokine CCL2; Flow cytometry; Transfection; Transformation.

INTRODUCTION

Inflammatory disorders, e.g. atherosclerosis, comprise a wide range of diseases with no definite cure. The initiation step of these diseases is leukocyte recruitment to the injured area, and their differentiation to macrophages after migrating to sub-endothelial tissue. Although, the migrated leukocytes actively remove dead cells from inflammation sites, their important role in the pathology of some diseases was illustrated. As monocyte chemoattractant protein-1 (MCP-1) accelerates the recruitment of monocytes to sites of inflammation, it is considered a key cytokine amongst several mediators involved in the inflammation process (1). Detecting abundant amounts of this chemokine in inflammatory tissue sites, human endothelial and smooth muscle cells, and even in atherosclerotic lesions, verified its crucial role in the initiation and development of inflammation-related disease (2-4).

*Corresponding authors:
S. Asgary, Tel: +98-3136115115, Fax: +98-3136115303
Email: sedighehasgary@gmail.com
H. Khanahmad, Tel: +98-3137922487, Fax: +98-313668859
Email: H_khanahmad@med.mui.ac.ir

Access this article online

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

DOI: 10.4103/1735-5362. 236836

Strategies targeting MCP-1 can therefore constitute an effective approach for the prevention and control of inflammatory diseases. Monoclonal antibodies (mAb) and polyclonal antibodies (pAb) against MCP-1 have been produced (5). The function of these agents is based on the interaction between these proteins and the catalytic sites or functional areas of the target molecules. Advances in antibody design allowed significant therapeutic developments. As human therapeutics, these types of drugs have to remain effective and their use cannot be associated with any side effects; however, as with other essential biomolecules, there are some limitations to their use, such as high immunogenicity, sensitivity to heat and high preparation costs. A new generation of drugs, nanobodies and, more recently aptamers, has been developed to overcome the limitations of antibodies (6).

Aptamers are short oligonucleotide sequences that can bind to a wide-range of target molecules with high affinity and specificity. Compared with mAbs, the immunogenicity of the aptamers is lower, with higher chemical stability. Also, aptamers can be easily manipulated and modified (7). Regarding the advantages of these molecules, aptamers against many targets has been generated (8-11). Because of the pivotal role of MCP-1 in the pathogenesis of inflammatory disorders, an aptamer against MCP-1 would effectively inhibit inflammation and has the potential to impressively revolutionize the control of these diseases.

Systematic evolution of ligands by exponential enrichment (SELEX) is a commonly used method for the selection of aptamers (12). Cell-SELEX is one of the various types of SELEX, used for the isolation of specific sequences against a cell of interest (13). This method is based on the interaction between an aptamer and specific marker on the cell surface. Therefore, cell-SELEX is mostly utilized to distinguish one type of cell from another using aptamers recognizing unique molecular signatures. Hence, lack of suitable cells is a major limitation of this method. In the case of secretory proteins such as MCP-1, expression vectors, e.g. pDisplay, have been designed to express them on the cell surface (14). To produce an aptamer-based drug, several steps of preclinical (animal) and clinical (human) trials have to be carried out. Thus, an aptamer recognizing both human and animal target molecules should be selected. The main SELEX method for selecting such an aptamer is toggle SELEX (15). Toggle SELEX is an *in vitro* selection strategy where aptamers that bind both human and animal target proteins are selected by "toggling" the target between human and animal species during alternating rounds of selection (16). Such selection process results in a set of aptamers that can bind both human and animal target proteins with high affinity. In toggle cell-SELEX, a combinatorial method of toggle- and cell-SELEX, an aptamer that can trap both targeted human and animal antigens expressed on the cell surface, is selected.

Since MCP-1 plays a key role in inflammatory disorders, generating an aptamer against this molecule using a novel combinatorial method based on employing the cell as a scaffold for the expression and anchoring of MCP-1 as the aptamer target would be useful. Using the principles of cell and bead-based SELEX, we developed a toggle cell-SELEX process. The type of the animal protein target depends on the animal model for each disorder. In the case of atherosclerosis and restenosis, the rabbit is one of the appropriate animal models available (17). We therefore aimed to generate two lines of human embryonic kidney (HEK 293T) cells stably displaying human or rabbit MCP-1 (HMCP-1, RMCP-1) on the cell surface to use in the selection of aptamers against both human and rabbit MCP-1.

MATERIALS AND METHODS

Plasmid construction and transformation

A 501 bp-long DNA fragment was synthesized containing *Nhe*I and *Xho*I restriction sites at its 5′ and 3′ ends, respectively; a 76 bp signal sequence of murine Igκ chain (P01837); a 26 bp-long HA tag (EP300); a 225 bp sequence of HMCP-1 (NC_000017.11, P13500) sandwiched between two *BamH*I recognition sites; and

150 bp intra-membrane domain of platelet-derived growth factor (PDGFR) (P09619). The fragment was cloned into *Nhe*I and *Xho*I sites of pcDNA plasmid generating pcDNA/HMCP-1 by GeneCust Company (Luxembourg) (Fig. 1). For the RMCP-1 construct (571d bp), a 303 bp sequence encoding rabbit MCP-1 (NC_013687.1, P28292) was analogously synthesized and cloned into *Nhe*I and *Xho*I sites of pcDNA plasmid, generating pcDNA/RMCP-1 (Fig. 1). The arrangement of the sequences within the construct was similar to pcDNA/HMCP-1, but instead of the HA tag, HMCP-1, and *BamH*I sequences, it contained a His tag, RMCP-1, and two *Kpn*I enzyme sites, respectively.

Escherichia coli (E. coli) cells strain TOP 10F' (*E.coli* TOP 10F') was purchased from the Pasteure Institute (Tehran, Iran). Competent *E. coli* TOP 10F' cells were prepared using the calcium chloride protocol (18), and pcDNA/HMCP-1 and pcDNA/RMCP-1 were used to separately transform the bacteria by a heat shock method. The transformants were cultured on Luria–Bertani (LB) agar (L2897, Sigma-Aldrich, USA) plates containing 50 μg/mL of ampicillin. The resultant colonies were verified by colony polymerase chain reaction (PCR) as follows. The colony PCR program started with an incubation at 94 °C for 4 min; and continued for 30 cycles of 94 °C for 30 s, 60 °C for 30 s and 72 °C for 1 min; and ended with a step at 72 °C for 5 min. The BioRad Thermocycler (Bio-Rad Laboratory, USA) was used. The reaction mixtures contained Taq DNA polymerase (EP0401, Thermo Scientific, USA) (0.25 μL, 1.25 U), 10× buffer (Thermo Scientific) (2.5 μL), 10 mM dNTPs (0.5 μL), 1.25 mM $MgCl_2$ (Thermo Scientific) (1 μL), double distilled water (ddW) (17.75 μL) and 1 μL of 10 mM forward (F) pcDNA backbone primer (5′-ACTAGAGAACCCACTGCTTAC TG-3′) and 1 μL of 10 mM reverse (R) pcDNA backbone primer (5′-ATGGCTGGCAACTA GAAGG-3′). PCR product sizes were verified by agarose gel (1%) electrophoresis and compared with 1 kb DNA ladder to verify their lengths.

Amplification and purification of pcDNA-human monocyte chemoattractant protein-1 and pcDNA-rabbit monocyte chemoattractant protein-1

Escherichia coli TOP 10F' transformants carrying pcDNA/HMCP-1 or pcDNA/RMCP-1 were grown in LB broth (L3152, Sigma-Aldrich, USA) containing 100 μg/mL of ampicillin overnight on a shaker set at 250 rpm and 37 °C. The plasmids were extracted using a SolGent Plasmid Mini Prep kit according to manufacturer's instructions (SPM01-C200, South Korea). The plasmids were linearized by digestion with *Bgl*II enzyme (ER0081, Thermo Scientific). The reactions contained 20 μL of 10× buffer (Thermo Scientific), 5 μL of *Bgl*II and 175 μL of plasmid DNA, i.e. 8 μg. The digestion products were resolved on a 1% agarose gel. The linearized plasmids were extracted using Accuprep Gel Purification Kit (K-3035-1, Bioneer, Korea). Finally, the sequences of the RMCP-1 and HMCP-1 in the plasmids were defined by sequencing (Bioneer).

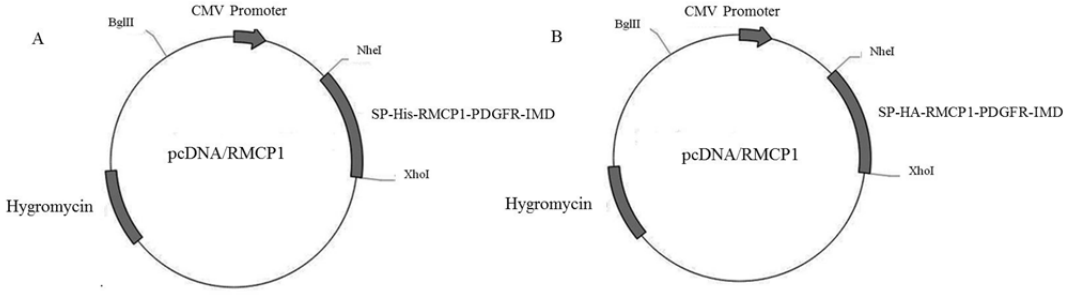

Fig. 1. Maps of expression vectors constructed for the display of human or rabbit monocyte chemoattractant protein 1 (MCP-1) on the HEK 293T cell surface. (A) pcDNA/RMCP-1. The vector includes the following: *Bgl*II restriction site, cytomegalovirus promoter (CMV), *Nhe*I restriction site, Igκ SP (murine Igκ chain signal peptide), His6 tag (an epitope tag for assessing MCP-1 expression levels), Rabbit MCP-1, PDGFR (transmembrane domain of human platelet-derived growth factor receptor), *Xho*I restriction site, and hygromycin resistance gene. (B) pcDNA/HMCP-1. The vector includes the following: *Bgl*II restriction site, CMV, *Nhe*I restriction site, Igκ SP, HA tag (an epitope tag for assessing MCP-1 expression levels), Human MCP-1, PDGFR, *Xho*I restriction site, and hygromycin resistance gene.

Transfection and production of stable cell lines

Cell culture

Human embryonic kidney 293T cell line was purchased from the Pasture Institute of Iran (Tehran, I.R. Iran). The cells were cultured in DMEM medium (D5796, Sigma-Aldrich) supplemented with 1% of penicillin-streptomycin mix (15140148, Gibco, USA) and 10% of fetal bovine serum (FBS) (F2442, Sigma-Aldrich) in a humidified incubator at 37 ºC and 5% CO_2.

Cell transfection

Human embryonic kidney 293T cells were sub-cultured and allowed to grow in density of 7×10^5 cells/mL for the day of transfection. Using a transfection protocol for TurboFect reagent (R0533, Thermo Scientific), 7.5 µg of linear plasmid was suspended in 750 µL of serum-free DMEM medium by gentle vortex-mixing for 1 to 2 min to equilibrate. TurboFect reagent (15 µL) was added to the tube containing the plasmid suspension, incubated at room temperature (22-24 °C) for 15-20 min, and then slowly added to a T_{25} cell culture flask, swirled and incubated 14-16 h at 37 °C. After 24 h of incubation, the culture medium was replaced with a fresh medium, and hygromycin (H3274, Sigma-Aldrich) treatment (150 µg/mL) was started after additional 48 h and continued for 3 weeks. A transient transfection of HEK 293T cells with a plasmid expressing green fluorescent protein (GFP) (pEGFP-N1) was carried out as a control reaction. The transfected cell lines were passaged for nearly 1 year.

Polymerase chain reaction using genomic DNA template

Following the transfection of HEK 293T cells with linear pcDNA/HMCP-1 and pcDNA/RMCP-1, the genomic DNA of the transfected and untransfected cells (negative control) was extracted using a Genetbio DNA extraction kit (USA). PCR was performed using pcDNA primers at 3 weeks, and 2, 3, and 4 months after the transfection. Finally, the sequences were confirmed by sequencing (Bioneer).

Absolute real-time polymerase chain reaction

Total RNA was extracted from transfected HEK 293T cells using a RNX-Plus kit (CinnaGen Co., I.R. Iran). DNA in the extracted RNA samples was removed by DNaseI (Thermo Scientific) treatment. The quality and quantity of the extracted RNA was assessed spectrophotometrically and by electrophoresis.

First strand cDNA was synthesized from 1 µg of RNA using first strand cDNA synthesis kit (K1622, Thermo Scientific). Serial dilutions of the pcDNA/RMCP1 and pcDNA/HMCP1 plasmids (from 10^2 to 10^9 copies/µL) were prepared to construct a standard curve. Real-time PCR reactions (20 µL final volume) containing 1 µL of cDNA, 1× SYBR green master mix (4385412, Thermo Scientific) and 10 µM of each forward and reverse primers were performed in triplicate for each sample. The following primers were used: HMCP-1 forward primer: 5′-TACTGCTGCTCTGGGTTCC-3′, HMCP1 reverse primer: 5′-GATTCTTCTATAGCTCG CGAG-3′; RMCP1 forward primer: 5′-AGCCAGACGCAGTGAATTC-3′, and RMCP1 reverse primer: 5′- TCACGGAGATG GTCTTGTTG-3′. The cycling conditions comprised initial 10 min incubation at 95 °C, followed by 40 cycles of 95 °C for 15 s and 60 °C for 1 min. After amplification, *HMCP-1* and *RMCP1* mRNA cycle threshold (Ct) values were determined for each sample using StepOnePlus software v 2.3 (Applied Biosystems, USA). The mRNA copy numbers per cell were calculated using the standard curves and were based on the number of transfected cells and dilution factor.

Flow cytometry

For each cell line (HMCP-1-HEK and RMCP-1-HEK), a suspension of 2×10^5 transfected cells in 400 µL of DMEM was prepared and divided equally between two flow cytometry tubes. The cells in one of the tubes were stained with 2 µL of a specific conjugated antibody (for HMCP-1-HEK: PE-conjugated monoclonal anti-MCP-1 antibody, Abcam, USA, ab95558; for RMCP-1-HEK: PE-conjugated monoclonal anti-His tag

antibody, Milteny Biotec, Germany, 130098810), (working dilution of 1/100) while the cells in another tube were stained with the appropriate isotype antibodies (PE-conjugated IgG antibody, Abcam, ab95558, and mouse PE-conjugated IgG1 antibody, Milteny Biotec, 130098845, respectively) as control. The tubes were incubated for 45 min at 4 °C in the dark, and the cells were then washed with phosphate buffer saline (PBS) (10010023, Gibco, USA). Finally, the cells from each tube were resuspended in 200 µL of PBS and analyzed for HMCP-1 and RMCP-1 expression using flow cytometry (BD Bioscience, USA) by accumulating up to 50,000 events per tube. The obtained data were analyzed by Cell Quest software version 8.6.1 (BD Bioscience). The flow cytometry was performed at 3 weeks and 2, 3, and 4 months after the transfection.

RESULTS

Plasmid construction and transformation

The results of *E. coli* TOP 10F' colony PCR from selected colonies revealed successful cloning of both expression vectors (Fig. 2).

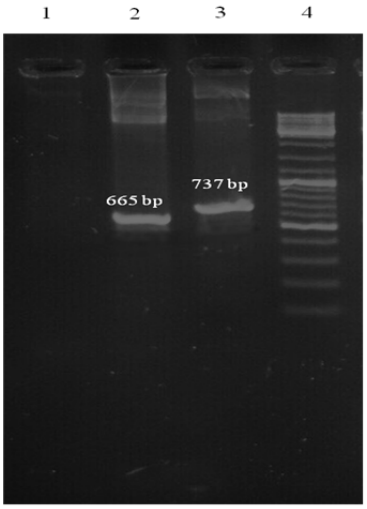

Fig. 2. Colony polymerase chain reaction (PCR) verification of human or rabbit monocyte chemoattractant protein-1 *(HMCP-1 and RMCP-1)* gene sequence integration into pcDNA vector. Lane 1, negative control; lane 2, pcDNA*/HMCP-1* clone (nearly 665 bp band related to a fragment containing *HMCP-1)*; lane 3, pcDNA/*RMCP-1* clone (nearly 737 bp band related to a fragment containing *RMCP-1)*; lane 4, DNA ladder mix.

For positive clones containing pcDNA/RMCP-1 or pcDNA/HMCP-1, a 737 bp or a 644 bp band was observed, respectively. Electropherogram obtained in sequencing of both RMCP1 and HMCP-1 confirmed the sequences of the fragments in pcDNA/RMCP-1 and pcDNA/HMCP-1 expression vectors extracted from transfected *E. coli* (Fig. 3).

Polymerase chain reaction using genomic DNA template

The products of PCR reactions with genomic DNA from HEK 293T cells transfected with pcDNA/HMCP-1 and pcDNA/RMCP-1 migrated in agarose gels as 644 bp and 737 bp bands, respectively, indicating the successful insertion of HMCP-1 and RMCP-1 into the HEK 293T genome (Fig. 4).

Absolute real-time polymerase chain reaction

According to the obtained standard curves prepared using serial dilutions of 10^2 to 10^9 copies/µL from the pcDNA/RMCP-1 plasmids, the copy numbers of *RMCP-1* and *HMCP-1* mRNA per transfected cells were 264 and 500, respectively (Fig. 5A and 5B).

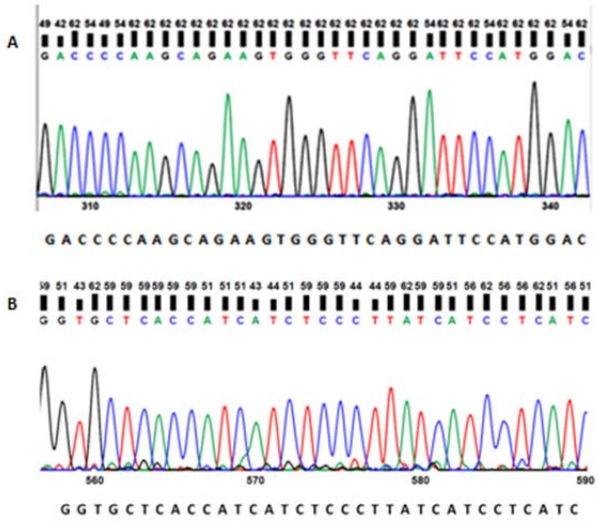

Fig. 3. A part of elecropherogram of rabbit and human monocyte chemoattractant protein-1 (RMCP-1 and HMCP-1) fragment of expression vectors. (A) RMCP-1 sequence of pcDNA/RMCP-1 expression vector extracted from transformed *Escherichia coli* TOP10. (B) HMCP-1 sequence of pcDNA/HMCP-1 expression vector extracted from transformed *Escherichia coli* TOP 10F'.

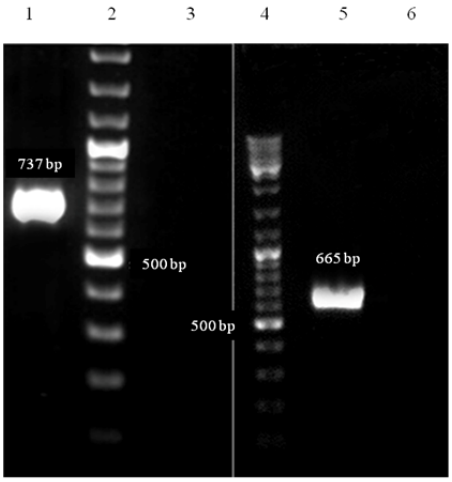

Fig. 4. Polymerase chain reaction (PCR) verification of the integration of monocyte chemoattractant protein-1 (*MCP-1)* expression constructs into the genomes of transfected cells. Genomic DNA of the transfected cells was used as the template in the PCR reactions. Lane 1, 737 bp band corresponding to the chromosome-integrated rabbit *MCP-1* sequence; lane 2, DNA ladder (1 Kb) with relevant size marker labelled; lane 3, negative control; lane 4, DNA ladder (1 Kb) with relevant size marker labelled; lane 5, 665 bp band corresponding to the chromosome-integrated human *MCP-1* sequence; lane 6, negative control.

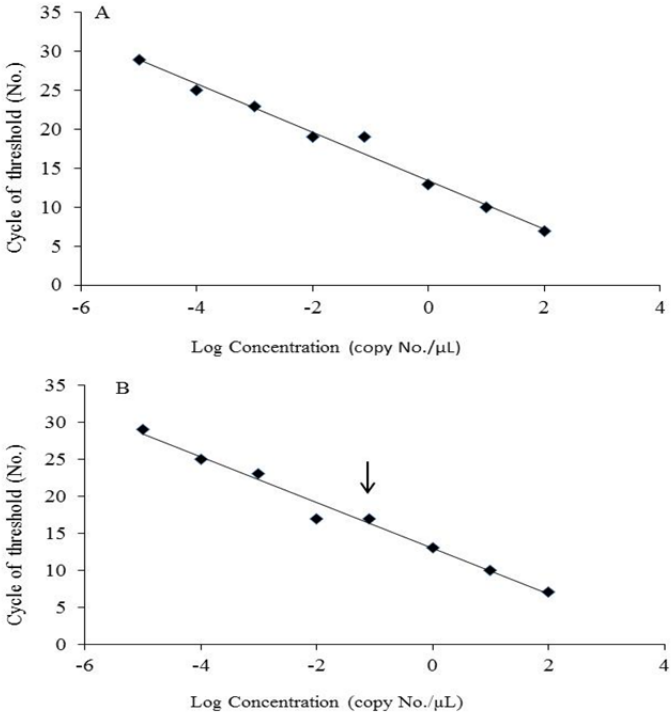

Fig. 5. Rabbit and human monocyte chemoattractant protein-1 (*RMCP-1* and *HMCP-1*) gene expression in the transfected HEK 293T cells. (A) Standard curve was prepared using serial dilutions of 10^2 to 10^9 copies/μL from the pcDNA/RMCP-1 plasmids. *RMCP-1* mRNA copy numbers per cell (black cluster) was 264. (B) Standard curve was prepared using serial dilutions of 10^2 to 10^9 copies/μL from the pcDNA-HMCP-1 plasmids. *HMCP-1* mRNA copy numbers per cell (black cluster) was 500.

Flow cytometry

Flow cytometry analysis revealed that, compared with cells stained with isotype antibodies, RMCP-1 was displayed on 76.5% and 75.8% of the transfected cells at 3 weeks and 4 months after transfection, respectively (Fig. 6A and 6B). For HMCP-1, 84.7% and 86.7% of the transfected cells showed HMCP-1 protein on their surfaces after 3 weeks and 4 months, respectively (Fig. 6C and 6D).

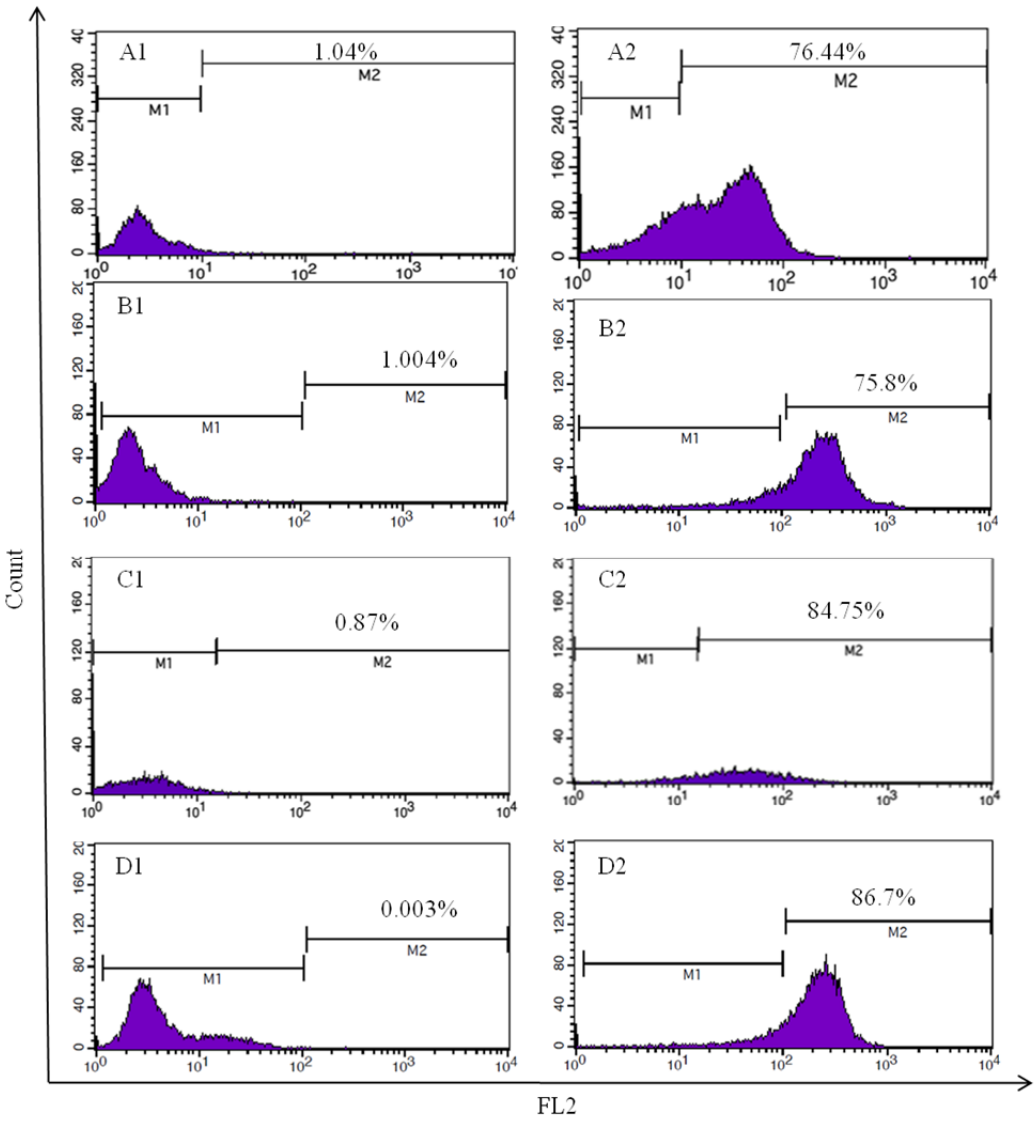

Fig. 6. Cell surface expression of the rabbit and human monocyte chemoattractant protein-1 (RMCP-1 and HMCP-1) proteins in HEK 293T cells. (A) Flow cytometry analysis of RMCP-1 expression in transfected cells after 3 weeks of transfection using (A1) isotypic control and (A2) PE-conjugated anti-His tag antibody for RMCP-1 detection. (B) Flow cytometry analysis of RMCP-1 expression in transfected cells after 4 months of transfection using (B1) isotypic control (B2) and PE-conjugated anti-His tag antibody for RMCP-1 detection. (C) Flow cytometry analysis of HMCP-1 expression in transfected cells after 3 weeks of transfection using (C1) isotypic control and (C2) PE-conjugated anti-HMCP-1 antibody for HMCP-1 detection. (D) Flow cytometry analysis of HMCP-1 expression in transfected cells after 4 months of transfection using (D1) isotypic control and (D2) PE-conjugated anti-HMCP-1 antibody for HMCP-1 detection.

DISCUSSION

In this study, we succeeded to create two cell lines displaying either human or rabbit MCP-1 on their surface. To achieve this aim, at first we constructed two expression vectors (pcDNA/HMCP-1 and pcDNA/RMCP-1) which were designed using important fragments of other major plasmids. Plasmid pDisplay is a specific expression vector for the display of secretory or intracellular proteins on the cell surface (19). In the current study, to enable secretion of the newly synthesized HMCP-1 or RMCP-1 proteins and to anchor them at the HEK 293T cell surface, the murine Ig kappa-chain signal peptide and the intra-membrane domain of PDGFR from pDisplay plasmid (20) were utilized in the construction of pcDNA/HMCP-1 or pcDNA/RMCP-1. A fluorescence gene-based reporter vector was

constructed using pDisplay, as a new tool for identifying cells with an activated Wnt signalling pathway (21). To study the anti-tumor effect of heat shock protein 70 (HSP70) of Bacille Calmette-Guérin (BCG), a recombinant eukaryotic expression vector has been generated by sub-cloning the *BCG HSP70* gene into the multiple cloning site of pDisplay (22). In addition, using recombinant phage display techniques, an intracellular immunization with a single chain Fv fragment (ScFv) of LC-1 antibody (LC-1 ScFv) has been performed to prevent the growth of lung cancer cells (23). As a general tool, pDisplay was utilized to produce a mammalian cell display for antibody engineering (14).

We used HEK 293T cells because the growth and manipulation of these cells is relatively facile and inexpensive. To confirm the integration of HMCP-1 or RMCP-1-coding sequences into the genome of the HEK 293T cells, PCR reactions on genomic DNAs of the transfected HEK 293T cells were carried out. MCP-1 expression in the transfected HEK 293T cells was checked at mRNA and protein levels. Real-time PCR revealed high copy numbers of *HMCP1* and *RMCP1* mRNAs in the respective transfected cells. Flow cytometry evaluations provided evidence that HMCP1 and RMCP1 are localized on the cell surface of nearly 85% and 87% of the transfected HEK 293T cells, respectively. Real time PCR and flow cytometric results demonstrated a correlation between mRNA and protein expression levels for HMCP-1 and RMCP-1. Moreover, repeating the flow cytometry up to 4 months after transfection yielded results. These findings verified the integration of *HMCP-1* or *RMCP-1* genes into the genomic DNA of HEK 293T cells and their stable expression.

In a primary study, we were able to overexpress RMCP-1 antigen on the HEK 293T cell surface with another tag as a pilot study (24).

This strategy has been utilized in many studies. For instance, Mirian *et al*. displayed human hepatitis B surface antigen on the surface of HEK293T cell (25). They used the mentioned cells for selecting a DNA aptamer against hepatitis B surface antigen (not published data). Also, human α4 integrin was overexpressed on the HEK 293T cell surface (26). Consequently, they generated a DNA aptamer against integrin α4 using this recombinant cell (not published data). In two early studies, researchers succeeded to overexpress human T-cell immunoglobulin mucin domain-1 (27) and domain-3 (28) on the HEK 293T cell surface. Similar to the present study, HEK 293T was considered as a host in the mentioned studies. Recently, Bruun *et al.* produced stable HEK 293 cell lines with HIV-1 Env variants on their surface to use in establishing a new technology for affinity-based selection of envelope variants from libraries (29). In 2014, for discovery and optimization of antibody therapeutics, a library of rearranged immunoglobulins with germline V-gene segments was displayed on the surface of HEK293 cells (30). King *et al*., also applied this system for discovery of human therapeutic antibodies (31). In these three projects, HEK 293 cells were used for cell display.

One limitation of our study was the absence of a specific anti-RMCP-1 antibody to verify surface expression of the protein. Instead, anti-His antibody was used in the flow cytometry experiments.

CONCLUSION

In this study, two different recombinant cell lines were prepared which human or rabbit MCP-1 proteins displayed on their surface. These two cell lines are essential for performing toggle cell-SELEX to generate one aptamer against HMCP-1 and RMCP-1. The use of the cells developed in this approach may lead to the selection of a cross-reactive aptamer, facilitating preclinical evaluation of a new generation of drugs for inflammatory disorders.

ACKNOWLEDGEMENT

This project was financially supported via the Grant No. 192148 by the Cardiovascular Research Institute and the Genetic and Molecular Biology Department, School of Medicine, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

REFERENCES

1. Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res. 2009;29(6):313-326.
2. Nelken NA, Coughlin SR, Gordon D, Wilcox JN. Monocyte chemoattractant protein-1 in human atheromatous plaques. J Clin Invest. 1991; 88(4):1121-1127.
3. Rayner K, Van Eersel S, Groot PH, Reape TJ. Localisation of mRNA for JE/MCP-1 and its receptor CCR2 in atherosclerotic lesions of the ApoE knockout mouse. J Vasc Res. 2000;37(2): 93-102.
4. Yla-Herttuala S, Lipton BA, Rosenfeld ME, Sarkioja T, Yoshimura T, Leonard EJ, *et al*. Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions. Proc Natl Acad Sci U S A. 1991;88(12):5252-5256.
5. Satonaka H, Suzuki E, Nishimatsu H, Oba S, Takeda R, Goto A, *et al*. Calcineurin promotes the expression of monocyte chemoattractant protein-1 in vascular myocytes and mediates vascular inflammation. Circ Res. 2004;94(5):693-700.
6. Song KM, Lee S, Ban C. Aptamers and their biological applications. Sensors (Basel). 2012; 12:612-631.
7. Mallikaratchy PR, Ruggiero A, Gardner JR, Kuryavyi V, Maguire WF, Heaney ML, *et al*. A multivalent DNA aptamer specific for the B-cell receptor on human lymphoma and leukemia. Nucleic acids research. 2011;39(6):2458-2469.
8. Boshtam M, Asgary S, Kouhpayeh S, Shariati L, Khanahmad H. Aptamers against pro- and anti-inflammatory cytokines: A review. Inflammation. 2017;40:340-349.
9. Mirian M, Khanahmad H, Darzi L, Salehi M, Sadeghi-Aliabadi H. Oligonucleotide aptamers: potential novel molecules against viral hepatitis. Res Pharm Sci. 2017;12(2):88-98.
10. Parashar A. Aptamers in therapeutics. J Clin Diagn Res. 2016;10(6):BE01-BE06.
11. Vater A, Sell S, Kaczmarek P, Maasch C, Buchner K, Pruszynska-Oszmalek E, *et al*. A mixed mirror-image DNA/RNA aptamer inhibits glucagon and acutely improves glucose tolerance in models of type 1 and type 2 diabetes. J Biol Chem. 2013;288(29):21136-21147.
12. Dua P, Kim S, Lee D-K. Patents on SELEX and therapeutic aptamers. Recent Pat DNA Gene Seq. 2008;2(3):172-186.
13. 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.
14. Ho M, Pastan I. Mammalian cell display for antibody engineering. Methods Mol Biol. 2009;525:337-352.
15. White R, Rusconi C, Scardino E, Wolberg A, Lawson J, Hoffman M, *et al*. Generation of species cross-reactive aptamers using" toggle" SELEX. Mol Ther. 2001;4(6):567-574.
16. Kim YS, Gu MB. Advances in aptamer screening and small molecule aptasensors. Adv Biochem Eng Biotechnol. 2014;140:29-67.
17. Kapourchali FR, Surendiran G, Chen L, Uitz E, Bahadori B, Moghadasian MH. Animal models of atherosclerosis. World J Clin Cases. 2014;2(5): 126-132.
18. Li X, Sui X, Zhang Y, Sun Y, Zhao Y, Zhai Y, *et al*. An improved calcium chloride method preparation and transformation of competent cells. Afr J Biotechnol.2010;9(50):8549-8554.
19. Dalton AC, Barton WA. Over-expression of secreted proteins from mammalian cell lines. Protein Sci. 2014;23(5):517-525.
20. Ho M, Pastan I. Display and selection of scFv antibodies on HEK-293T cells. Methods Mol Biol. 2009;562:99-113.
21. Apfel J, Reischmann P, Muller O. A new fluorescence-based reporter gene vector as a tool for analyzing and fishing cells with activated wnt signaling pathway. ISRN Oncol. 2013;2013:603129.
22. Li XL, Zhao YX, Sun LR, Yang J, Xu HJ. The preparation of HL-60 cells vaccine expressing BCG heat shock protein 70 and detection of its immunogenicity in vitro. Hum Vaccin Immunother. 2012;8(10):1376-81.
23. Chen L, Li G, Tang L, Wang J, Ge XR. The inhibition of lung cancer cell growth by intracellular immunization with LC-1 ScFv. Cell Res. 2002;12:47-54.
24. Rahimmanesh I, Khanahmad H, Boshtam M, Kouhpayeh S, Hejazi Z. Cell surface display of rabbit MCP1 on human embryonic kidney 293T cell line. IJBT. 2017;16(3):284-288.
25. 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.
26. 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.
27. 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.
28. Moballegh M, Khanahmad H, Homayouni V, Ganjalikhani H, Salehi M, Rahimmanesh I, *et al*. Producing recombinant HEK293 T-cells with high expression of T-cell immunoglobulin and mucin domain-3 (TIM3) protein. JIMS. 2015;32(317):2312-2323.
29. Bruun TH, Grassmann V, Zimmer B, Asbach B, Peterhoff D, Kliche A, *et al*. Mammalian cell surface display for monoclonal antibody-based FACS selection of viral envelope proteins. MAbs. 2017;9(7):1052-1064.

30. Bowers PM, Horlick RA, Kehry MR, Neben TY, Tomlinson GL, Altobell L, *et al*. Mammalian cell display for the discovery and optimization of antibody therapeutics. Methods. 2014;65:44-56.

31. King DJ, Bowers PM, Kehry MR, Horlick RA. Mammalian cell display and somatic hypermutation in vitro for human antibody discovery. Curr Drug Discov Technol. 2014;11:56-64.