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Optimization of monoclonal antibody expression in CHO cells by employing epigenetic gene regulation tools

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Abstract: Monoclonal antibodies (mAbs) are crucial in pharmaceutical biotechnology. Mammalian cell lines are the most preferred for their production. One of the significant challenging issues of mammalian expression systems is epigenetic gene silencing. Employing epigenetic gene regulation tools can increase the productivity of the mammalian cell lines. Sodium butyrate (NaBut) and valproic acid (VPA) regulate gene expression by inhibiting histone deacetylase. A ubiquitous chromatin-opening element (UCOE) can improve expression by reducing DNA methylation. Here, the separate and combined effects of NaBut and VPA histone deacetylase inhibitors (iHDACs) and UCOE on mAb synthesis were studied. Stable cell lines were generated by non-UCOE (CHO-HL) and UCOE-containing vectors (CHO-UHUL) and cultured in the presence and absence of NaBut or VPA. Expression analysis showed that CHO-UHUL gave a 4-fold greater yield than non-UCOE CHO-HL. Antibody production levels of the CHO-HL and CHO-UHUL cells increased 2-fold and 2.5-fold after NaBut and VPA treatment, respectively. These results indicate that UCOE has more impact on antibody expression than iHDACs. iHDAC treatment exhibited at least a 0.5-fold higher antibody yield in UCOE containing CHO-UHUL cells. Thus utilization of NaBut and VPA (iHDACs) and UCOE resulted in antibody expression improvement, and the combined use of them had a synergistic effect on antibody synthesis.

Key words: Monoclonal antibodies, Chinese hamster ovary, epigenetic gene silencing, chromatin-opening element, sodium butyrate, valproic acid

1. Introduction

Recombinant monoclonal antibodies (mAbs) are the best-selling class of biopharmaceutical drugs (Aggarwal, 2011). They are used in the treatment of a variety of diseases, including many types of cancers, autoimmune diseases, and cardiovascular disorders (Li and Zhu, 2010; Ecker et al., 2015). Approval and sales rates of therapeutic antibodies have increased progressively and reached nearly $75 billion in 2013 (Walsh, 2014).

To acquire proper biological functions, cultivated mammalian cell lines are the most preferred hosts for their production, especially Chinese hamster ovary (CHO) cells (Wurm, 2004). Following random integration of a transgene into the host genome, the rate of gene transcription is related to the chromosomal site of integration, resulting in a position effect (Wilson et al., 1990). Since most of the mammalian genome is transcriptionally inactive heterochromatin, transgene silencing occurs in large numbers of cells. Several studies have tried to target transgenes in genomic hotspots where high gene expression occurs due to the endogenous sites of open chromatin (Fukushige and Sauer, 1992; Gilbertson, 2003; Ahmadi et al., 2016). Even if a transgene integrates into the transcriptionally active euchromatin regions, over cultivation time it is silenced as a result of epigenetic processes, such as histone modifications and DNA methylation (Richards and Elgin, 2002; Kwaks and Otte, 2006). Therefore, achieving high-yielding mammalian cell lines is a challenging issue in the field of cell culture technology. In an effort to overcome this challenge, epigenetic gene regulation tools can be used to protect transgenes from such adverse epigenetic events.

Sodium butyrate (NaBut) and valporonic acid (VPA) are histone deacetylation inhibitors, widely used for recombinant protein production by mammalian cells (Backliwal et al., 2008; Jiang and Sharfstein, 2008). Histone deacetylase inhibitors (iHDACs) regulate gene expression through histone hyperacetylation, thereby facilitating gene accessibility to transcription factors and improving gene expression (Kwaks and Otte, 2006).

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Ubiquitous chromatin-opening elements (UCOEs) are cis-acting epigenetic regulatory elements, derived from promoter regions of ubiquitously expressed housekeeping genes. They are methylation-free CpG islands and prevent heterochromatin formation and transgene silencing by reducing DNA methylation (Antoniou et al., 2003). A2UCOE from the human HNRPA2B1-CBX3 locus is one of the most effective UCOEs, and it has been shown that its incorporation into expression vectors can enhance transgene expression levels in mammalian cells (Benton et al., 2002; Williams et al., 2005; Allen and Antoniou, 2007; Boscolo et al., 2012; Nematpour et al., 2017).

The present study set to compare the influence of NaBut and/or VPA iHDACs and A2UCOE on model mAb omalizumab production in CHO cells. Moreover, the study tried to investigate whether combining these epigenetic tools would also increase mAb production. To this end, first, non-UCOE and UCOE eukaryotic expression plasmids of target antibody were constructed. Then stable CHO cell lines were developed by different vector combinations: non-UCOE vectors (CHO-HL) and UCOE vectors (CHO-UHUL). Antibody expression was analyzed using western blot analysis, and binding activity of purified antibody was tested using enzyme-linked immunosorbent assays (ELISA). Omalizumab is a recombinant anti-IgE antibody that interacts with circulating IgE and interrupts the allergic cascade. Recombinant test mAb function was compared with commercial omalizumab using direct ELISA. Finally, the cells were cultured in the presence or absence of NaBut and VPA and mAb production was compared using ELISA.

2. Materials and methods

2.1. Construction of antibody-coding vectors

In this investigation, the humanized IgG_1 mAb omalizumab (drug bank database ID: DB00043) was used as a model antibody. The antibody consists of two identical heavy and two identical light polypeptide chains. The sequences of both heavy and light chains were commercially synthesized by Genscript (Piscataway, NJ, USA). The A2UCOE sequence was obtained from NCBI epigenomics browser (accession number: NC_000007.13) and was commercially synthesized by Genscript.

Each of the antibody chains were cloned into separate expression vectors under the control of the same human cytomegalovirus (CMV) immediate–early enhancer and promoter. The heavy chain sequence was ligated into pTracer-CMV2 vector (Invitrogen, Carlsbad, CA, USA) using KpnI and NotI sites. The light chain sequence was ligated into the pIRES2-DsRed2 vector (Clontech, Mountain View, CA, USA) using NheI and EcoRI sites on the vector and insert sequence. Heavy and light chain coding vectors were named PH and PL, respectively. The A2UCOE sequence was obtained from the pUC-57 vector using Genscript. It was ligated into the upstream of the CMV promoter in the PH and PL vectors using SgrDI and NruI sites and ApaLI and NdeI sites, respectively. These UCOE-containing vectors were termed PUH and PUL, respectively. All cloning steps were done based on standard cloning methods (Sambrook and Russell, 2001). The cloned sequences were confirmed by DNA sequencing.

2.2. Cell culture

CHO-DG44 host cell line (Life Technologies, Carlsbad, CA, USA) (Catalog no: A10971-01) was used for mAb production. Cells were cultivated in chemically defined, protein-free CD CHO medium (Gibco, Carlsbad, CA, USA), supplemented with 8 mM L-glutamine (Gibco), 0.1% Pluronic F-68 (Gibco), and 1% penicillin/streptomycin (100 µg/mL) (Gibco) at 37 °C and 5% CO_2 in disposable vented T75 flasks and subcultured every 3 days at a density of 3–5 × 10^5 cells/mL. The cell concentration and viability were determined by 0.4% Trypan Blue (Sigma–Aldrich, St. Louis, MO, USA) staining assay using hemacytometer, and all counts were performed in duplicate and averaged (Tennant, 1964).

2.3. Transfection and generation of stable cell lines

Transfection was carried out in duplicate in 6-well tissue culture plates; for each well, 2 × 10^6 cells were seeded and transfected with 3 µg of linearized plasmid DNA, using Xtream HD reagent (Roche, Basel, Switzerland) according to the manufacturer's instructions.

To generate stable cell lines, CHO cells were first transfected with LC-expressing vectors (PL or PUL) and selected with G418 (400 µg/mL) (Sigma–Aldrich) 72 h posttransfection over 14 days. Then the resulting LC-expressing stable cells were transfected with HC-expressing vectors (PH or PUH) and selected with zeocin (500 µg/mL) (Gibco) 72 h posttransfection over 14 days. Stable cell lines were generated using different antibody vector combinations: 1) CHO-HL transfected with PH and PL vectors and 2) CHO-UHUL transfected with PUH and PUL vectors.

2.4. NaBut and VPA treatment

Stocks of 1 M NaBut and 500 mM VPA (Sigma–Aldrich) were prepared in PBS and sterilized with a 0.22-mm filter. The working concentrations of NaBut and VPA were optimized and further added at concentrations of 2 mM and 500 µM, respectively. To analyze antibody productivity, 1 × 10^6 cell/mL were seeded in 6-well tissue culture plates in ProCHO5 medium (Lonza, Basel, Switzerland) supplemented with 100 mM hypoxanthine, 16 mM thymidine (Sigma–Aldrich), and 8 mM glutamine and grown under shaking at 125 rpm. CHO-HL and CHO-UHUL cell lines were cultured in the presence and absence of NaBut or VPA, and antibody yields were determined using ELISA after 5 days of treatment.

2.5. Antibody purification

mAb was purified using Protein A affinity chromatography. Aliquots with a density of 1×10^6 cell/mL were seeded in 125 mL shake flasks in ProCHO5 medium (Lonza) with supplements and grown under shaking at 125 rpm for 5 days. The culture media of stable cell lines were collected at the end of batch culture, centrifuged at 7000 rpm for 20 min, filtered, and then loaded on a Protein A column packed with MabSelect (GE Healthcare, Piscataway, NJ, USA). The column was washed with washing buffer (PBS, pH of 7) and eluted with elution buffer (sodium citrate, pH of 4) (Merck, Kenilworth, NJ, USA). Fractions were collected in 1-mL tubes and stored at –20 °C for further analysis.

2.6. Western blot analysis

The quality and specificity of antibody expression were characterized using western blot analysis. Culture supernatants or purified samples of stably transfected CHO pools were subjected to standard reducing or nonreducing 12% polyacrylamide gel SDS-PAGE, followed by electrophoretic transfer onto nitrocellulose membrane (GE Healthcare) using a Trans-Blot SD semidry transfer cell (BioRad, Hercules, CA, USA). The nitrocellulose membrane was washed with PBS supplemented with 0.025% [v/v] Tween 20 and blocked overnight with 5% skimmed milk in PBS at 4 °C. After the washing step, the samples were stained with horseradish peroxidase (HRP)-conjugated goat antihuman IgG at 1:5000 in PBS (Sigma–Aldrich) at room temperature for 2 h and developed using DAB Peroxidase Substrate (Sigma–Aldrich). Standard human IgG with known concentration was used as a control.

2.7. Binding activity of purified antibody

Model mAb omalizumab is an anti-IgE and interrupts the allergic cascade via binding to circulating IgE. Specific binding of the recombinant test mAb to the IgE antigen was investigated using direct ELISA and compared with a commercial mAb. Briefly, 100 µL of the purified recombinant test mAb, commercial omalizumab as a positive control, rituximab as a nonspecific control antibody, and bovine serum albumin (BSA) as a negative control were applied to the microtest wells containing IgE antibody (Abcam, Cambridge, UK) in triplicate and incubated for 1 h at 37 °C. After the washing step, 100 µL of polyclonal HRP-conjugated goat antihuman IgG detector antibody (Sigma–Aldrich) was added and incubated for 1 h at 37 °C. Then the substrate was added to each well and incubated at room temperature for 30 min. Finally, the reaction was stopped and the samples were read at 450 nm using a PowerWave XSTM (BioTek, Winooski, VT, USA) microplate reader.

2.8. Antibody quantification

Recombinant mAb concentration was measured using sandwich ELISA. Multiwell strips (Thermo Fisher Scientific, Boston, MA, USA; Nunc) were coated with 100 µL of polyclonal rabbit antihuman IgG Fc capture antibody (Thermo Fisher Scientific, Pierce) at a 1:16,000 dilution in coating buffer (50 mM $NaHCO_3$, pH 9) and incubated overnight at 4 °C. The wells were washed three times with washing buffer (PBS supplemented with 0.025% [v/v] Tween 20), and blocked with 150 µL of PBS containing 1% (w/v) BSA for 1 h at 37 °C. Following five washes, 100 µL of sample culture supernatant (diluted in PBS if necessary) was loaded into wells in duplicate and incubated for 1 h at 37 °C. After another washing step, 100 µL of polyclonal HRP-conjugated goat antihuman IgG detector antibody (Sigma) at a 1:32,000 dilution in washing buffer was added and incubated for 1 h at 37 °C. The washing step was repeated and 100 µL of tetramethylbenzidine peroxidase substrate was added and proceeded at room temperature in the dark for 30 min. To stop the reaction, 100 µL 1N H_2SO_4 was added, and absorbance at 450 nm was measured using a PowerWave XSTM (BioTek) microplate reader. In all experiments, a standard curve was generated using standard human IgG (Genscript).

3. Results

3.1. Expression of the recombinant mAb

In this study, two stable cell lines were generated by different vector combinations: CHO-HL generated via PL and PH vectors, and CHO-UHUL generated via PUL and PUH vectors. After several passages, the expression of transfected cells in culture supernatants was examined via western blot analysis. Under reducing conditions, the appearance of bands with the expected size of 25 KD for light chain and 50 KD for heavy chain verified the presence of antibody (Figure 1).

3.2. Binding activity of the purified antibody

It is crucial that the recombinant test mAb have its correct biological function. To investigate the biological activity of expressed antibody in generated cell lines, the antibody was purified by Protein A affinity chromatography. In SDS-PAGE analysis, the presence of 25 KD light chain and 50 KD heavy chain bands (Figure 2A) in reducing conditions and a 150 KD whole antibody band in nonreducing conditions confirmed the quality of purified antibody (Figure 2B). The binding activity of purified antibody to IgE was tested and compared with that of commercial omalizumab using direct ELISA. BSA was used as a negative control and Rituximab was used as a nonspecific control antibody. Figure 3 shows that the recombinant test mAb and commercial omalizumab have similar binding activities in 5, 10, and 20 ng/mL concentrations, confirming the valid activity of the proposed antibody. The activity of produced

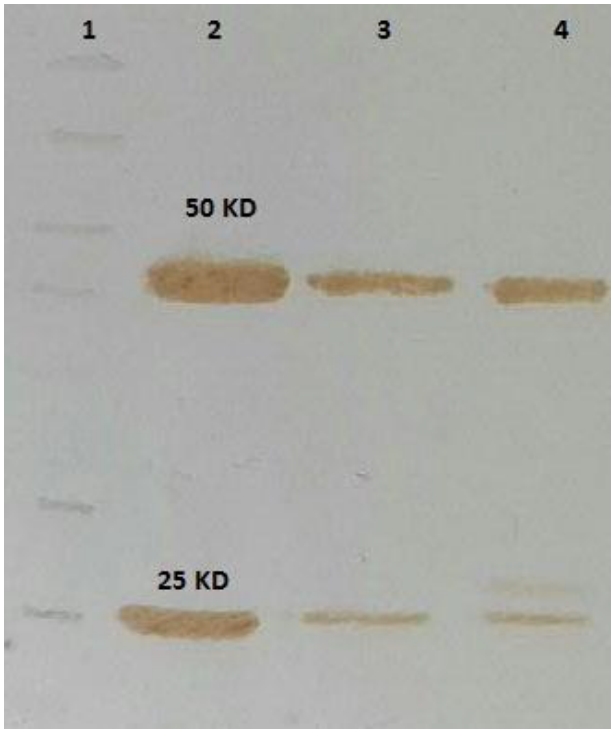

Figure 1. Western blot analysis of reduced supernatants from developed cell lines using HRP-conjugated goat antihuman IgG. CHO-DG44 cells were transfected with non-UCOE HC and LC plasmids (CHO-HL) and UCOE HC and UCOE LC (CHO-UHUL). The transfected cell pools were selected with G418 and zeocin. After several passages, the expression of cells was examined using western blot analysis on culture supernatants [Section 2.6]. Protein molecular weight marker (lane 1), standard human IgG (lane 2), sample supernatants of CHO-HL (lane 3), and sample supernatants of CHO-UHUL (lane 4).

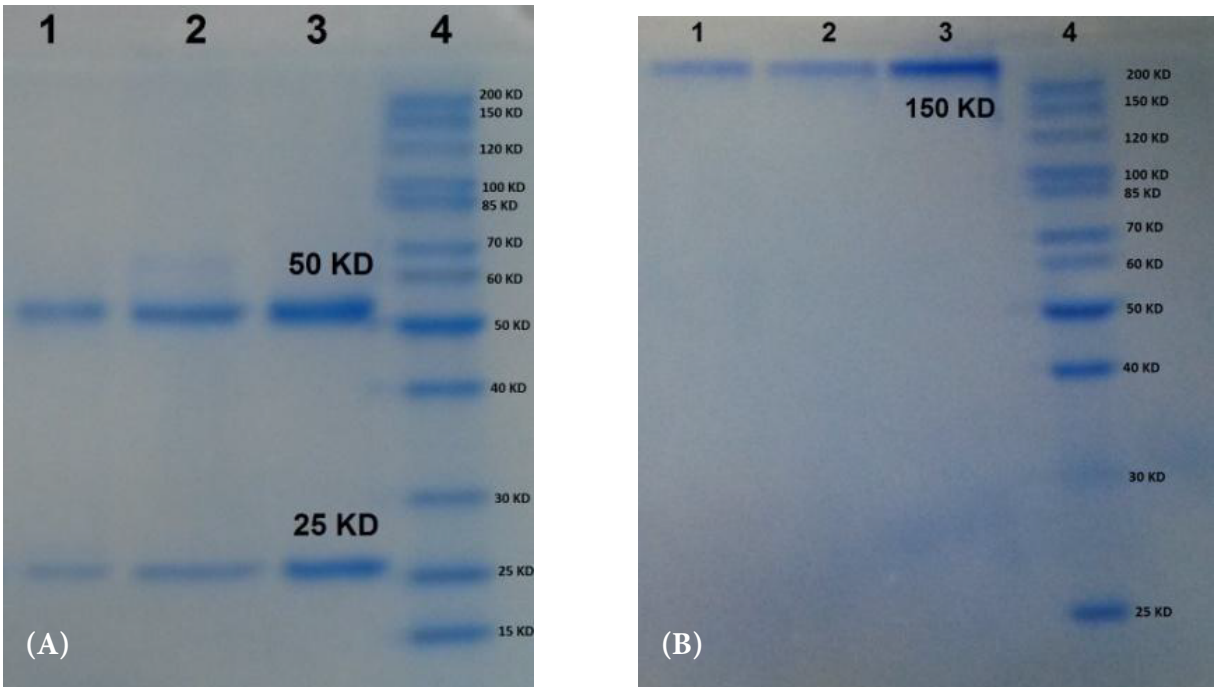

Figure 2. Reduced (A) and nonreduced (B) SDS-PAGE analysis of purified antibody. mAb was purified using Protein A affinity chromatography and subjected to standard 12% polyacrylamide gel SDS-PAGE [Sections 2.5 and 2.6]. CHO-HL (lane 1), CHO-UHUL (lane 2), standard human IgG (lane 3), and protein molecular weight marker (lane 4).

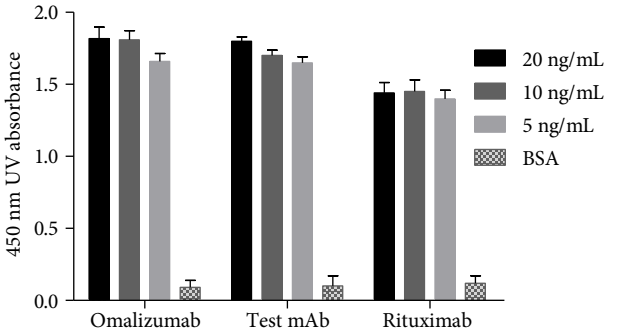

Figure 3. Comparison of binding activity of recombinant test mAb with commercial omalizumab at 20, 10, and 5 ng/mL concentration. BSA was used as a negative control and rituximab was used as a nonspecific control antibody [Section 2.7]. The error bars represent standard deviation of triplicate ELISA measurements of each sample.

mAb from both CHO-HL and CHO-UHUL was the same (data not shown).

3.3. The effect of UCOE on mAb expression

To evaluate the impact of UCOE on antibody production in CHO cells, non-UCOE– and UCOE-expressing cells were constructed. The non-UCOE CHO-HL expressed both antibody chains without UCOE regulation and was used as a control. The CHO-UHUL expressed HC and LC under the UCOE control and was used as a UCOE system. Following stable transfection and selection with G418 and zeocin, expression levels were compared using ELISA. Cells were maintained at a density of 1×10^6 cells/mL in 6-well tissue culture plates in ProCHO5 medium (Lonza) with supplements, and cell-free supernatants were collected after 5 days. Figure 4 indicates that UCOE-containing cells have significantly higher antibody expression than non-UCOE cells. The expression levels of CHO-HL and CHO-UHUL were 0.34 mg/L and 1.2 mg/L, respectively. Moreover, the cell viability of UCOE and non-UCOE was similar: about 60%. In accordance with previous investigations, these data indicate that the inclusion of UCOE on antibody chains leads to substantially higher mAb productivity in CHO cells.

3.4. The effect of NaBut and VPA iHDACs on mAb expression

In order to assess the effect of iHDACs on antibody production, CHO-HL and CHO-UHUL cells were cultivated in the presence and absence of NaBut (2 Mm) or VPA (500 μM). A total of 1×10^6 cells/mL were grown in 6-well tissue culture plates in ProCHO5 medium (Lonza) with supplements and antibody titers were calculated after 5 days using ELISA. Treatment of cells with NaBut and VPA improved mAb yields about 2-fold in CHO-HL and 2.5-fold in CHO-UHUL cell lines (Figure 5). As depicted in Figure 5, VPA treatment increased antibody expression levels slightly more than NaBut, and the cell viability for VPA treated cells was similar to that of the untreated control, but the viability of NaBut-treated cells dramatically decreased. Therefore, it seems that VPA treatment is more beneficial than NaBut for antibody production. Moreover, antibody production from UCOE-containing cells generally remained higher than non-UCOE cell pool over iHDACs treatment (Figure 5), which represents the synergic effect of A2UCOE, NaBut, and/or VPA.

4. Discussion

Chemical iHDACs, including NaBut and VPA, are frequently employed for the production of recombinant proteins by mammalian cells (Backliwal et al., 2008; Jiang and Sharfstein, 2008; Rodrigues Goulart et al., 2010; Hong et al., 2014). These compounds nonspecifically regulate gene expression and have various effects on cell metabolism (Wippermann et al., 2016). UCOE acts as a cis-acting DNA element and may specially improve the expression of surrounded transgene (Williams et al., 2005). In this study, the responses of mAb-expressing CHO cells to NaBut or VPA iHDACs and UCOE were evaluated, and in the second part of the study, the combined effect of iHDACs and UCOE on antibody production was investigated. For this purpose, UCOE and non-UCOE vectors were constructed for an antibody expression model and evaluated in stably transfected CHO-DG44 cells. CHO-HL expressed both heavy and light chains without UCOE regulation, and CHO-UHUL expressed both antibody chains in the presence of UCOE.

Following the generation of stable cell lines, antibody expression was confirmed at the protein level using western blot. The recombinant test mAb function from both generated cell lines was similar to the commercial antibody. Comparison of antibody levels using ELISA revealed that the insertion of UCOE in mAb-coding vectors enhances antibody yields at least four-fold without influencing the viability of CHO cells.

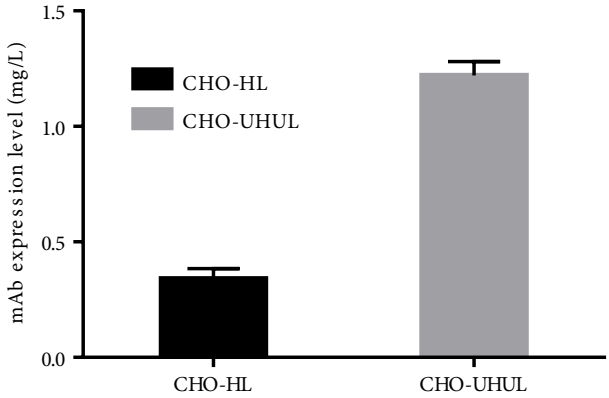

Figure 4. Antibody expression levels of generated cell lines. CHO-HL and CHO-UHUL cells were seeded in 6-well tissue culture plates at a density of 10^6 cell/mL and antibody yields were examined using ELISA [Section 2.8]. The error bars represent standard deviation of two independent assays of each sample.

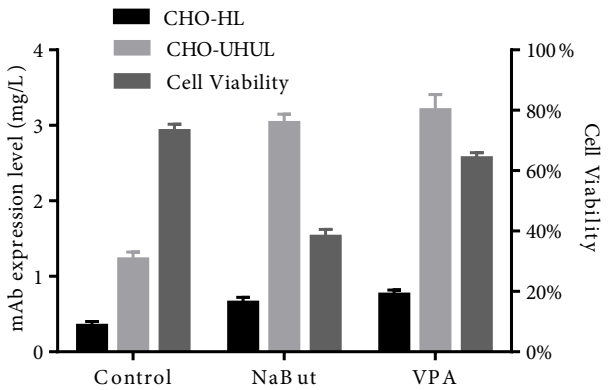

Figure 5. Antibody expression levels in untreated control and NaBut- and VPA-treated CHO-HL and CHO-UHUL cells. Stable cell lines were cultured in the presence and absence of NaBut or VPA, and antibody yields were determined using ELISA [Section 2.4]. The error bars represent standard deviation of two independent assays of each sample.

Further antibody expression was investigated in the presence or absence of iHDACs in the developed cell lines. Treatment of antibody-expressing CHO-HL and CHO-UHUL cells with NaBut and VPA increased expression 2- and 2.5-fold greater compared to control cells, respectively. UCOE as a cis-acting enhancer improved antibody expression more efficiently without any side effects on cell growth characteristics, which is crucial in industrial antibody production. On the other hand, iHDACs regulated gene expression nonspecifically and decreased cellular density and viability. NaBut decreased cell viability about 60%, which may have been due to its cytotoxic effect on these cells. In some studies, NaBut treatment frequently led to apoptosis and decreased cell growth (Davie, 2003; Mariani et al., 2003; Kobayashi et al., 2004). Since expression enhancement was slightly higher in VPA treatment than in NaBut and because of the cytotoxic effect of NaBut, it was suggested that VPA is more beneficial for antibody expression upregulation than NaBut.

UCOE and iHDACs could raise antibody expression, and expression enhancement of UCOE has been reported to be 2-fold greater than NaBut and VPA treatment. Furthermore, there was an additive effect when NaBut or VPA was used in combination with UCOE. Accordingly, the use of UCOE combined with VPA is recommended as an optimized system for mAb expression enhancement in CHO cells. Moreover, the findings may have significant implications for the design of mAb expression systems through employing epigenetic tools.

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References

Aggarwal S (2011). What's fueling the biotech engine—2010 to 2011. Nat Biotechnol 29: 1083-1089.

Ahmadi M, Mahboudi F, Eidgahi A, Reza M, Nasr R, Nematpour F, Ahmadi S, Ebadat S, Aghaeepoor M, Davami F (2016). Evaluating the efficiency of phiC31 integrase-mediated monoclonal antibody expression in CHO cells. Biotechnol Prog 32: 1570-1576.

Allen ML, Antoniou M (2007). Correlation of DNA methylation with histone modifications across the HNRPA2B1-CBX3 ubiquitously-acting chromatin open element (UCOE). Epigenetics 2: 227-236.

Antoniou M, Harland L, Mustoe T, Williams S, Holdstock J, Yague E, Mulcahy T, Griffiths M, Edwards S, Ioannou PA (2003). Transgenes encompassing dual-promoter CpG islands from the human TBP and HNRPA2B1 loci are resistant to heterochromatin-mediated silencing. Genomics 82: 269-279.

Backliwal G, Hildinger M, Kuettel I, Delegrange F, Hacker DL, Wurm FM (2008). Valproic acid: a viable alternative to sodium butyrate for enhancing protein expression in mammalian cell cultures. Biotechnol Bioeng 101: 182-189.

Benton T, Chen T, McEntee M, Fox B, King D, Crombie R, Thomas TC, Bebbington C (2002). The use of UCOE vectors in combination with a preadapted serum free, suspension cell line allows for rapid production of large quantities of protein. Cytotechnology 38: 43-46.

Boscolo S, Mion F, Licciulli M, Macor P, De Maso L, Brce M, Antoniou MN, Marzari R, Santoro C, Sblattero D (2012). Simple scale-up of recombinant antibody production using an UCOE containing vector. New Biotechnology 29: 477-484.

Davie JR (2003). Inhibition of histone deacetylase activity by butyrate. Journal Nutr 133: 2485S-2493S.

Ecker DM, Jones SD, Levine HL (2015). The therapeutic monoclonal antibody market. MAbs 7: 9-14.

Fukushige S, Sauer B (1992). Genomic targeting with a positive-selection lox integration vector allows highly reproducible gene expression in mammalian cells. Proc Natl Acad Sci U S A 89: 7905-7909.

Gilbertson L (2003). Cre–lox recombination: Cre-ative tools for plant biotechnology. Trends Biotechnol 21: 550-555.

Hong JK, Lee SM, Kim KY, Lee GM (2014). Effect of sodium butyrate on the assembly, charge variants, and galactosylation of antibody produced in recombinant Chinese hamster ovary cells. Applied Microbiology and Biotechnology 98: 5417-5425.

Jiang Z, Sharfstein ST (2008). Sodium butyrate stimulates monoclonal antibody over-expression in CHO cells by improving gene accessibility. Biotechnol Bioeng 100: 189-194.

Kobayashi H, Tan EM, Fleming SE (2004). Acetylation of histones associated with the p21WAF1/CIP1 gene by butyrate is not sufficient for p21WAF1/CIP1 gene transcription in human colorectal adenocarcinoma cells. Int J Cancer 109: 207-213.

Kwaks TH, Otte AP (2006). Employing epigenetics to augment the expression of therapeutic proteins in mammalian cells. Trends Biotechnol 24: 137-142.

Li J, Zhu Z (2010). Research and development of next generation of antibody-based therapeutics. Acta Pharmacologica Sinica 31: 1198-1207.

Mariani MR, Carpaneto EM, Ulivi M, Allfrey VG, Boffa LC (2003). Correlation between butyrate-induced histone hyperacetylation turn-over and c-myc expression. J Steroid Biochem Mol Biol 86: 167-171.

Nematpour F, Mahboudi F, Vaziri B, Khalaj V, Ahmadi S, Ahmadi M, Ebadat S, Davami F (2017). Evaluating the expression profile and stability of different UCOE containing vector combinations in mAb-producing CHO cells. BMC Biotechnol 17: 18.

Richards EJ, Elgin SC (2002). Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. Cell 108: 489-500.

Rodrigues Goulart H, Arthuso FdS, Capone MVN, Oliveira TL, Bartolini P, Soares CRJ (2010). Enhancement of human prolactin synthesis by sodium butyrate addition to serum-free CHO cell culture. J Biomed Biotechnol 2010.

Sambrook J, Russell D (2001). Molecular Cloning. A Laboratory Manual. 3rd ed. 1: 1.32-1.34, Cold Spring Harbor, NY, USA: Laboratory Press.

Tennant JR (1964). Evaluation of the trypan blue technique for determination of cell viability. Transplantation 2: 685-694.

Walsh G (2014). Biopharmaceutical benchmarks 2014. Nat Biotechnol 32: 992-1000.

Williams S, Mustoe T, Mulcahy T, Griffiths M, Simpson D, Antoniou M, Irvine A, Mountain A, Crombie R (2005). CpG-island fragments from the HNRPA2B1/CBX3 genomic locus reduce silencing and enhance transgene expression from the hCMV promoter/enhancer in mammalian cells. BMC Biotechnol 5: 17.

Wilson C, Bellen HJ, Gehring WJ (1990). Position effects on eukaryotic gene expression. Annu Rev Cell Biol 6: 679-714.

Wippermann A, Rupp O, Brinkrolf K, Hoffrogge R, Noll T (2016). Integrative analysis of DNA methylation and gene expression in butyrate-treated CHO cells. J Biotechnol (in press).

Wurm FM (2004). Production of recombinant protein therapeutics in cultivated mammalian cells. Nat Biotechnol 22: 1393-1398.