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Short communication

Digital mRNA profiling of *N*-glycosylation gene expression in recombinant Chinese hamster ovary cells treated with sodium butyrate

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ABSTRACT

To understand the effects of sodium butyrate (NaBu) on protein glycosylation, recombinant Chinese hamster ovary (rCHO) cells producing Fc-fusion glycoprotein were subjected to 3 mM NaBu. The addition of NaBu to the cultures reduced the relative proportion of acidic isoforms and sialic acid content of the glycoprotein. Fifty-two *N*-glycosylation-related gene expressions were also assessed by the NanoString nCounter system, which can provide a direct digital readout using custom-designed color-coded probes. Among them, ten genes (*ugp*, *slc35a2*, *ganc*, *man1a*, *man1c*, *mgat5a*, *st3gal5*, *glb1*, *neu1*, and *neu3*) were up-regulated and three genes (*b4galt2*, *st3gal3*, and *neu2*) were down-regulated significantly. Altered expression patterns in *st3gal3*, *neu1*, and *neu3*, which have roles in the sialic acid biosynthesis pathway, correlated with reduced sialic acid content of the glycoprotein by NaBu. Taken together, the results obtained in this study provide a better understanding of the detrimental effect of NaBu on *N*-glycosylation in rCHO cells.

1. Introduction

For high-level expression of recombinant proteins, sodium butyrate (NaBu), which is known to increase specific protein productivity (q_p), is often used in rCHO cell cultures (Jiang and Sharfstein, 2008; Kim et al., 2009, 2012; Sung et al., 2004). However, its use at a relatively high concentration is limited, because it is also known to be detrimental to cell growth by inducing apoptotic cell death (Jiang and Sharfstein, 2008; Kim et al., 2009) and protein quality by affecting glycosylation of the protein (Sung et al., 2004).

In the production of a therapeutic glycoprotein using rCHO cells, the glycosylation of the protein is a critical factor in terms of efficacy and safety. It is thus important to understand the effect of NaBu on protein glycosylation. However, studies on the effect of NaBu on the glycosylation of the proteins produced from rCHO cells, particularly in regard to transcriptomes of *N*-glycosylation-related genes, have not been fully substantiated yet.

Most comparative transcriptomic analyses on rCHO cells have been conducted with a genome-wide microarray followed by validation via a targeted quantitative polymerase chain reaction (PCR) (Baik et al., 2006; Nissom et al., 2006; Wong et al., 2006; Yee et al., 2009). Although a quantitative real-time PCR (qRT-PCR) is a sensitive method to examine the transcription level of genes, its application is limited due to its low scalability in terms of the number of genes analyzed. Therefore, qRT-PCR has been used to monitor a limited number of genes in specific pathways, such as the *N*-glycan biosynthesis pathway, in rCHO cells (Chen and Harcum, 2006; Wong et al., 2010).

The NanoString nCounter system is a currently emerging technology that measures the number of different individual mRNA expression levels via a direct digital readout by custom-designed color-coded probes (Geiss et al., 2008). It is advantageous in that it can detect low-abundant mRNA that is not detected on a microarray and it does not require any step for gene amplification or enzyme reactions usually required in PCR-based methods. Furthermore, an additional validation step by a qRT-PCR method is not required, as evidenced from the high level of concordance of NanoString nCounter analysis results with TaqMan RT-PCR (Geiss et al., 2008). Despite the benefits of NanoString nCounter analysis, little work has been published, and there has been no application of this technology to rCHO cell culture in particular.

In an effort to understand the influence of NaBu on the glycosylation of the proteins produced from rCHO cells, we investigated the changes of *N*-glycosylation-related genes in Fc-fusion glycoprotein-producing rCHO cells treated with NaBu. For an

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accurate comparison, we used the NanoString nCounter system to quantify the mRNA transcripts.

2. Results and discussion

To determine the effects of NaBu on cell growth and recombinant glycoprotein production, rCHO cells producing Fc-fusion glycoprotein were cultivated in the absence as well as in the presence of NaBu. NaBu was added to the culture medium at the final concentration of 3 mM which allowed the best production performance for this particular cell line (data not shown).

As observed with other rCHO cell lines (Jiang and Sharfstein, 2008; Kim et al., 2009), the presence of NaBu inhibited cell growth (Supplementary Fig. S1) and enhanced the q_p. To determine the effect of NaBu on the isoform distribution of glycoprotein, Fc-fusion glycoproteins were purified from culture supernatants collected after three days of cultivation in the absence and the presence of NaBu using protein A chromatography.

Fig. 1 shows the isoform distribution of purified Fc-fusion glycoprotein by a Coomassie blue-stained isoelectric focusing (IEF) gel analysis. The proportion of acidic isoforms in the glycoproteins expressed in the presence of NaBu was much lower than that in the absence of NaBu. Furthermore, the sialic acid content was determined from technical triplicate samples as described previously (Yoon et al., 2003). The sialic acid content of glycoprotein in the absence of NaBu was 21.9 ± 0.1 mol sialic acid/mol protein, while it decreased to 9.7 ± 0.2 mol sialic acid/mol protein in the presence of NaBu. Thus, 3 mM NaBu decreased the portion of Fc-fusion protein with a higher content of sialic acid.

To quantify the mRNA expression levels of *N*-glycosylation-related genes in rCHO cells, NanoString nCounter codesets were designed to include fifty-two *N*-glycosylation-related genes selected on the basis of transcriptomic studies of rCHO cells (Chen and Harcum, 2006; Wong et al., 2010; Zhang et al., 2010b). These selected genes are categorized into nucleotide sugar synthesis (8 genes), nucleotide sugar transporter (6 genes), *N*-glycan chain extension (18 genes), galactosylation (7 genes), sialylation (6 genes), fucosylation (1 gene), *N*-glycan degradation (6 genes),

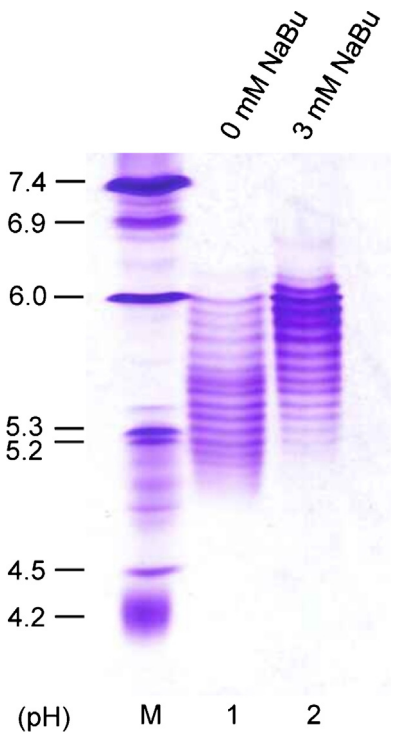

Fig. 1. Isoelectric focusing (IEF) gel analysis for purified Fc-fusion glycoprotein cultivated in the presence and absence of NaBu. The Fc-fusion glycoprotein-producing rCHO cells were provided by ISU ABXIS Co., Ltd. (Seoul, Korea). They were adapted to grow in a suspension culture with a serum-free medium (HyQ SFM4CHO; HyClone). Cells were inoculated at 2×10^5 cells/mL into a serum-free medium. After three days of cultivation, cells were harvested and resuspended to a fresh serum-free medium supplemented with NaBu or without NaBu. Culture supernatants were harvested after three days of cultivation and the protein was purified by protein A affinity chromatography using MabSelect SuRe™ (GE Healthcare) and a chromatography system (ÄKTA explorer 100Air; GE Healthcare). Equal amounts of purified protein were analyzed on Novex® pH 3–7 IEF gels (Invitrogen) and the gels were stained with InstantBlue™ (Expedeon). Lane M, pI marker in pH; lane 1, purified Fc-fusion glycoprotein produced in the absence of NaBu; lane 2, purified Fc-fusion glycoprotein produced in the presence of 3 mM NaBu.

Table 1
List of *N*-glycosylation-related genes in CHO cells. These selected genes are categorized into seven groups: nucleotide sugar synthesis, nucleotide sugar transporter, *N*-glycan chain extension, galactosylation, sialylation, fucosylation, and *N*-glycan degradation.

Group	Name	Accession No.	Gene
Nucleotide sugar synthesis	*dpagt1*	CGU09453	UDP-GlcNAc:dolichyl-phosphate GlcNAc-1 phosphate transferase
	dpm1	AF121895	Dolichol-phosphate-mannose synthase
	ugp	AF004368	UDP-glucose pyrophosphorylase
	gpi	Z37977	Glucose phosphate isomerase
	gnpda	X94699	Glucosamine-6-phosphate isomerase
	gale	EGW04772	UDP-glucose 4-epimerase, UDP-galactose 4-epimerase
	gne	AB107226	UDP-*N*-acetylglucosamine-2 epimerase
	nans	EGW07929	Sialic acid synthase
Nucleotide sugar transporter	*slc35a1*	EGW05061	CMP-sialic acid transporter
	slc35a2	EGW06098	UDP-galactose translocator
	slc35a3	EGW02964	UDP-*N*-acetylglucosamine transporter
	slc35b4	EGV98807	UDP-xylose/UDP-*N*-acetylglucosamine transporter
	slc35c1	EGW03916	GDP-fucose transporter 1
	slc35d2	EGW12899	UDP-*N*-acetylglucosamine/UDP-glucose/GDP-mannose transporter
N-Glycan chain extension	*gcs1*	EGW11798	Mannosyl-oligosaccharide glucosidase (glucosidase I)
	ganab	EGV92756	Neutral α-glucosidase AB (glucosidase II α subunit)
	ganc	EGV97236	Neutral α-glucosidase C (glucosidase II β subunit)
	man1b1	EGW14976	Endoplasmic reticulum mannosyl-oligosaccharide 1,2-α-mannosidase
	manea	EGV92778	Glycoprotein endo-α-1,2-mannosidase
	man1a	EGV92774	Mannosyl-oligosaccharide 1,2-α-mannosidase IA
	man1b	EGW05808	Mannosyl-oligosaccharide 1,2-α-mannosidase IB
	man1c	EGW10727	Mannosyl-oligosaccharide 1,2-α-mannosidase IC
	man2a1	EGW04032	α-Mannosidase 2 (mannosidase II)
	man2a2	EGW12798	α-Mannosidase 2x (mannosidase IIx)
	mgat1	EGV96592	α-1,3-Mannosyl-glycoprotein 2-β-*N*-acetylglucosaminyltransferase
	mgat2	EGW09264	α-1,6-Mannosyl-glycoprotein 2-β-*N*-acetylglucosaminyltransferase