Research in Pharmaceutical Sciences, August 2019; 14(4): 335-342
Received: October 2018
Accepted: June 2019

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Enhanced transient expression of an anti-CD52 monoclonal antibody in CHO cells through utilization of miRNA sponge technology

Morvarid Sadat Pairawan[1], Azam Bolhassani[2], and Azam Rahimpour[3,4,*]

[1]Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences and Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, I.R. Iran.
[2]Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, I.R. Iran.
[3]Medical Nano-Technology and Tissue Engineering Research Center, Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran.
[4]Department of Tissue Engineering and Applied Cell Sciences, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran.

Abstract

Chinese hamster ovary (CHO) cells are the dominant mammalian host system for the production of recombinant therapeutic proteins. Improving the viable cell density during culture of recombinant CHO cells can greatly affect the production yield. MicroRNAs (miRs) -15a and 16-1 are known as negative regulators of multiple genes involved in cell cycle progression and apoptotic inhibition. miR sponges, which act as decoy targets, are transcripts which contain complementary binding sites to the seed region of related miRs. Stably expressed miR sponges are known as efficient tools for miR loss of function studies. In this study, stable CHO cell pools and clones expressing miRs-15a and 16-1 specific decoy transcript downstream of an enhanced green fluorescent protein reporter gene was developed. Analysis of cell growth during 12 days of batch culture indicated improved maximum viable cell density of CHO cells and clones expressing the decoy transcript. In addition, transient expression of a recombinant anti-CD52 monoclonal antibody was significantly improved in a decoy harboring CHO cell clone, representing a 3.37-fold increase in yield after 4 days of culture. Our results indicated that miR sponge technology can be successfully applied for the improvement of cell viability and transient monoclonal antibody expression in CHO host cells.

Keywords: Chinese hamster ovary cells; MicroRNA sponge; Decoy; miR-15a; miR-16-1; Monoclonal antibody.

INTRODUCTION

Recombinant therapeutic proteins are among the most invested drugs in pharmaceutical industry (1). As the demand for recombinant therapeutic proteins is increasing, more studies are focused on developing efficient strategies for generation of more potent host cell lines. Since post-translational modifications of recombinant protein can greatly affect their activity and immunogenicity, mammalian cell lines are the preferred host systems for manufacturing of these products (2). Despite the availability of a number of mammalian cell lines such as baby hamster kidney, mouse myeloma-derived NS0, human embryonic kidney 293, and human retina-derived PerC6, nearly 70% of all recombinant therapeutic proteins are produced in Chinese hamster ovary (CHO) cells (3).

A number of stresses including nutrients and growth factors depletion, shear and oxidative stresses, metabolic by-products accumulation, pH, and osmolality during CHO cell culture can trigger activation of the apoptosis cascade. Apoptosis induction can affect viable cell density, productivity and the quality of recombinant proteins (4,5).

Several anti-apoptotic proteins including Bcl-xL, Bcl-2, and Mcl-1 have significant roles in protecting cells from apoptosis (6-8).

*Corresponding author: A. Rahimpour
Tel: +98-9127954384, Fax: +98-2122439847
Email: rahimpour@sbmu.ac.ir

Access this article online

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

DOI: 10.4103/1735-5362. 263626

It has been shown that overexpression of the anti-apoptotic proteins can affect the performance and productivity of therapeutic proteins produced in CHO cells (9,10).

MicroRNAs (miRs) are small non-coding RNAs (~22 nucleotides in length) that can regulate post-transcriptional gene expression through degradation of target mRNAs or inhibition of protein translation (11). Many important biological processes including cell cycle control and apoptosis are regulated by miRs. In addition, miRs have the ability to affect several genes in a given pathway which makes them even more attractive for CHO cell engineering (12,13). miR sponges are RNA transcripts harboring multiple binding sites for specific miRs which can bind and sequester miRs from their endogenous targets. The inhibitory activity of a miR sponge not only depends on the affinity and avidity of binding sites, but also on the efficiency of its expression (14).Expression of miR-15a and miR-16-1 is associated with apoptosis induction and reduced cell proliferation. It has been shown that miR-15a and miR-16-1 target multiple cell cycles and apoptosis related genes including cyclins D1, D3, E1, CDK6, Bcl-2, and Mcl-1 (15-17). Indeed, miR-15a and miR-16-1 have been identified as tumor suppressors in some type of cancers including chronic lymphocytic leukemia and HBV-related hepatocellular carcinoma (18,19). In a study conducted by Bonci *et al*. it was shown that inhibition of miR-15a and miR-16-1 using a sponge decoy encoding vector can inhibit apoptosis in LNCaP prostate cancer cell lines (16).

Monoclonal antibodies (mAbs) are known as the most diverse and successful category of recombinant therapeutic proteins due to their high efficacy and specificity (2). CD52 is a cell-surface glycopeptide expressed by human lymphocytes and monocytes. Anti-CD52 monoclonal antibodies are potent lymphocyte depleting agents which have shown substantial benefits for the treatment of chronic lymphocytic leukemia and multiple sclerosis (20,21).

Here we have described development of CHO-K1 stable cells expressing a miRs-15a and 16-1 specific decoy transcript. The growth performance and protein expression productivity of the resulting cells were evaluated in transient expression assay using an anti-CD52 IgG1 mAb as a model. To our knowledge, this is the first report on utilization of miRs-15a and 16-1 specific sponges for the development of engineered CHO host cells.

MATERIALS AND METHODS

Vector construction

Oligonucleotides encompassing the complementary sequences for miR-15a were designed and synthesized (Genfanavaran, I.R. Iran). NotI restriction enzyme site was added at the ends of the oligonucleotides. The sequences of oligonucleotides are shown in Table 1. Ten µL of upper and lower oligonucleotides were hybridized and phosphorylated using T4 polynucleotide kinase. The decoy encoding vector was constructed by cloning of the sponge bearing fragment in NotI site of the enhanced green fluorescent protein expression vector, pEGFP. The resulting vector was designed as pEGFP-SP. The light chain (LC) and heavy chain (HC) expression vector, pLCHC, which encodes anti-CD52 IgG1 monoclonal antibody LC and HC has been described previously (2).

Cells culture

Adherent CHO-K1 cells (Iranian Biological Resource Center, I.R. Iran) were routinely passaged in Dulbecco's modified eagle medium (DMEM)/F-12 medium (Inoclon, I.R. Iran) supplemented with 10% fetal bovine serum (FBS) (Inoclon, I.R. Iran) and 2 mM L-glutamine (Inoclon, I.R. Iran) at 37 °C and 5% CO_2, in a humidified incubator (New Brunswick Scientific, Connecticut, USA). Cells were maintained at the density of 2×10^5 viable cells/mL. Trypan blue exclusion method was employed to determine cell density and viability.

Table 1. Sequences of the oligonucleotides containing the miR-15a complimentary region.

Oligo Name	Sequence
Upper	GGCCGCGCACAAACCATTATGTGCTGCTAAGAGAACTTAGAGAACTTCACAAACCATTATGTGCTGCTATGC
Lower	GGCCGCATAGCAGCACATAATGGTTTGTGAAGTTCTCTAAGTTCTCTTAGCAGCACATAATGGTTTGTGCGC

Development of stable Chinese hamster ovary cell pools and clones

Twenty four h prior to transfection, 1 ×10^5 CHO-K1 cells were seeded in 24-well plates (SPL Life Sciences, Korea). The following day, cells were transfected with either pEGFP or pEGFP-SP expression vectors, using TurboFect transfection reagent (Thermo Fisher scientific, Massachusetts, USA) according to manufacturer's instruction. After 48 h, transfectants were diluted in a 1:10 ratio and cultured in the medium containing 5 µg/mL of puromycin for 14 days. After several passages in non-selective medium, stable cell pools were analyzed for EGFP expression using fluorescence microscopy (Nikon Instruments, New York, USA) and flow cytometry (BD Bioscience, New Jersey, USA).

Single cell cloning

Single cell isolation was performed using serial dilution cloning. Cells were diluted to the density of 40, 20, 10, and 5 cells/mL in nutrient mixture F-12 medium supplemented with 15% FBS and nonessential amino acid mixture (Thermo Fisher Scientific, Massachusetts, USA). One hundred µL of each dilution was seeded in 96-well plates and incubated for 14 days. Clonal cells were subsequently identified and assessed using fluorescent microscope. EGFP positive clones were expanded to 24-well plates and 5 clones were selected for further analysis.

Cell viability assay

Cells were plated at a density of 0.5 × 10^5 cells/well in 24-well plates. Cell viability was assessed using trypan blue dye exclusion assay every 2 days during 12 days of culture.

Analysis of mAb expression

Cells were transfected with pLCHC anti-CD52 antibody expression vector. Cell culture supernatants were collected 2 and 4 days post-transfection and viable cell density was measured. The antibody titers were analyzed using a sandwich IgG1 specific ELISA assay in which a goat polyclonal anti-human IgG1 antibody (Agrisera, Sweden) was used as the capture antibody in 1 µg/well concentration and a goat polyclonal anti-kappa HRP conjugate antibody (Agrisera, Sweden) was applied as the secondary antibody in 1/10000 dilution.

Quantitative reverse transcriptase polymerase chain reaction

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was employed to assess the decoy transcript expression in single cell clones derived from EGFP-SP pool. Total RNA was isolated from 1 × 10^6 cells using RNA extraction kit (Favorgen, Taiwan) followed by DNase treatment using RNase free DNase enzyme (Thermo Fisher Scientific, Massachusetts, USA). Total cDNA was prepared using first-strand cDNA synthesis kit (Vivantis, Korea). qRT-PCR was performed in a StepOnePlus system (Applied Biosystems, Massachusetts, USA) using SYBR green PCR master mix. Beta-actin housekeeping gene was used for normalization. The amplification program was set at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. Relative quantification analysis was performed using the comparative CT (ΔΔCT) method. Primers used in qRT-PCR are shown in Table 2.

Statistical analysis

Statistical analysis was performed using SPSS 18 software (SPSS Inc., USA). Differences in means were compared using Student's t test.

RESULTS

Vector design and construction

The pEGFP expression vector which provides high level expression of the EGFP reporter gene under human cytomegalovirus promoter was selected for construction of the decoy vector.

Table 2. Primer sequences used for quantitative reverse transcriptase polymerase chain reaction.

Primer name	Sequence (5' > 3')
EGFP (forward)	ACAACCACTACCTGAGCACC
SEED (revers)	GTTCTCTTAGCAGCACATAATGG
ACTB (forward)	TCCCAGCACCATGAAGATCAAG
ACTB (revers)	GCTTGCTGATCCACATCTCCTG

An oligonucleotide containing two miR-15a complementary sequences was used as the decoy element and cloned downstream of the EGFP coding sequence in pEGFP vector to obtain pEGFP-SP vector. Since miR-15a and miR-16-1 share a common seed sequence, this element can also sequester miR-16-1. pLCHC which encodes the anti-CD52 antibody LC and CH under the control of separate cytomegalovirus promoters was employed for antibody expression. Figure 1 shows the schematic representation of the expression vectors used in this study.

Development of stable cell pools and clones

Stable CHO-K1 cell pools harboring either pEGFP or pEGFP-SP vectors were developed through selection of transfected cells in selective medium. Analysis of the pEGFP or pEGFP-SP derived stable cell pools with fluorescent microscopy indicated presence of the GFP positive cells in both cell pools. Evaluation of the cell pools using flow cytometry also indicated presence of 59.4% and 75.3% of GFP positive cells in the EGFP and EGFP-SP stable cell pools, respectively (Fig. 2). Five cell clones were isolated from pEGFP-SP transfected pools using limiting dilution cloning.

Analysis of cell viability

Viability of CHO-K1, EGFP pool, EGFP-SP pool, and EGFP-SP selected clones were examined during 12 days of batch culture. As it is shown in Fig. 3, CHO-K1 cells and EGFP pool were reached the peak viable cell density of 14.5×10^4 cells/mL and 15×10^4 cells/mL at day 6. In contrast, EGFP-SP pool and clones EGFP-SP1 to EGFP-SP4 attained the peak viable cell density of 35-53. 5×10^4 cells/mL at day 8. Interestingly, clone EGFP-SP5 showed the peak viable cell density of 31×10^4 cells/mL at day 6. While improved viability was observed for EGFP-SP pool and all clones compared with CHO-K1 and EGFP pool ($P < 0.001$), 3.55- and 3.33-fold enhancement in viability was observed in clone EGFP-SP2 compared with CHO-K1 and EGFP pool at day 8, respectively. Based on these results, this clone was selected for further analysis.

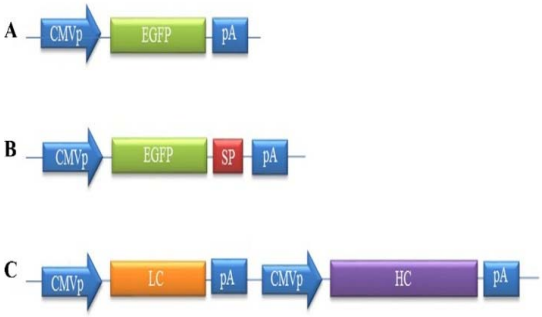

Fig. 1. Schematic representation of the expression vectors used in this study. (A) pEGFP, (B) pEGFP-SP, (C) pLCHC. CMVp, Cytomegalovirus promoter; EGFP, enhanced green fluorescent protein; pA, polyadenylation signal; SP, sponge decoy; LC, light chain; HC, heavy chain.

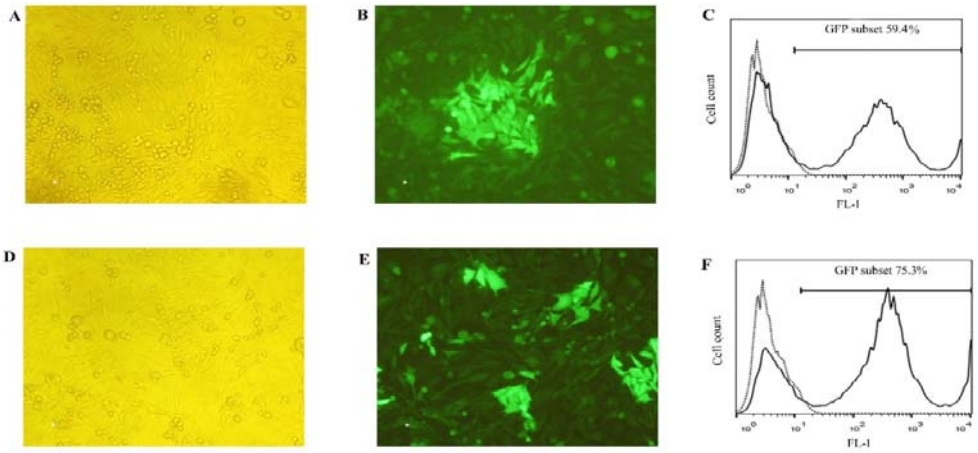

Fig. 2. The assessment of EGFP expression in EGFP and EGFP-SP pools. (A and D) light microscope, (B and E) fluorescence microscope, (C) flow cytometry plot of EGFP expression in EGFP pool (solid line) compared with un-transfected CHO-K1 cells (dotted line), and (F) flow cytometry plot of EGFP expression in EGFP-SP pool (solid line) compared with un-transfected CHO-K1 cells (dotted line). EGFP, enhanced green fluorescent protein; SP, sponge decoy; CHO, Chines hamster ovary.

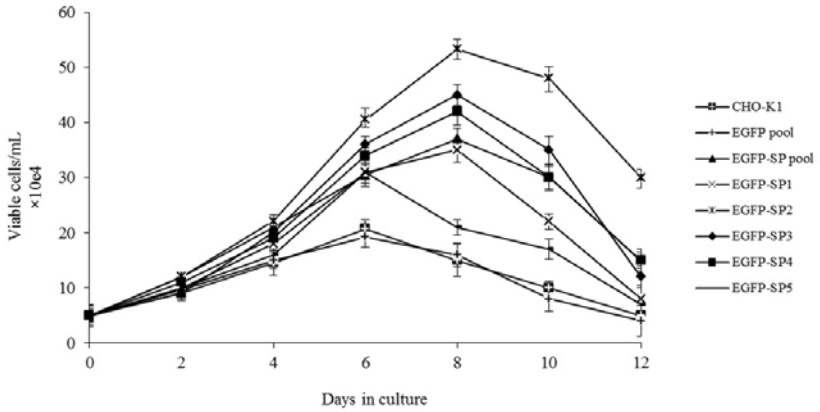

Fig. 3. Evaluation of viability of CHO-K1, EGFP pool, EGFP-SP pool, and EGFP-SP selected clones during 12 days of batch culture indicate significant differences in viable cell density of EGFP-SP pool and clones 1-4 compared with EGFP pool and CHO-K1 cells at day 8 ($P < 0.001$). EGFP, enhanced green fluorescent protein; SP, sponge decoy; CHO, Chines hamster ovary.

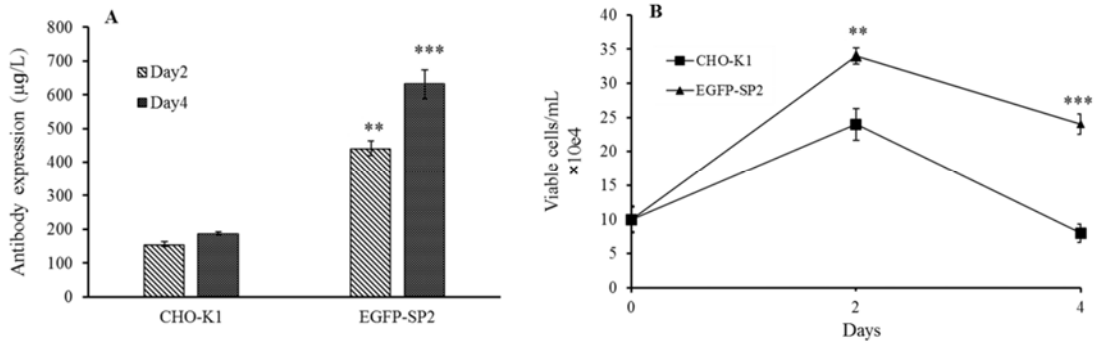

Fig. 4. (A) Analysis of mAb transient expression in CHO-K1 and EGFP-SP2 clone at days 2 and 4 post transfection and (B) numbers of viable CHO-K1 and EGFP-SP2 cells at days 0, 2, and 4. ** ($P < 0.01$) and *** ($P < 0.001$) show significant differences compared with CHO-K1 cells, n = 3. mAb, monoclonal antibody; EGFP, enhanced green fluorescent protein; SP, sponge decoy; CHO, Chines hamster ovary.

Transient expression of mAb

To evaluate the efficiency of pEGFP-SP2 clone in transient mAb expression, pLCHC expression vector was transfected to EGFP-SP2 as well as CHO-K1 cells. mAb titers were analyzed on days 2 and 4 post-transfection. As indicated in Fig. 4A, the expression level of mAb in EGFP-SP2 cells transfected with the pLCHC vector reached to 441.42 and 632.32 µg/L at days 2 and 4, respectively; which was 2.83-fold and 3.37-fold higher compared with the titers obtained from parental CHO-K1 cells ($P < 0.001$). Not surprisingly, the viable cell density of pEGFP-SP2 cells also showed up to 1.41-fold and 3-fold increase compared with CHO-K1 cells during 2 and 4 days of culture, respectively ($P < 0.001$, Fig. 4B).

Evaluation of the sponge expression in single cell clones

qRT-PCR was employed to further evaluate the correlation between decoy transcript expression and the observed improvement in cell viability in single cell clones. The decoy expression in single cell clones was compared with EGFP-SP pool. As it is shown in Fig. 5, while all clones showed increased expression of the decoy transcript compared to the EGFP-SP pool, significant increase was observed in clones pEGFP-SP2 and pEGFP-SP3 with up to 7.3-fold and

6.9-fold enhancement in decoy transcript expression, respectively.

DISCUSSION

Cell line engineering represents a promising tool for the development of novel host systems. The conventional approach of targeting a single gene has been extensively employed for CHO cell engineering. However, given the complexity of mammalian cell gene expression, targeting multiple genes in related pathways is desirable. In line with this premise, miR based cell engineering has evolved as a novel approach for the development of improved CHO host cells (22).

Several studies have focused on modulation of the apoptosis pathway in CHO cells through over-expression or down-regulation of the anti-apoptosis and pro-apoptosis genes, respectively (23). The introduction of engineered nucleases has also facilitated deletion of pro-apoptosis genes from the genome of host cells (24). miRs-based anti-apoptosis engineering has also been utilized as an effective strategy for the improvement of recombinant protein expression in CHO cells (12,25). Fischer *et al.* showed that overexpression of miR-30 improves recombinant protein expression and the maximal cell density of CHO cells by 2-fold (26). In another report, Druz *et al.* indicated that stable inhibition of miR-466h using siRNA technology resulted in 1.2- and 1.4-fold improvement in maximal cell density and recombinant protein productivity of CHO cells, respectively (27).

miR sponge technology has appeared as a reliable strategy for the inhibition of miRs in mammalian cells (14). miRs-15a and 16-1 are known as pro-apoptotic miRs which can target cell survival and apoptosis related genes including Bcl-2 (16,28). The purpose of this study was to evaluate the effects of miRs-15a and 16-1 specific decoy expression on CHO-K1 cells viability and monoclonal antibody productivity. The comparison of the growth of EGFP-SP pool and clones with CHO-K1 and pEGFP control pool during 12 days of batch culture showed marked increase in the peak viable cell density in EGFP-SP pool and related clones. Improved viability of EGFP-SP cells in cell culture could be a result of increased Bcl-2 anti-apoptotic gene expression, although further research has to be undertaken to confirm this speculation. In fact, the positive effect of Bcl-2 over-expression on CHO cell growth has been shown in other studies. Tey *et al*. indicated that ectopic expression of Bcl-2 resulted in a 75% increase in maximum viable cell density compared with the control culture (29).

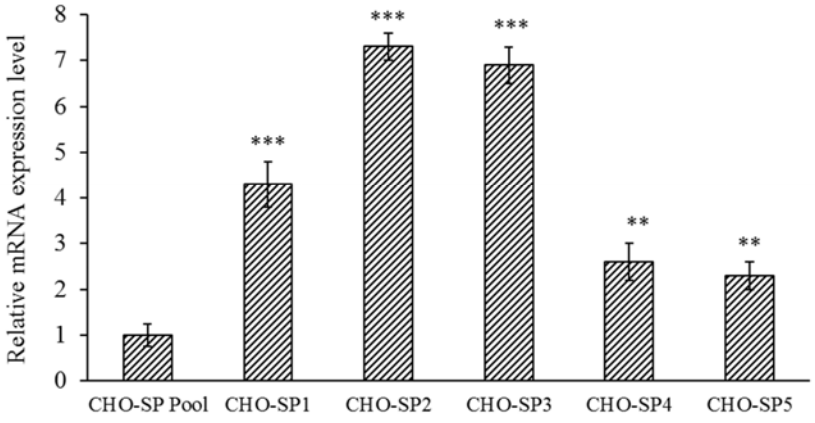

Fig. 5. Evaluation of the decoy transcript level in EGFP-SP clones relative to the EGFP-SP pool. ** ($P < 0.01$) and *** ($P < 0.001$)Shows significant differences compared with CHO-K1 cells, n = 3. EGFP, enhanced green fluorescent protein; SP, sponge decoy; CHO, Chines hamster ovary.

One of the clones derived from EGFP-SP pool showed significant enhancement in viable cell density (3.55-fold) compared to CHO-K1 cells. Therefore, it was selected for analysis of mAb transient expression. Not surprisingly, up to 3.37-fold increase in mAb transient expression was observed in EGFP-SP2 clone compared to CHO-K1 cells which could be a result of higher viable cell density of this clone. Analysis of sponge transcript expression also indicated higher levels of sponge expression in EGFP-SP2 clone. It is worth mentioning that sponge transcript level was also significantly increased in clone EGFP-SP3 which showed improved viability during the batch culture, as well. It can be argued that miR inhibition occurs when the decoy transcript is abundant in the cells.

Our study indicated successful utilization of the miR sponge technology for the enhancement of cell growth and productivity of CHO cells which is in agreement with previous studies. In a report by Sanchez *et al*. inhibition of miR-7 activity in CHO cells using sponge vectors resulted in up to 40% enhancement in cell density and around 2-fold increase in recombinant protein expression (30). Costello *et al*. examined the efficiency of the sponge decoy technology for stable depletion of five miRs, miR-204-5p, 338-3p, 378-3p, 409-3p, 455-3p, and 505-3p, which are associated with cell growth (31). According to their findings, inhibition of miR-378-3p function improved peak cell density of CHO cells by 59%. In another study, Kelly *et al*. indicated that depletion of miR-23 which is involved in glutamate metabolism using a miR-sponge decoy leads to 3-fold enhancement in specific productivity of CHO cells (32).

CONCLUSION

This study is the first report on expression of miRs-15a and 16-1 specific decoy transcript in CHO cells and evaluation of their effects on cells viability and mAb transient expression. Our results here showed that utilization of miR sponge technology can provide a reliable strategy for CHO host cell engineering. More studies are needed to evaluate the performance of EGFP-SP clones in stable expression of monoclonal antibodies.

ACKNOWLEDGEMENTS

This study was financially supported under Grant No. 8817 by Deputy of Research and Technology, Shahid Beheshti University of Medical Sciences, Tehran, I.R. Iran.

REFERENCES

1. Lalonde ME, Durocher Y. Therapeutic glycoprotein production in mammalian cells. J Biotechnol. 2017;251:128-140.
2. Bayat H, Hossienzadeh S, Pourmaleki E, Ahani R, Rahimpour A. Evaluation of different vector design strategies for the expression of recombinant monoclonal antibody in CHO cells. Prep Biochem Biotechnol. 2018;48(2):160-164.
3. Wurm FM. Production of recombinant protein therapeutics in cultivated mammalian cells. Nat Biotechnol. 2004;22(11):1393-1398.
4. Han S, Rhee WJ. Inhibition of apoptosis using exosomes in Chinese hamster ovary cell culture. Biotechnol Bioeng. 2018;115(5):1331-1339.
5. Rangan S, Kamal S, Konorov SO, Schulze HG, Blades MW, Turner RFB, et al. Types of cell death and apoptotic stages in Chinese Hamster Ovary cells distinguished by Raman spectroscopy. Biotechnol Bioeng. 2018;115(2):401-412.
6. Kim JY, Kim Y-G, Lee GM. CHO cells in biotechnology for production of recombinant proteins: current state and further potential. Appl Microbiol Biotechnol. 2012;93(3):917-930.
7. Majors BS, Betenbaugh MJ, Pederson NE, Chiang GG. Mcl-1 overexpression leads to higher viabilities and increased production of humanized monoclonal antibody in Chinese hamster ovary cells. Biotechnol Prog. 2009;25(4):1161-1168.
8. Zustiak MP, Jose L, Xie Y, Zhu J, Betenbaugh MJ. Enhanced transient recombinant protein production in CHO cells through the co-transfection of the product gene with Bcl-xL. Biotechnol J. 2014;9(9):1164-1174.
9. Jeon MK, Yu DY, Lee GM. Combinatorial engineering of ldh-a and bcl-2 for reducing lactate production and improving cell growth in dihydrofolate reductase-deficient Chinese hamster ovary cells. Appl Microbiol Biotechnol. 2011;92(4):779-790.
10. Meents H, Enenkel B, Eppenberger HM, Werner RG, Fussenegger M. Impact of coexpression and coamplification of sICAM and antiapoptosis determinants bcl-2/bcl-x(L) on productivity, cell survival, and mitochondria number in CHO-DG44 grown in suspension and serum-free media. Biotechnol Bioeng. 2002;80(6):706-716.

11. Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell. 2009;136(2):215-233.
12. Barron N, Sanchez N, Kelly P, Clynes M. MicroRNAs: tiny targets for engineering CHO cell phenotypes? Biotechnol Lett. 2011;33(1):11-21.
13. Jadhav V, Hackl M, Druz A, Shridhar S, Chung CY, Heffner KM, et al. CHO microRNA engineering is growing up: recent successes and future challenges. Biotechnol Adv. 2013;31(8):1501-1513.
14. Ebert MS, Sharp PA. MicroRNA sponges: progress and possibilities. RNA. 2010;16(11):2043-2050.
15. Liu Q, Fu H, Sun F, Zhang H, Tie Y, Zhu J, et al. miR-16 family induces cell cycle arrest by regulating multiple cell cycle genes. Nucleic Acids Res. 2008;36(16):5391-5404.
16. Bonci D, Coppola V, Musumeci M, Addario A, Giuffrida R, Memeo L, et al. The miR-15a–miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. Nat Med. 2008;14(11):1271-1277.
17. Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proc Natl Acad Sci U S A. 2005;102(39):13944-13949.
18. Pekarsky Y, Croce CM. Role of miR-15/16 in CLL. Cell Death Differ. 2015;22(1):6-11.
19. Lian YF, Huang YL, Wang JL, Deng MH, Xia TL, Zeng MS, et al. Anillin is required for tumor growth and regulated by miR-15a/miR-16-1 in HBV-related hepatocellular carcinoma. Aging (Albany NY). 2018;10(8):1884-1901.
20. Havrdova E, Horakova D, Kovarova I. Alemtuzumab in the treatment of multiple sclerosis: key clinical trial results and considerations for use. Ther Adv Neurol Disord. 2015;8(1):31-45.
21. Rommer PS, Dudesek A, Stüve O, Zettl UK. Monoclonal antibodies in treatment of multiple sclerosis. Clin Exp Immunol. 2014;175(3):373-384.
22. Fischer S, Handrick R, Otte K. The art of CHO cell engineering: A comprehensive retrospect and future perspectives. Biotechnol Adv. 2015;33(8): 1878-1896.
23. Wells E, Robinson AS. Cellular engineering for therapeutic protein production: product quality, host modification, and process improvement. Biotechnol J. 2017;12(1).: DOI: 10.1002/biot.201600105. 1600105.
24. Grav LM, Lee JS, Gerling S, Kallehauge TB, Hansen AH, Kol S, et al. One-step generation of triple knockout CHO cell lines using CRISPR/Cas9 and fluorescent enrichment. Biotechnol J. 2015;10(9):1446-1456.
25. Druz A, Chu C, Majors B, Santuary R, Betenbaugh M, Shiloach J. A novel microRNA mmu-miR-466h affects apoptosis regulation in mammalian cells. Biotechnol Bioeng. 2011;108(7):1651-1661.
26. Fischer S, Buck T, Wagner A, Ehrhart C, Giancaterino J, Mang S, et al. A functional high-content miRNA screen identifies miR-30 family to boost recombinant protein production in CHO cells. Biotechnol J. 2014;9(10):1279-1292.
27. Druz A, Son YJ, Betenbaugh M, Shiloach J. Stable inhibition of mmu-miR-466h-5p improves apoptosis resistance and protein production in CHO cells. Metab eng. 2013;16:87-94.
28. Diniz MG, Gomes CC, de Castro WH, Guimarães ALS, De Paula AMB, Amm H, et al. miR-15a/16-1 influences BCL2 expression in keratocystic odontogenic tumors. Cell Oncol (Dordr). 2012;35(4):285-291.
29. Tey BT, Singh RP, Piredda L, Piacentini M, Al-Rubeai M. Influence of bcl-2 on cell death during the cultivation of a Chinese hamster ovary cell line expressing a chimeric antibody. Biotechnol Bioeng. 2000;68(1):31-43.
30. Sanchez N, Kelly P, Gallagher C, Lao NT, Clarke C, Clynes M, et al. CHO cell culture longevity and recombinant protein yield are enhanced by depletion of miR-7 activity via sponge decoy vectors. Biotechnol J. 2014;9(3):396-404.
31. Costello A, Coleman O, Lao NT, Henry M, Meleady P, Barron N, et al. Depletion of endogenous miRNA-378-3p increases peak cell density of CHO DP12 cells and is correlated with elevated levels of ubiquitin carboxyl-terminal hydrolase 14. J Biotechnol. 2018;288:30-40.
32. Kelly PS, Breen L, Gallagher C, Kelly S, Henry M, Lao NT, et al. Re-programming CHO cell metabolism using miR-23 tips the balance towards a highly productive phenotype. Biotechnol J. 2015;10(7): 1029-1040.