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Original Article

Expression of the Mouse HSP27 Chaperone in CHO-K1 Cells for the Enhancement of Viable Cell Density in Batch Culture

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HIGHLIGHTS

- Cell engineering is an effective strategy to cope with apoptosis in CHO cells.
- HSP27 is involved in mammalian cell apoptosis.
- Expression of the mouse HSP27 increased the viability and cell density of CHO-K1 cells.

ABSTRACT

Chinese hamster ovary (CHO) cells are extremely vulnerable to cell viability loss in culture despite the availability of different nutrients supplementation strategies. As a result, extending the culture lifetime can profoundly increase recombinant protein expression. Overexpression of HSP27 and its anti-apoptotic effects have been shown in human cell lines in previous studies. In the current study, mouse HSP27 (mHSP27) was cloned in pcDNA 3.1 hygro expression vector and was expressed in CHO-K1 cells to assess its impacts on cell viability and growth. Expression of mHSP27 in CHO-K1 cells was confirmed using RT-PCR. A 3-fold enhancement in peak viable cell density of mHSP27 transfected clones was observed, and culture viability loss was delayed by 2 days compared to un-transfected cells. In future studies, the resulting mHSP27 CHO-K1 cells could be employed as a novel host system for the transient and stable expression of therapeutic recombinant proteins.

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Introduction

Chinese hamster ovary (CHO) cells are the main host cells for manufacturing recombinant therapeutic protein products (Lalonde and Durocher, 2017). Improved CHO cell lines with optimal product titers are required due to

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the introduction of biosimilars and newly-developed biologics (Walsh, 2018; Grilo and Mantalaris, 2019).

Several reports exist on the detrimental effects of cell death resulting from apoptosis during late culture phases despite nutrients supplementation through fed-batch approaches. This situation can be addressed by uncovering the key pathways triggering apoptosis and employing cell engineering approaches to develop robust cell lines with improved resistance to apoptosis. Prevention of cell death in culture through various genetic and physical methods in industrial cell lines has been addressed in previous studies (Vives et al., 2003; Arden and Betenbaugh, 2004).

Heat Shock Protein 27 (HSP27) is a small 27 kDa HSP located in the cytosol. It acts as a molecular chaperone to aid in inhibiting proteins aggregation and participates in misfolded proteins refolding through other molecular chaperones (Garrido et al., 2012). It was also shown that during various apoptotic insults, the higher HSP27 expression levels modulated the apoptosis signaling pathways leading to the incremented resistance against apoptotic cell death (Concannon et al., 2003, Lanneau et al., 2008). The capability of HSP27 at intervening at the mitochondrial pathway of apoptotic was found through interacting with cytochrome *C* in the cytosol for inhibiting caspases 3 and 9 activations (Bruey et al., 2000, Pandey et al., 2000). Incremented tolerance of adapted CHO cells to hyper acidic and hyperthermic culture circumstances was attributed to the cellular HSP27 elevation (Coss et al., 2002).

Initial HSP27 overexpressing in CHO-DxB11 and CHO-DG44 cells led to an extension of fed-batch culture duration in bioreactor along with enhancement of recombinant human interferon-gamma (IFN-ɣ) and monoclonal antibody (mAb) production yield (Lee et al., 2009, Tan et al., 2015). However, cell line engineering approaches based on the expression of HSP27 in parental CHO cells have not been explored yet. This study represents the development of the novel mouse HSP27 (mHSP27) expressing CHO-K1 cell lines and evaluating their growth and viability during batch culture. The capability of the resulting cell lines in efficient expression of the recombinant proteins in transient and stable expression settings can be evaluated in future studies.

Materials and Methods

Cell culture

Adherent CHO-K1 cells (ATCC CCL-61) were utilized as parental cells. DMEM/F12 medium was supplemented with 1 % penicillin/streptomycin (100 $\mu g.mL^{-1}$) and 10 % fetal bovine serum (FBS) (Biosera, France). The cells were maintained as monolayer cultures in a 5 % CO_2 humidified incubator at 37 °C, in filter caped flasks and were passaged every 2 to 3 days. Trypan blue exclusion assay was employed for the analysis of cell count and viability.

Generation of expression vectors

The codon-optimized *Mus musculus* HSP27 gene (GenBank accession NM_024441.2) was commercially synthesized (Genfannavaran, Iran). To construct the expression vector, the mHSP27 coding gene (576 bp) was cloned in pcDNA3.1 (-) Hygro vector using *Xho*I and *Bam*HI restriction enzymes (Thermofisher Scientific, Germany). The cloning process was confirmed by the restriction digestion analysis and sequencing.

Development of stable mHSP27 expressing CHO cells

CHO-K1 mHSP27 stable cell pools were developed using pcDNA3.1 (-)-mHSP27 expression vector. In short, to perform transfection, Lipofectamine 2000 reagent (Thermofisher Scientific, USA) was used according to the instructions of the manufacturer in 12-well culture plates under static conditions. Cells were maintained in selective medium containing hygromycin (200 $\mu g.mL^{-1}$) (Thermofisher Scientific, USA) for 14-days. To acquire single-cell clones, the stably transfected cell pools were diluted and seeded at 96-well plates at 1 cell/well density. Single-cell clones were expanded to 24 well plates after 1 week.

Reverse-transcriptase PCR (RT-PCR)

To further confirm the expression of the mouse HSP27 chaperone mRNA in CHO-K1-HSP27 cells, total RNA was isolated from stable cells pools and parental cells, and cDNA was synthetized.

PCR was performed using codon-optimized mHSP27 gene specific primers (forward primer 5′-AGACTCGAGATGTCCGGAAGAA -3′, and reverse primer 5′- TCATCATGGCTCCACTCTAGCAA -3′). PCR program was as follows: Primary denaturation for 30 s at 95 °C, followed by 30 amplification cycles (30 s at 95 °C; 30 s at 60 °C; 50 s at 72 °C). A 1 % agarose gel was used to analyze the amplified RT-PCR products.

Analysis of cell concentration and viability

Two single clones, which had reached confluency faster than other clones in 96-well plates, were selected for cell growth and viability studies. The mHSP27 clones and parental CHO-K1 cells were cultured in a 24 wells plate

(20000 cells per well) in duplicate. The cells were counted every 24 h over 12 days.

Results

Vector construction

The restriction enzyme digestion of the recombinant vector pcDNA3.1 (-) Hygro carrying the mHSP27 gene was performed using *Bam*HI and *Xho*I enzymes. As expected, the 576 bp mHSP27 gene fragment was removed from the vector (Fig.1).

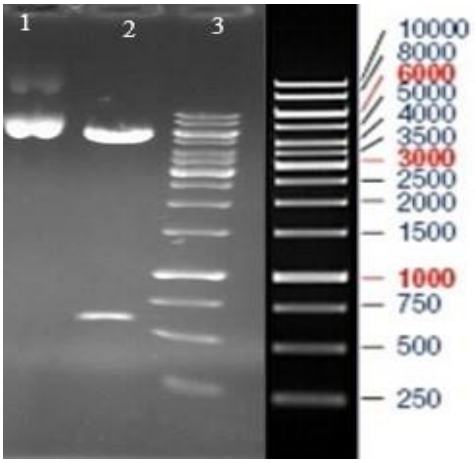

Figure 1. The analysis of restriction digestion of pcDNA3.1/Hygro-mHSP27. Line 1: un-digested pcDNA3.1/Hygro. Line 2: digested pcDNA3.1/Hygro-mHSP27 vector using XhoI and BamHI. Line 3: DNA ladder.

Establishment of CHO-mHSP27 stable cell pool and RT-PCR

The expression of mHSP27 was verified in a stable cell pool using RT-PCR analyses. In comparison to the parental cell pool, expression of mHSP27 at mRNA level was detected in a stable mHSP27 cell pool (Fig 2).

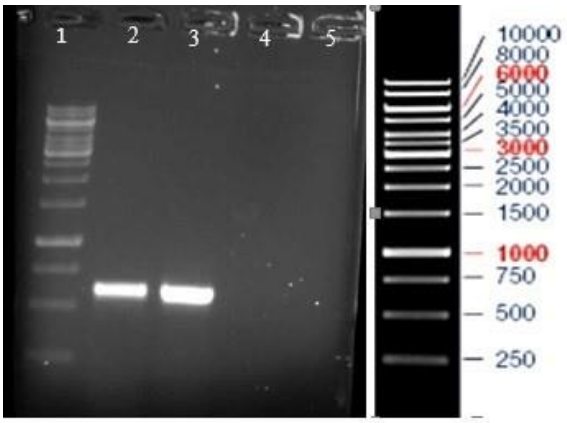

Figure 2. Expression of the mHSP27 chaperone in CHO-K1-mHSP27 cells analyzed at mRNA level. Line1: DNA ladder. Lines 2,3: CHO-K1-mHSP27. Line 4: un-transfected CHO-K1 cells. Line 5: Negative control.

Evaluation of growth and viability in HSP27 single-cell clones

Single-cell clones provide homogenous cell populations as opposed to cell pools which are inherently heterogeneous. Thus, limiting dilution was performed, and two single clones were selected from CHO-mHSP27 pool for viability test. Un-transfected CHO-K1 cells were used as the control. Evaluation of cell count and viability was performed every 24 hours during 12 days.

mHSP27 clones showed considerable enhancement in culture durations compared to CHO-K1 cells (Fig.3A), with a 3-fold increase in maximum viable cell concentration in 5 days (Fig.3B). These results suggest that apoptosis inhibition by mHSP27 could be a probable mechanism for the observed growth enhancements in the mHSP27-CHO cell pool.

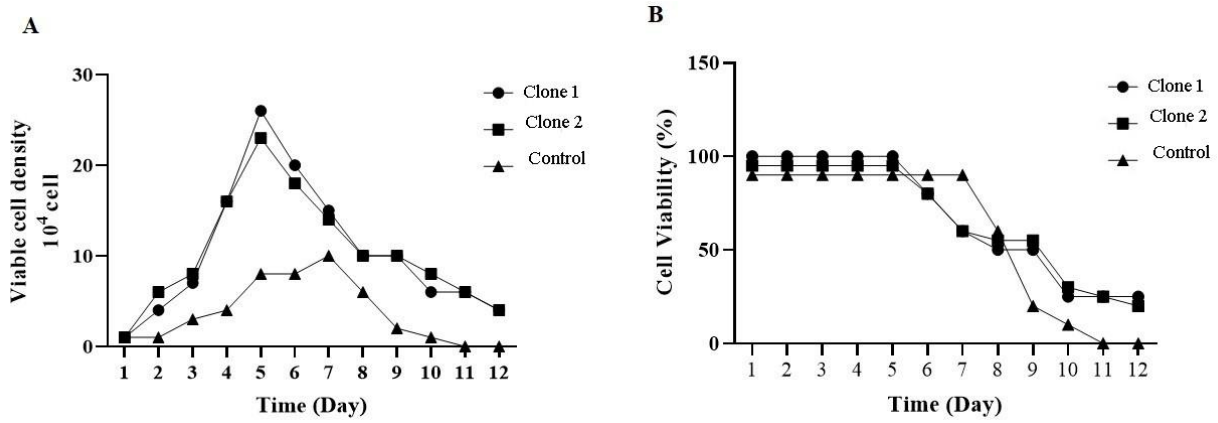

Figure 3. Characterization of CHO-K1-mHSP27 clones and un-transfected CHO-K1 cells in batch culture. The cells were seeded at 2×10^4 cell/well concentration in duplicate and the viable cells were measured every day for 12 days. (A) The viable cell concentration. (B) Culture viability.

Discussion

Apoptosis is one of the main obstacles for the production of recombinant therapeutic proteins. several anti-apoptosis cell engineering approaches have been employed, including overexpression of BCL-2 (Jeon et al., 2011), BCL-x (Ha et al., 2013), XIAP (Kim et al., 2009), AKT (Hwang and Lee 2009) as well as down-regulation or knock out of caspase 3, caspase 7 (Sung et al., 2007), and Bak and Bax (Misaghi et al., 2013).

Increased expression of chaperones such as HSP70 and HSP27 is also one strategy that increases cell resistance to adverse environmental conditions and delays the onset of apoptosis. Numerous studies have been conducted on this subject, and the results of these studies show the same results (Lee, Wong et al. 2009, Tan, Lee et al. 2015).

In a previous study conducted by Lee et al. (2009), it was shown that overexpression of HSP27 in IFN-γ expressing cells improved culture extension time 36–72 h, delayed the activity of caspases 2, 3, 8, and 9, and resulted in a 2.5-fold enhancement in IFN-γ expression in fed-batch culture.

Tan et al. (2015) investigated the effects of HSP27 overexpression on monoclonal antibody (mAb) producing CHO cells. A 2.2-fold increase was observed in maximum viable cell density in bioreactor fed-batch culture along with a 2.3-fold enhancement in mAb volumetric productivity. These effects were attributed to delayed activation of caspases in CHO cells.

Although the effects of mHSP27 expression on apoptosis in engineered clones were not evaluated in the current study, modulation of the apoptosis pathways by this chaperone could be the main reason for the improved culture duration, and cell density observed in this study.

Conclusion

In the present study, the effects of mouse mHSP27 expression on the growth and viability of adherent CHO-K1 cells was evaluated. The results showed a positive effect on cell growth as well as enhancement in culture duration. This study highlights the utility of an mHSP27-based cell engineering approach for developing the CHO host cells with improved performance in longer culture durations. Employment of the current platform for the expression of recombinant proteins will further reveal its potential as an expression system.

Ethical Statement

This article does not contain any studies with human and animal subjects performed by any of the authors.

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Competing Interests

The authors declare no conflict of interest.

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Authors Contributions

Mohammad Reza Amini performed literature search, experimental studies, data acquisition, and manuscript preparation; Azam Rahimpour did literature search, data analysis, manuscript editing and review; Reyhaneh Hoseinpoor collaborated on experimental studies, data acquisition, Masoumeh Rajabibazl supervised and conducted literature search, data analysis, manuscript editing and review.

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