Evaluation of Chemical Chaperones on the Transient Expression in Chinese Hamster

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Abstract

Recombinant proteins play an important role in biomedical and basic science researches. In this regard, mammalian cells have widely use in monoclonal antibodies. Improper aggregation complex recombinant proteins have been known as a bottleneck in successful production of active biotherapeutics. Anti-CD52 is efficient immunosuppressive agents of therapeutic applications especially in autoimmune diseases. In this study, effects of four chemical Chaperones including DMSO and 4-phenyl butyrate (4-PBA) on transient of a recombinant evaluated during six days. Analysis of the expression level showed up to 2 fold in DMSO treated cells, while 4-PBA treatment improved antibody expression up to 50%, 30% and 10%, respectively.

Keywords: Monoclonal antibody, Transient expression, Chemical chaperone.

Introduction

The advent of hybridoma was a milestone in technologies and has led the way to emergence of the monoclonal antibodies. The global sales was estimated $75 and until 2014, frothy-seven antibody based therapeutics were approved [1]. Features which make an appropriate host cell proteins particularly include:

The possibility of suspension growth in high density culture, the feasibility of genetic modification, the ability to generate human-like post-translational modifications such as glycosylation and the safety of cells with regard to viral infections [3, 4]. Several studies do in different aspects of gene expression optimization and analysis including optimization of culture medium, culture mode and detection methods [6, 7]. It has been known that proportional expression and heavy chain has a successful expression [4]. IRES elements provide an alternative translation initiation system initiation in which the cellular translation machinery can recognize the IRES. Therefore, using IRES elements eliminates the need for a secondary promoter and thus enables taking less space on the expression vector.

However, there are also some shortcomings including the lower rate of the downstream gene which it mediated through IRES [9].IRES sequences provides a useful method for the multicistronic of three transgenes which multiunit heterologous proteins. Multicistronic expression of transgenes and selection markers speed and efficiency of clone selection [10]. Since antibodies chains, the bicistronic expression vectors can be efficiently molecules.
It that the arrangement and heavy chains in upstream and IRES can lead to proper active antibody molecules [11]. Transient gene expression (TGE) is strategy for a short time frame for laboratory and preclinical studies [12].

Anti-CD52 is known as immunosuppressive agents for hematopoietic cancers autoimmune diseases GVHD [14]. Chaperones are a set of proteins which correct folding of inappropriate aggregation of proteins. Likewise, chemical chaperones are improved the folding and stability of proteins [15]. In the current study used as the model molecule to investigate effects of different chemical chaperones on transient expression.

Materials and Methods

Cell Culture

CRL-9661 is DMEM/F12 medium containing serum, 100 units per ml penicillin, 1 microgram per ml streptomycin and 2 mM glutamine (Biosera, France). The cultures maintained with 5% CO₂ and 85% humidity. Cultures two days with 0.2×10⁶ cells/ml. Cell counting method.

Plasmid Preparation

The bicistronic antibody expression vector was constructed by subcloning the Light chain-IRES-Heavy chain fragment from an intermediate vector to a pcDNA3.1 based expression (Figure 1a). The pEGFP plasmid containing was used for monitoring the transfection efficiency. Plasmid a plasmid extraction kit (Qiagen, Germany) and preparations with 260/280 ratio higher than 1.8 were used for transfection.

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Results

Transient Transfection and Chaperone Treatment

Transient transfection was performed in CHO-K1 cell line (Figure 2). To evaluate chemical chaperones on CHO cells during culture with the density of 5×10⁴ cells/mL in 6 well plates. Chaperone treatment was started 6 hours post transfection.

Effects of the Chemical Chaperones on Antibody Production

The antibody in chaperone was evaluated every 48 hours using ELISA. The antibody titer during cultures. DMSO treated cells with up to 2 fold enhancement at 96 hours post culture. The maximum enhancement of
4-PBA was observed at 96 hours post culture and estimated as 50%, 30% and 10%, respectively.

![Figure 4: The antibody treated with comparison to un-treated cells](image)

Discussion

Monoclonal antibodies, due to their high specificity, have been known as valuable agents in biomedical research applications [16]. In order increasing demand for clinical applications, different optimization [17]. In this regards, miRNA based cell engineering strategies [18] novel tools such as CRISPR/Cas system have been successfully utilized for generation of the cells [19]. Using a selection/augmentation protocols, the ability of producing monoclonal antibody levels more than 1gr/lit [20]. several studies have investigates effects of recombinant CHO cells. Most of these studies have focused on stable protein producing cells.

Onitsuka et al. [21] reported the positive effects of trehalose on bi-specific antibody production up to 4 fold in CHO cells cultured in bioreactor. Rezaei et al. [22] observed 1.7-2.1 fold enhancements in human growth hormone production level in CHO cells. Hwang et al. [23] investigated four different chemical chaperones (DMSO, proline, glycerol and 4-PBA) on aggregation and COMP-angiopoietin 1 cells. In this work different chaperones showed variable levels of enhancement between 2.5-5 folds. target protein and its expression level, the concentration of the chaperone and the culture mode and conditions. Our results here also indicate positive effects of different chemical chaperones on transient

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