Journal of Biotechnology 231 (2016) 136–140

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Short communication

# Limitations to the development of recombinant human embryonic kidney 293E cells using glutamine synthetase-mediated gene amplification: Methionine sulfoximine resistance

Da Young Yu, Soo Min Noh, Gyun Min Lee *

*Department of Biological Sciences, KAIST, 373-1 Kusong-Dong, Yusong-Gu, Daejon 305-701, South Korea*

ARTICLE INFO

*Article history:*
Received 6 January 2016
Received in revised form 16 May 2016
Accepted 6 June 2016
Available online 8 June 2016

*Keywords:*
HEK293 cells
Methionine sulfoximine
Glutamine synthetase
CHO cells

ABSTRACT

To investigate the feasibility of glutamine synthetase (GS)-mediated gene amplification in HEK293 cells for the high-level stable production of therapeutic proteins, HEK293E cells were transfected by the GS expression vector containing antibody genes and were selected at various methionine sulfoximine (MSX) concentrations in 96-well plates. For a comparison, CHOK1 cells were transfected by the same GS expression vector and selected at various MSX concentrations. Unlike CHOK1 cells, HEK293E cells producing high levels of antibodies were not selected at all. For HEK293E cells, the number of wells with the cell pool did not decrease with an increase in the concentration of MSX up to 500 μM MSX. A q-RT-PCR analysis confirmed that the antibody genes in the HEK293E cells, unlike the CHOK1 cells, were not amplified after increasing the MSX concentration. It was found that the GS activity in HEK293E cells was much higher than that in CHOK1 cells ($P < 0.05$). In a glutamine-free medium, the GS activity of HEK293E cells was approximately 4.8 times higher than that in CHOK1 cells. Accordingly, it is inferred that high GS activity of HEK293E cells results in elevated resistance to MSX and therefore hampers GS-mediated gene amplification by MSX. Thus, in order to apply the GS-mediated gene amplification system to HEK293 cells, the endogenous GS expression level in HEK293 cells needs to be minimized by knock-out or down-regulation methods.



## 1. Introduction

Mammalian cells are the dominant expression system to produce complex therapeutic glycoproteins that require authentic glycosylation or other post-translational modifications (PTMs) (Zhu, 2012). Although Chinese hamster ovary (CHO) cells have been the most widely used mammalian host for the commercial production of therapeutic glycoproteins, non-human glycan structures at the terminal, such as N-glycolylneuraminic acid and Galα1-3Galβ1-GlcNAc-R, in CHO cells which both risk increased immunogenicity of glycoproteins, remain a safety concern (Ghaderi et al., 2012). As an alternative, human cell lines such as human embryonic kidney (HEK) 293 cells, human embryonic retinoblasts (PER.C6), and F2N78 have emerged for the production of therapeutic glycoproteins due to their human glycosylation machinery (Seo et al., 2014; Swiech et al., 2012).

HEK293 cells, generated by the transformation of HEK cells by adenovirus type 5 DNA (Graham et al., 1977), have been widely used as hosts for gene expression in scientific research (Cervera et al., 2013; Liu et al., 2014). However, despite the superior PTM ability of HEK293 cells, they have rarely been used for the commercial production of therapeutic proteins. Unlike CHO cells, HEK293 cells lack an efficient gene amplification and selection system for stable foreign protein production, which hampers their industrial use.

For CHO cells, dihydrofolate reductase (DHFR)-mediated and glutamine synthetase (GS)–mediated gene amplification systems are used for the high-level production of therapeutic protein (Noh et al., 2013). Between them, the use of the GS system is increasing because the GS system, unlike the DHFR system, typically requires only a single round of selection for amplification in the presence of a GS inhibitor, methionine sulfoximine (MSX). Furthermore, the GS system can be used in cell lines with an endogenous GS gene such as CHOK1 cells, although it was initially developed for murine myeloma cell types lacking GS activity, such as the NS0 and Ag14 cell lines (Brown et al., 1992).

In contrast with CHO cells, the potential of the GS system in HEK293 cells has yet to be examined. With the availability of an effi-

* Corresponding author.
*E-mail address:* gyunminlee@kaist.ac.kr (G.M. Lee).

http://dx.doi.org/10.1016/j.jbiotec.2016.06.003
0168-1656/

cient gene amplification and selection system, the use of HEK293 cells for the commercial production of therapeutic proteins is likely to increase.

In this study, to investigate the feasibility of the GS system in HEK293 cells for the stable production of therapeutic proteins, HEK293E cells were transfected by the GS expression vector containing antibody genes and selected at various MSX concentrations. The selection efficiency, along with the endogenous GS expression and GS activity levels, of recombinant HEK293E (rHEK293E) cells expressing an antibody was determined. For comparison, the selection efficiency of recombinant CHOK1 (rCHOK1) cells expressing an antibody was also investigated at various MSX concentrations.

## 2. Results and discussion

The GS-mediated gene amplification system is based on the GS enzyme, which catalyzes the formation of glutamine from glutamic acid and ammonia and provides the critical pathway for the synthesis of glutamine (Liaw et al., 1995). Selection in the absence of glutamine may be applicable to cell lines which produce active endogenous GS in the presence of MSX. Cells containing the transfected GS gene can develop resistance to MSX with the amplification of the GS gene. The specific gene of interest, which is co-linked to the GS gene in the same expression vector or adjacently located in the host cell chromosome, is co-amplified with the GS gene (Bebbington et al., 1992).

To construct rHEK293E cell lines producing Rituximab, HEK293E cells (ATCC number: CRL-10852) were transfected by a GS expression vector containing antibody genes (Supplementary Fig. S1A) according to Invitrogen FreeStyle™ MAX 293 protocol. Selection was performed by seeding $2 \times 10^3$ cells/well in five 96-well plates in a selection medium containing various MSX concentrations. For comparison, CHOK1 cells (ATCC number: CCL-61) were also transfected by the same GS expression vector according to the Invitrogen FreeStyle™ MAX CHO protocol, after which the rCHOK1 cells were selected at various MSX concentrations. The term "cell pool" indicates the cells which survived in the 96-wells after selection, and "selection efficiency" indicates a percentage of wells with a cell pool to the number of wells in which transfected cells were seeded. A flow cytometric analysis of the intracellular antibody in transfected cells, just before being seeded, confirmed that the GS expression vector containing antibody genes was transfected into CHOK1 and HEK293E cells (Supplementary Fig. S2).

Fig. 1 shows the selection efficiency and antibody concentration in the wells with a cell pool at various MSX concentrations. For CHOK1 cells, the number of wells with a cell pool decreased significantly with an increase in the MSX concentration. In the absence of MSX, all 480 wells contained a cell pool, whereas only three wells contained a cell pool at 100 μM (Fig. 1A). When MSX concentration was increased further to 500 μM, transfected CHO cells could not survive at all. As expected, antibody production was increased with an increased concentration of MSX (Fig. 1B). The average antibody concentration in the three wells with a cell pool at 100 μM MSX was 9.7 μg/mL, which was approximately 121 times higher than that in the absence of MSX.

Surprisingly, for HEK293E cells, the number of wells with a cell pool did not decrease with an increase in the MSX concentration (Fig. 1A). Even at 500 μM MSX, all 480 wells contained a cell pool. When the MSX concentration was increased further to 1000 μM, 125 wells out of 480 wells still contained a cell pool. Unlike rCHO cells, the antibody concentration did not increase with an increase in the MSX concentration (Fig. 1B). Regardless of the MSX concentration used, the antibody concentration, except for only one well at 50 μM MSX, was lower than 1 μg/mL, suggesting that the antibody genes were not amplified by increasing the MSX concentration. The q-RT-PCR analysis confirmed that the antibody genes in rHEK293E cells, unlike rCHOK1 cells, were not amplified by increasing the MSX concentration (Supplementary Fig. S3). GS-mediated gene expression for the production of other antibody (Trastuzumab, Supplementary Fig. S4) in both HEK293E and CHOK1 cells were attempted. GS-mediated gene expression system did not result in enhanced Trastuzumab production in HEK293E cells unlike CHOK1 cells (Supplementary Fig. S5).

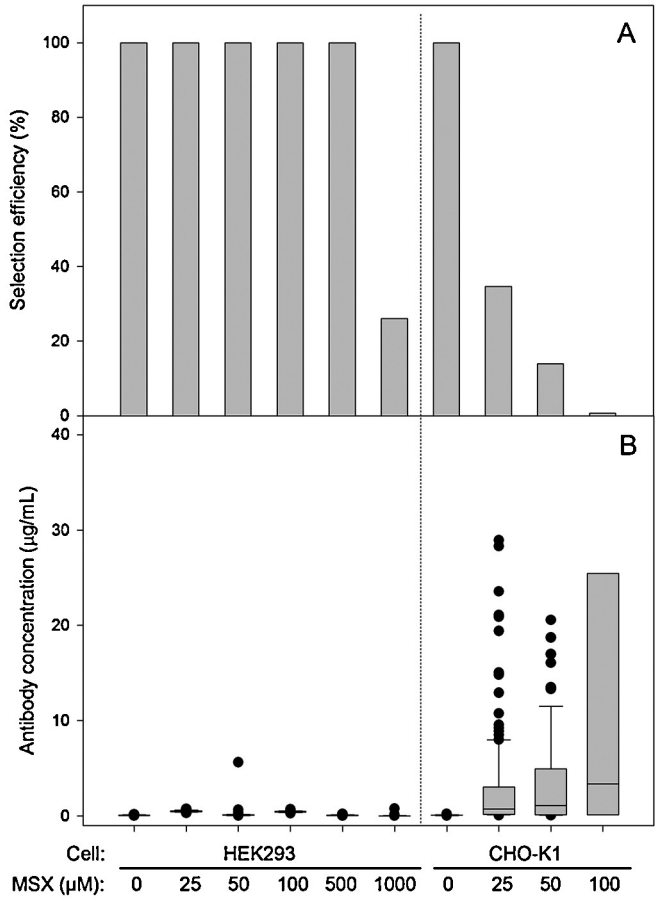


**Fig. 1.** Selection efficiency (A) and antibody concentration in the wells with a cell pool (B) at various MSX concentrations. The selection medium for the HEK293E cells was composed of an 80% EX-CELL CHO cloning medium (Sigma-Aldrich, St. Louis, MO), a 20% EX-CELL 293 SFM (Sigma) and 1× GS expression medium supplement (GSEM, Sigma). For the CHOK1 cells, the selection medium was composed of an 80% EX-CELL CHO cloning medium, 20% PowerCHO2-CD (Lonza, Verviers, Belgium) and 1× GSEM. Upon exceeding 30% confluence, the culture supernatants in the wells with a cell pool were harvested to quantify the antibody concentration by an enzyme-linked immunosorbent assay (ELSIA) as described previously (Jeon et al., 2011).

To understand the differences in the selection efficiency levels at a high MSX concentration between the HEK293E and the CHOK1 cells, cells were cultivated at various MSX concentrations in the absence of glutamine. As a control, cells were also cultivated in the presence of 4 mM glutamine. Cell cultures were performed three separate times for each condition.

Fig. 2 shows the cell growth and viability profiles during the cultures. For both cell lines, glutamine depletion decreased cell growth. Compared with the control cultures in the presence of 4 mM glutamine, the maximum viable cell concentration (MVCC) of the HEK293E cells and the CHOK1 cells in the absence of glutamine showed decreases of 58% and 33%, respectively. The addition of MSX in the absence of glutamine further decreased the cell growth in a dose-dependent manner. However, HEK293E cells were more resistant to MSX than CHOK1 cells. CHOK1 cells scarcely survived