

## Identification of the Pig $\beta$ -1,3-*N*-acetylglucosaminyltransferase 1 (pB3GNT1) that is Involved in Poly-*N*-acetylactosamine (poly-LacNAc) Synthesis

Ji-Youn Kim<sup>1†</sup>, Hwan-Jin Hwang<sup>1†</sup>, Hak-Jae Chung<sup>2</sup>, Shinichi Hochi<sup>3</sup>, Mi-Ryung Park<sup>1</sup>, Sung June Byun<sup>1</sup>, Keon Bong Oh<sup>1</sup>, Hyeon-Yang<sup>1</sup> and Kyung-Woon Kim<sup>1\*</sup>

<sup>1</sup>Animal Biotechnology Division, National Institute of Animal Science, Rural Development Administration, Wanju-Gun 55365, Korea

<sup>2</sup>Swine Science Division, National Institute of Animal Science, Rural Development Administration, Cheonan 31000, Korea

<sup>3</sup>Faculty of Textile Science and Technology, Shinshu University, Ueda, Nagano 386-8567, Japan

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The structure of glycan residues attached to glycoproteins can influence the biological activity, stability, and safety of pharmaceutical proteins delivered from transgenic pig milk. The production of therapeutic glycoprotein in transgenic livestock animals is limited, as the glycosylation of mammary gland cells and the production of glycoproteins with the desired homogeneous glycoform remain a challenge. The  $\beta$ -1,3-*N*-acetylglucosaminyltransferase1 (B3GNT1) gene is an important enzyme that attaches *N*-acetylglucosamine (GlcNAc) to galactose (Gal) residues for protein glycosylation; however, there is limited information about pig glycosyltransferases. Therefore, we cloned the pig B3GNT1 (pB3GNT1) and investigated its functional properties that could attach *N*-acetylglucosamine to galactose residue. Using several different primers, a partial pB3GNT1 mRNA sequence containing the full open reading frame (ORF) was isolated from liver tissue. The ORF of pB3GNT1 contained 1,248 nucleotides and encoded 415 amino acid residues. Organ-dependent expression of the pB3GNT1 gene was confirmed in various organs from adult and juvenile pigs. The pB3GNT1 mRNA expression level was high in the muscles of the heart and small intestine but was lower in the lungs. For functional characterization of pB3GNT1, we established a stable expression of the pB3GNT1 gene in the porcine kidney cell line (PK-15). As a result, it was suggested that the glycosylation pattern of pB3GNT1 expression in PK-15 cells did not affect the total sialic acid level but increased the poly *N*-acetylactosamine level. The results of this study can be used to produce glycoproteins with improved properties and therapeutic potential for the generation of desired glycosylation using transgenic pigs as bioreactors.

**Key words** : *N*-acetylglucosamine (GlcNAc), *N*-acetylactosamine (LacNAc), pig  $\beta$ -1,3-*N*-acetylglucosaminyltransferase 1 (pB3GNT1), PK-15

### Introduction

Many physiologically active proteins contain various carbohydrate structures whose formation is catalyzed by glycosyltransferases [17, 19]. The glycosylation process is considered to be important for intrinsic and extrinsic cellular signal transduction [12, 13], as various types of cancer avoid apoptosis through glycan modifications. When Fas is hyper- $\alpha$ -2,6 sialylated by  $\beta$ -galactoside  $\alpha$ 2,6-sialyltransferase 1 (ST6Gal1),

apoptotic signaling is precluded, as both death-inducing signal complex formation and Fas internalization are suppressed [27]. Conversely, proliferation is significantly inhibited, and apoptosis is induced in human colorectal cancer cells treated with *O*-glycosylation inhibitors [22]. The ability of cells to adhere to the extracellular matrix is also altered by modifying glycan structures [16, 18, 25]. Poly *N*-acetylactosamine (poly-LacNAc) is a chain of disaccharides composed of galactose (Gal) and *N*-acetylglucosamine (GlcNAc) that provides a unique structure for cell-type-specific glycans when attached to glycoproteins and glycolipids [4, 6]. The linear form of poly-LacNAc is dominant in fetal erythrocytes, while the branched form is dominant in adult erythrocytes [7].  $\beta$ -1,4-*N*-galactosyltransferase (B3GALT) of the  $\beta$ -1,3-*N*-acetylglucosaminyltransferase (B3GNT) family is a key enzyme in the synthesis of poly-LacNAc and the formation of i antigen, a human alloantigen [28]. Further mod-

<sup>†</sup>Authors contributed equally.

\*Corresponding author

Tel : +82-63-238-7264, Fax : +82-63-238-7297

E-mail : kw72kim@korea.kr

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ification of poly-LacNAc induces the display of various terminal epitopes [20]. Sialyl Lewis X, a terminal epitope, plays an important role in leukocyte adhesion [8, 23].

The B3GNT1 gene has been identified in many mammalian species, including humans, mice, rats and cows [24]. Functional analysis of B3GNT1<sup>-/-</sup> genetic knock-out mice revealed inhibited neuronal migration, and the observed abnormality was attributed to decreased terminal lactosamine levels [3]. Impaired sexual behavior has also been reported in male B3gnt1<sup>-/-</sup> knock-out mice [2]. Conversely, over-expression of the B3GNT gene appears to decrease tumor formation rate, probably due to increased synthesis of laminin-binding glycans that can interact with adhesion molecules in the epithelial cell basement membrane [1].

Pigs have received significant attention as animal bioreactors in transgenic research as well as transplantable organ donors in regenerative medicine. The data on B3GNT1 in pigs is currently very limited but could be beneficial for understanding the equivalent enzymes in humans. Therefore, in the present study, we cloned the pig B3GNT1 gene (pB3GNT1), and its enzymatic activity was measured using CD39L3, which hydrolyzes phosphate groups from uridine diphosphate (UDP) [29]. The expression of pB3GNT1 in various organs from adult and juvenile pigs, the sialic acid and poly-LacNAc levels in pB3GNT1-overexpressing porcine kidney cells (PK-15) and the catalytic activity of this enzyme toward the *O*-glycan core were also investigated.

## Materials and Methods

### Ethics approval and consent to participate

All experimental procedures were examined and approved by the Animal Research Committee at National Institute of Animal Science (NIAS 2015-593).

### RNA extraction and cDNA synthesis

Various tissues were homogenized in the presence of TRIzol (Invitrogen, San Diego, CA, USA), and RNA was isolated. The obtained RNA fragments were utilized for cDNA synthesis using a First-Strand cDNA Synthesis Kit (GE Healthcare, Pittsburgh, PA, USA).

### Cloning of the pig B3GNT1 gene

Primers were designed for the untranslated region (UTR) through homology comparisons with equivalent sequences from other species obtained from NCBI, such as humans (NM 006876.2), mice (NM 175383.2), cows (NM 001034808.1), and rats (NM 001106324.1). The forward and reverse primers were 5'-ATG CAR ATG TCS TAC GCC ATC-3' and 5'-TRG CCT TCA ACT CCT GYT TG-3', respectively. Degenerate PCR was performed under the following conditions: 94°C for 3 min; 30 cycles of 94°C for 30 s, 42°C for 30 s, 72°C for 90 s; and 72°C for 5 min (Fig. 1).

Total RNA was extracted from pig liver tissue, and the GeneRacer Kit (Invitrogen, San Diego, CA, USA) was used for 5' and 3' RACE. The 5' RACE primers consisted of a 5'-anchor primer: 5'-GAA ATA TTG GTC TTG CTC CTC CTG C-3', and the GeneRacer 5' primer. The 3' RACE primers consisted of a 3'-anchor primer: 5'-ACG AAG GTT TCC TGG TTC ATA AAG G-3', and the GeneRacer 3' primer (Fig. 1). The amplified PCR products were analyzed through agarose gel (Takara, Kyoto, Japan) electrophoresis and cloned using the TOPO-TA cloning kit (Invitrogen, San Diego, CA, USA). Finally, selected clones were identified via DNA sequence analysis (Macrogen, Seoul, Korea).

### Quantitative real-time polymerase chain reaction (qPCR)

For quantitative analysis of the expression levels of pB3GNT1 and pig beta-actin gene were assessed in various organs from adult pigs (n=3) and a juvenile piglet (n=1).

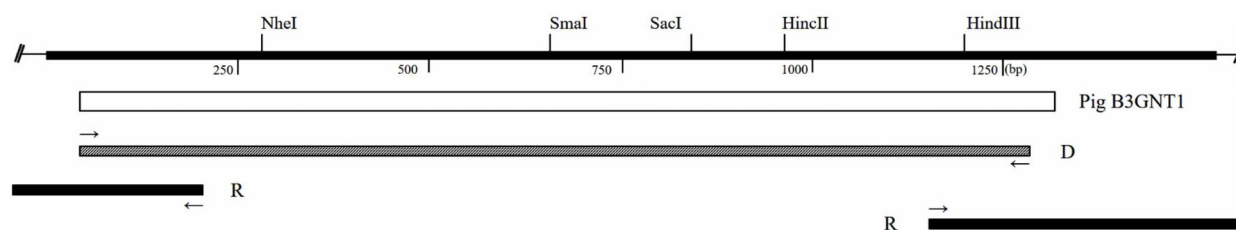


Fig. 1. Schematic diagram of pig B3GNT1 cDNA clones. The open bar represents the protein coding region, and thick lines indicate the noncoding regions of B3GNT1. D, Degenerate PCR clone, R, RACE clones. The small, thin arrows indicate the position and direction of primers.

qPCR was performed using LightCycler FastStart DNA Master SYBR Green I (Roche, Basel, Switzerland). The primer sets used in these assays were as follows: for pB3GNT1, 5'-AGC CCG GGA TCA ATT ATG CAC-3' (forward) and 5'-ACC ATG TCC ACG TCG ATT ACC-3' (reverse); and for beta-actin, 5'-CAT CAC CAT CGG CAA CGA GC-3' (forward) and 5'-TAG AGG TCC TTG CGG ATG TC-3' (reverse). The thermal profile consisted of the following steps: pre-incubation at 95°C for 10 min and 45 cycles of amplification at 95°C for 10 s, 58°C for 5 s, and 72°C for 4 s. The  $2^{-\Delta\Delta CT}$  method was used to analyze pB3GNT1 expression.

### Construction of the pB3GNT1 expression vector

For the cloning of the pB3GNT1 gene with a flag tag at the N-terminal region, the following primers were designed: 5'-GAA TTC **GCC** ACC ATG **GAT TAC AAG GAT GAC GAC GAT AAG** ATG CAG ATG TCG TAC GCC ATC-3' and 5'-GCG GCC GCT CAG CAG TGA CGT GGG GAG-3' (the *EcoRI* and *NotI* sites are underlined, and the flag tag sequences are highlighted in bold). The amplified PCR products and the pcDNA3.1(+) vector (Invitrogen, San Diego, CA, USA) were sequentially digested with *EcoRI* (Takara, Kyoto, Japan) and *NotI* (Takara, Kyoto, Japan) and then ligated together with the Mighty Mix DNA Ligation Kit (Takara, Kyoto, Japan).

### pB3GNT1 transfection into PK-15 cells

Porcine kidney cells (PK-15) were maintained in high-glucose Dulbecco's Modified Eagle Medium (DMEM; Invitrogen, San Diego, CA, USA) containing 10% fetal bovine serum (FBS; Invitrogen, San Diego, CA, USA), 50 U/ml penicillin, and 50 µg/ml streptomycin (Invitrogen). The cells were maintained at 37°C under 5% CO<sub>2</sub> and 95% air. The pB3GNT1 expression vector was linearized with *PvuI* (Takara, Kyoto, Japan) and transfected into PK-15 cells using Lipofectamine (Invitrogen, San Diego, CA, USA). Transformed cells were selected via treatment with 800 µg/ml G418 (Sigma-Aldrich Corp., St. Louis, MO, USA) for 2 weeks.

### Western blotting to detect FLAG-tagged pB3GNT1

Cells were harvested and lysed with M-PER buffer (Thermo Fisher Scientific, West Palm Beach, FL, USA). Proteins were separated using SDS-PAGE and blotted onto 0.45 µm nitrocellulose membranes. The membranes were blocked in 5% non-fat dry milk in TBST buffer (Tris-buffered

saline, 0.05 M Tris, pH 7.4; 0.2 M NaCl; 0.1% Tween 20) for 1 h at room temperature (RT) with shaking and then incubated in 0.1% TBST containing a monoclonal FLAG antibody (Sigma-Aldrich Corp., St. Louis, MO, USA) overnight at 4°C with shaking. On the following day, the membranes were washed 3 times with 0.1% TBST for 10 min and further incubated in 0.1% TBST containing an horseradish peroxidase (HRP)-conjugated anti-mouse secondary antibody (Invitrogen, San Diego, CA, USA) for 1 hr at RT. Finally, the membranes were washed 3 times with 0.1% TBST for 10 min and developed using an enhanced chemiluminescence solution (GE Healthcare, Pittsburgh, PA, USA) to visualize the targeted proteins.

### Immunofluorescence microscopy analysis

Cells were seeded onto Lab-Tek chamber slides and cultured for 24 hr. Subsequently, the cells were fixed with 3.7% formaldehyde for 15 min, washed 3 times with phosphate-buffered saline (PBS) for 3 min, and then incubated for 10 min at -20°C with ice-cold MeOH. Next, the cells were washed 3 times with PBS and incubated in PBS containing 1% bovine serum albumin (BSA) and 0.2% Triton-X 100 (blocking buffer) for 1 hr. After 3 washes in PBS, the cells were incubated with a FLAG antibody (diluted 1:1,000 in dilution buffer consisting of PBS with 1% BSA and 0.05% Triton-X 100) overnight at 4°C. The cells were subsequently washed 3 times in PBS and incubated with fluorescein isothiocyanate-conjugated mouse IgG (1:500 in dilution buffer) (Sigma-Aldrich Corp., St. Louis, MO, USA) for 1 hr in dark conditions. After 3 washes, cell nuclei and Golgi complexes were stained using 4',6-diamidino-2-phenylindole (DAPI; Roche, Basel, Switzerland) and BODIPY® TR ceramide (Invitrogen, San Diego, CA, USA), respectively. Finally, the cells were observed under a fluorescence microscope (Olympus, Tokyo, Japan).

### Enzyme-linked immunosorbent assay (ELISA)

ELISA was performed using the ELISA Starter Accessory Package (Bethyl Laboratories, Montgomery, TX, USA) according to the Vectorlabs method. Total protein lysates (10 - 30 µg) from PK-control and PK-pB3 cells were coated onto 96-well plates with coating buffer (0.05 M carbonate bicarbonate, pH 9.6) for 1 hr at 37°C. The plates were subsequently washed 3 times and filled with post-coat solution (50 mM Tris, 1% BSA, pH 8.0) for 30 min at RT. After 3 washes, each well was incubated with 10 µg/ml of bio-

tinylated *Lycopersicon esculentum* agglutinin (LEA; EY Laboratories, San Mateo, CA, USA) or *Limax flavus* agglutinin (LFA; EY Laboratories, San Mateo, CA, USA) for 1 hr at RT. The plates were next washed with Tris/Tween 20 buffered saline (pH 8.0) and incubated with streptavidin-HRP (1:2,000; GE Healthcare, Pittsburgh, PA, USA). Then, the plates were incubated for 30 min at RT and washed 3 times and the 3,3',5,5'-tetramethylbenzidine substrate was used as the chromogen. After the solution turned blue, stop solution (1 N H<sub>2</sub>SO<sub>4</sub>) was added to each well. The optical density (OD) value was finally determined at 450 nm.

**Measurement of pB3GNT1 activity**

pB3GNT1 activity was assessed using a Glycosyltransferase Activity Kit (R&D Systems, Minneapolis, MN, USA) and 0.6 mM *p*-Nitrophenyl galacto-*N*-bioside (Gal-β1,3-GalNAc-

pNP; Sigma-Aldrich Corp., St. Louis, MO, USA), 200 μM UDP-GlcNAc (Sigma-Aldrich Corp., St. Louis, MO, USA), and 20 mg/ml of asialo-fetuin type I (Sigma-Aldrich Corp., St. Louis, MO, USA). To prepare the reaction, 10 μl of donor substrate solution (UDP-GlcNAc), 10 μl of acceptor substrate solution (Gal-β1,3-GalNAc-pNP or asialo-fetuin type I), and 5 μl of coupling phosphatase 1 solution were mixed in each well. Coupling phosphatase (ENTPD3/CD39L3) can remove a phosphate group from free-UDP. To initiate the reaction, glycosyltransferase solution (10 μg of cell protein lysate) was added to each well, and assay buffer (3.125 mM Tris HCl [pH 7.5], 1.25 mM CaCl<sub>2</sub>, 12.5 mM MnCl<sub>2</sub>) was then added to a total volume of 50 μl. As the negative control, assay buffer alone was used. The plate contents were mixed via gentle tapping and incubated at 37°C for 2 hr. Then, 30 μl of Malachite Green Reagent A was added to each well. The

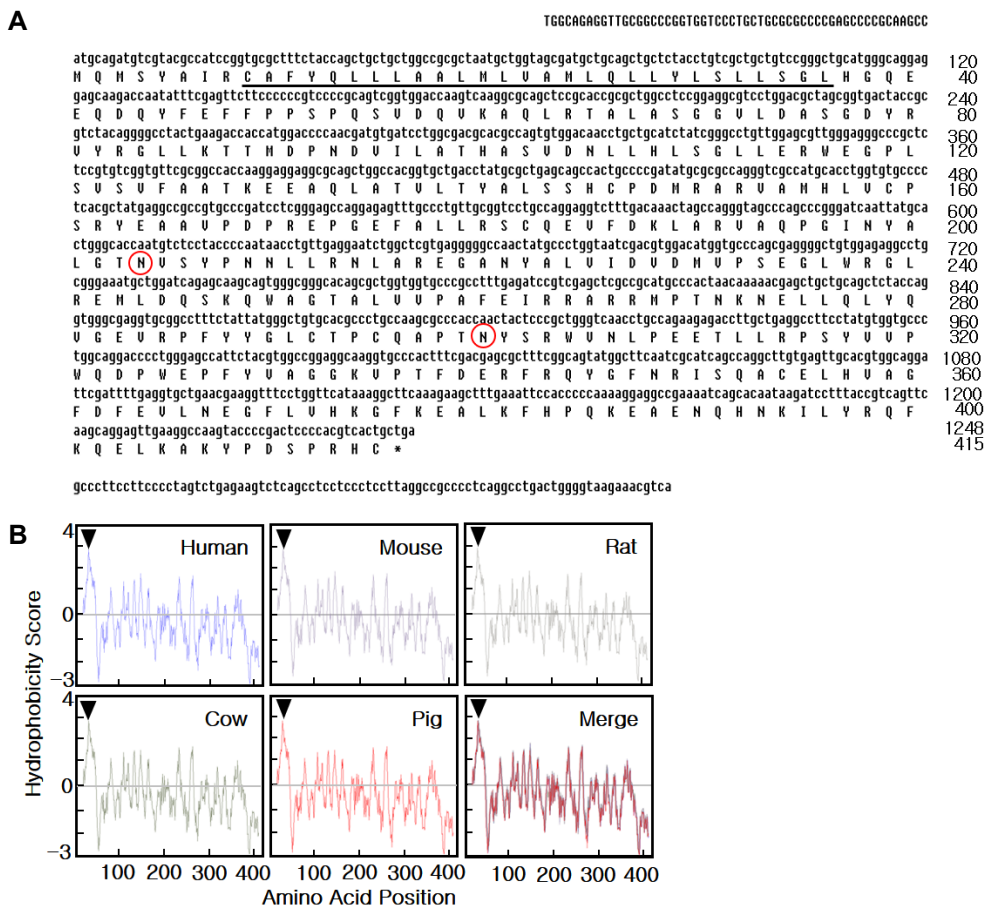


Fig. 2. Characterization of the pB3GNT1 gene. A - The cDNA sequence of the pB3GNT1 gene derived from pig liver cDNA and its deduced amino acid sequence. The underlined text indicates a putative transmembrane domain, and open circles indicates putative *N*-glycosylation sites. The sequence data have been submitted to the NCBI GenBank databases under accession No. JN975036. B - Hydrophobicity of B3GNT1 proteins from various species, calculated with the Kyte and Doolittle program (<http://www.expasy.ch/cgi-bin/protscale.pl>). High hydrophobicity in the N-terminal region (arrowhead) predicts that 1,3-*N*-acetylglucosaminyltransferase 1 protein shows a type II transmembrane topology.

plate contents were mixed by gentle tapping, and 100  $\mu$ l of deionized water was added. Finally, 30  $\mu$ l of Malachite Green Reagent B was added, followed by mixing by tapping. The color reaction was allowed to develop for 20 min at RT, and the OD value was determined at 655 nm. A standard curve was generated through 2-fold serial dilution, where the highest concentration was 1 mM phosphate saline.

## Results

### Characterization of pB3GNT1 cDNA

Through degenerate PCR and RACE PCR targeting pB3GNT1, a partial pB3GNT1 sequence containing the full open reading frame (ORF) was identified (Fig. 1). The ORF consisted of 1,248 nucleotides (nt), encoding 415 amino acids (aa) (Fig. 2A). The pB3GNT1 gene sequence was highly homologous to those of *Homo sapiens* (92% nt, 98% aa), *Mus musculus* (88% nt, 94% aa), *Bos taurus* (95% nt, 98% aa), and *Rattus norvegicus* (87% nt, 94% aa) (data not shown). Moreover, the N-terminal region of pB3GNT1 exhibited high hydrophobicity (Fig. 2B). Comparison of the hydropathy plots of the hydrophobicity scores for B3GNT1 proteins from several species indicated high conservation across species

(Fig. 2B). These results confirm that the B3GNT1 gene is highly conserved in all investigated species.

### pB3GNT1 expression in various organs

As B3GNT1 is known to be expressed in diverse tissues, we analyzed the relative expression levels of this gene in many organs through RT-PCR and quantitative PCR (Fig. 3A, Fig. 3B). In adult pig samples, pB3GNT1 mRNA levels were found high in the heart, small intestine and muscle, while lower in the lungs, stomach and bladder (Fig. 3C). On the other hand, the piglet samples showed high level of pB3GNT1 mRNA in liver tissue, while low in lung and splenic tissues (Fig. 3D).

### pB3GNT1 activity

In RT-PCR of PK-15 cells transfected with the pB3GNT1 expression vector, the expression of the introduced gene was confirmed (Fig. 4A), and qPCR indicated that the transcription and translation of pB3GNT1 were stronger in transfected cells (PK-pB3) than non-transfected control cells (PK-control) (Fig. 4B). Cells were double-labeled with a FLAG antibody and BODIPY<sup>®</sup> TR ceramide, and pB3GNT1 localization was identified within the Golgi complex (Fig.

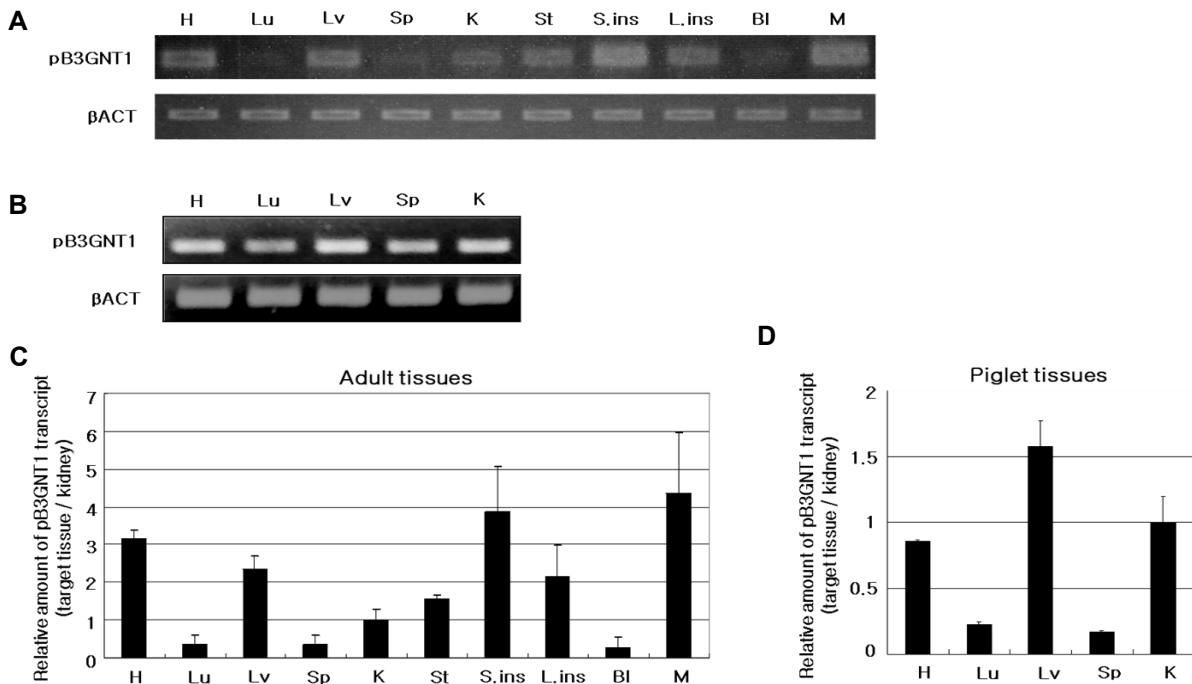


Fig. 3. qPCR analysis of pB3GNT1 mRNA levels in various pig organs. Data from duplicate samples of 3, 2-years-old adult pigs (A, C) and one 5-day-old piglet (B, D). H: heart, Lu: lung, Lv: liver, Sp: spleen, K: kidney, St: stomach, S.ins: small intestine, C: colon, Bl: bladder, M: muscle. The expression levels of pB3GNT1 were normalized to that of  $\beta$ -actin and subsequently normalized to kidney expression levels. The data represent the mean  $\pm$  S.D.

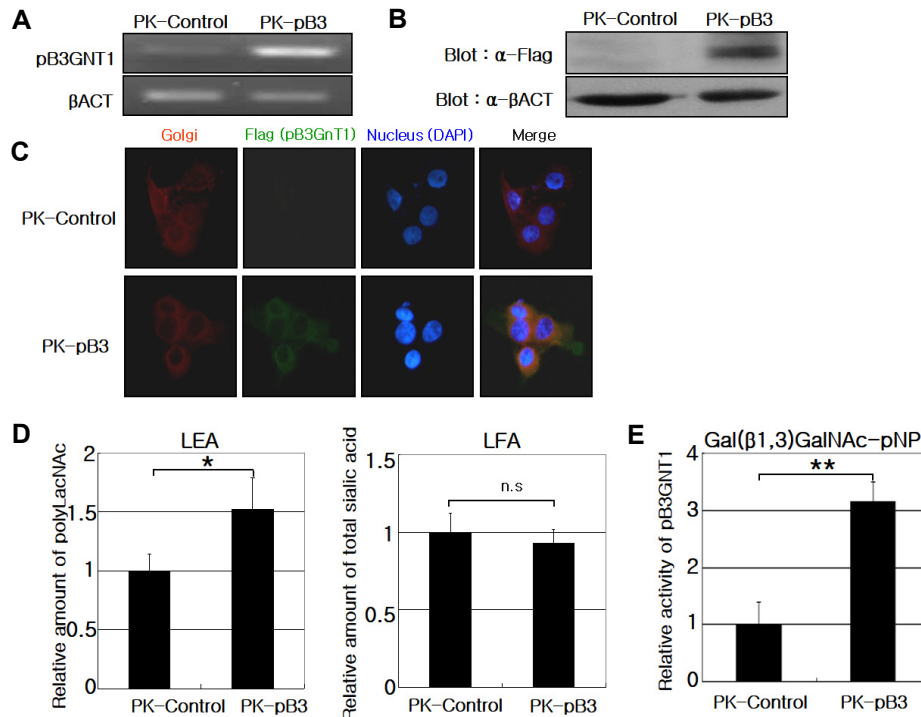


Fig. 4. Functional test of pB3GNT1 gene in PK-15 cell line. A - qPCR performed on PK-control and PK-pB3 cells to assess pB3GNT1 expression levels. B - Western blot analysis performed using a specific FLAG antibody. C - Immunofluorescence labeling for FLAG (green), the Golgi complex (red) and nuclei (blue), indicating pB3GNT1 localization within the Golgi complex. D - ELISA for poly-LacNAc and total sialic acid levels. The levels of poly-LacNAc structures, recognized by LEA, were higher in PK-pB3 than in PK-control cells. The total sialic acid levels, determined by LFA, were not significantly different between the PK-pB3 and PK-control cells. E - The specific activity of pB3GNT1 toward Gal(β1,3)GalNAc, a core structure of O-glycan, was analyzed using CD39L3. The activity was higher in PK-pB3 cells than in PK-control cells. The data represent the mean  $\pm$  S.D. (\* $p$ <0.05, \*\* $p$ <0.005 by Student's  $t$ -test, n.s: not significant).

4C).

As B3GNT1 plays an important role in the synthesis of poly-LacNAc, the intracellular poly-LacNAc content is indicative of B3GNT1 catalytic activity and can be measured using LEA lectin with a high affinity for LacNAc oligomers, rather than monomers or dimers. The poly-LacNAc level was approximately 1.5-fold higher in PK-pB3 cells than in PK-control cells ( $p$ <0.05). The sialic acid content, measured using a sialic acid-specific LFA lectin, was not significantly affected by pB3GNT1 expression in PK-15 cells (Fig. 4D).

*p*-Nitrophenyl galacto-*N*-bioside (Gal-β1,3-GalNAc-pNP) contains Gal residues and was employed as an acceptor substrate. As expected, the PK-pB3 cells showed approximately 3-fold higher catalytic activity than the PK-control cells (Fig. 4E). The specific activity of the PK-control cells was  $0.325 \pm 0.158$  pmol·min<sup>-1</sup>·μg<sup>-1</sup>, whereas, that of PK-pB3 was  $1.194 \pm 0.149$  pmol·min<sup>-1</sup>·μg<sup>-1</sup> (data not shown).

## Discussion

In this study, we cloned pig's B3GNT1 gene and analyzed its function. pB3GNT1 exhibited a type II membrane topology, which is a common feature of many glycosyltransferases [1, 5, 14, 24]. A 28-aa transmembrane domain in the N-terminal region was suggested to be a Golgi-anchoring region 253 based on sequence alignment with homologous proteins from other species, while the C-terminal region facilitates catalytic activity in the lumen. As most glycosyltransferases in the B3GNT family are glycoproteins, we searched for putative N-linked glycosylation sites in pB3GNT1. N-linked glycosylation usually occurs at the specific peptide sequence Asn-X-Ser/Thr, where X can be any amino acid other than proline (Fig. 2A) [31]. The identified regions coincided with potential N-glycosylation sites in human B3GNT1 [24].

qPCR analysis to detect pB3GNT1 mRNA levels in various pig organs indicated that the expression level in the liver

was higher than in the kidneys and spleen of pigs, whereas human B3GNT1 is more highly expressed in the kidneys and spleen than in the liver [24]. The pB3GNT1 activity of double labelled PK-15 cells was consistent with previous reports showing that enzymes in the B3GNT family are generally localized to the Golgi complex [17].

As sialic acids are added to Gal or GalNAc residues by various sialyltransferase families (ST3Gal, ST6Gal, ST6GalNAc, ST8Sia, etc.), the donor substrates for B3GNT1 may be competitively used by dominant sialyltransferases. Over-expression of sialyltransferases in Chinese hamster ovary (CHO) cells has been shown to significantly decrease poly-LacNAc levels [30].

Therapeutic proteins are the fastest growing industry in the pharmaceutical. These proteins can be recombinantly produced in mammalian cells capable of glycosylation such as human. We have tried to produce safe and useful glycoprotein drugs using transgenic pigs that secrete recombinant drugs proteins such as von Willebrand factor (vWF) [15] and erythropoietin (EPO) [21], into their milk. However, there were some limitations that prevented the development of these production methods. Based on these studies, we found that the secreted proteins require proper glycan modification to function properly.

Modification of glycan structures has been attempted in the pharmaceutical industry [10, 11] to regulate the biological activity and stability of glycoprotein biomedicines with the sialic acid content of *N*-glycan. When the sialic acid content of EPO is elevated, the biological activity and stability increase [9]. Some key enzymes in the B3GNT family that extend and diversify glycan structures could affect the stability of glycoproteins [26]. Therefore, efficient regulation of B3GNT family members and sialyltransferases in the mammary glands during lactation is critical to improve the biological activity of secreted recombinant proteins.

In conclusion, we characterized the molecular structure and function of pB3GNT1, an enzyme that attaches *N*-acetylglucosamine to galactose residues. The ORF contained 1,248 nt and encoded 415 aa. Furthermore, different expression levels of pB3GNT1 were confirmed via RT-PCR and qPCR in piglet organs and adult pig organs. We further concluded that the over-expression of the gene in a porcine kidney cell line (PK-15) did not affect the total sialic acid level but increase the poly *N*-acetylglucosamine level. This enzyme exhibited catalytic activity toward the *O*-glycan core; however, the glycoprotein stability could be affected by poly-LacNAc

structures. These results will serve as a useful basis for the development of a new, efficient bioreactor in the pharmaceutical industry.

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## 초록 : Poly-N-acetyllactosamine (poly-LacNAc) 합성에 관여하는 돼지 $\beta$ -1,3-N-acetylglucosaminyltransferase I (pB3GNT1) 유전자 동정

김지윤<sup>1\*</sup> · 황환진<sup>1\*</sup> · 정학재<sup>2</sup> · 신이치 호치<sup>3</sup> · 박미령<sup>1</sup> · 변승준<sup>1</sup> · 오건봉<sup>1</sup> · 양 현<sup>1</sup> · 김경운<sup>1\*</sup>  
(<sup>1</sup>국립축산과학원 동물바이오통학과, <sup>2</sup>국립축산과학원 양돈과, <sup>3</sup>신슈대학)

당 단백질에 붙어 있는 당사슬 구조는 형질전환 돼지 유즙으로 분비되는 의약품 단백질의 생물학적 활성, 안정성 그리고 안전성에 영향을 줄 수 있다. 형질전환 동물을 이용한 치료용 당 단백질 생산은 유선 세포에서 이루어지는 당사슬 부가능력에 의해 제한되며, 균일한 당사슬 형태를 가지는 당 단백질 생산은 도전 과제로 남아있다.  $\beta$ -1,3-N-acetylglucosaminyltransferase1 (B3GNT1) 유전자는 N-아세틸글루코사민에 갈락토오스 잔기를 부착시키는 단백질 당화기작에 중요한 효소이지만, 돼지 당 전이효소에 대한 정보는 매우 제한적이다. 따라서, 돼지 B3GNT1 (pB3GNT1) 유전자를 클로닝하고 N-아세틸글루코사민에 갈락토오스 잔기를 부착시키는 기능적 특성을 조사하였다. 몇가지 다른 프라이머를 사용하여 전체 전사영역(ORF)을 함유하는 부분적인 pB3GNT1 mRNA 염기서열을 간 조직으로부터 분리하였다. 클로닝 된 pB3GNT1의 ORF는 1,248개의 뉴클레오티드를 가지며, 415개 아미노산 잔기로 구성되어 있었다. pB3GNT1 유전자의 장기별 발현특성은 성돈 및 자돈의 여러 기관에서 분석하였다. pB3GNT1 mRNA 발현 수준은 심장, 소장 보다는 근육에서 높았지만 폐에서는 낮았다. pB3GNT1의 기능적 특성 분석을 위해 돼지 신장 세포주(PK-15)에서 pB3GNT1 유전자의 안정적인 발현을 확립하였다. 그 결과, PK-15 세포에서 pB3GNT1 발현에 의한 당화 패턴은 총 시알산 증가에는 영향을 미치지 않지만, poly-N-아세틸글루코사민은 증가하는 것으로 나타났다. 본 연구는 생물반응기로 형질전환 돼지를 이용할 때 희망하는 당사슬을 부가하여 치료 가능성을 높이며 개선된 활성을 나타내는 당단백질 생산에 도움이 될 것이다.