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Loss of expression of *N*-acetylglucosaminyltransferase Va results in altered gene expression of glycosyltransferases and galectins

Hua-Bei Guo, Alison Nairn, Kyle Harris, Matthew Randolph, Gerardo Alvarez-Manilla, Kelley Moremen, and Michael Pierce*

Department of Biochemistry and Molecular Biology, Complex Carbohydrate Research Center, 315 Riverbend Road, University of Georgia, Athens, GA 30602, United States

Abstract

We isolated mouse embryo fibroblasts (MEFs) from *N*-acetylglucosaminyltransferase Va (GnT-Va) knockout mice and studied the effects of loss of expression of GnT-Va on asparagine-linked glycans (*N*-glycan) synthesis and the gene expression of groups of glycosyltransferases and galectins. Loss of GnT-Va expression caused aberrant expression of several *N*-glycan structures, including *N*-linked $\beta(1,6)$ branching, poly-*N*-lactosamine, bisecting *N*-acetylglucosamine (GlcNAc) and sialic acid. Using quantitative reverse transcriptase-PCR (qRT-PCR), altered gene expression of several groups of glycosyltransferases and galectins was observed in GnT-Va null MEFs, supporting the observed changes in *N*-glycan structures. These results suggest that genetic disruption of GnT-Va ultimately resulted in altered MEFs gene expression and decreased tumor progression associated with loss of GnT-Va observed may result in part from a combination of effects from these altered gene expressions.

Keywords

Mouse embryo fibroblasts (MEFs); GnT-Va (Mgat5); Knockout; Glycosyltransferase; Galectin; qRT-PCR

1. Introduction

Many studies show that alterations in *N*-linked oligosaccharides of tumor cells are associated with tumorigenesis, progression and metastasis. *N*-linked $\beta(1,6)$ -*N*-acetylglucosamine (GlcNAc) synthesized by *N*-acetylglucosaminyltransferase Va (GnT-Va or Mgat5) is one of the more common glycans up-regulated during malignant transformation [1]. Studies have proposed a close relationship between increased GnT-Va activity and its glycan products with enhanced cell invasiveness, and in some cases metastatic potential [2–4]. In GnT-Va deficient mice, the progression of mammary tumors induced by polyoma middle T oncoprotein (PyMT) expression was significantly suppressed [5].

It is well-documented that modification of cell surface adhesion molecules and growth factor receptors by GnT-Va is implicated in GnT-Va mediated, invasiveness-related phenotypes [2,6–8]. A recent report showed that aberrant expression of GnT-Va caused altered expression of surface sialyl Lewis X (Sle^x), a sialylated fucose-containing antigen related to tumor metastasis, by changing the expression of Sle^x biosynthesis-related

glycosyltransferases [9]. We found that deletion of GnT-Va in mouse embryo fibroblasts (MEFs) causes up-regulation of β 1 integrin transcripts, which consequently increased fibronectin- induced cell adhesion and reduced cell motility [10]. These results suggest that aberrant glycosylation caused by GnT-Va inhibition might regulate the gene expression of other proteins.

To further elucidate the molecular mechanisms whereby GnT-Va-modified asparagine-linked glycans (*N*-glycans) may affect tumor invasiveness-related phenotypes in MEFs and mammary tumor cells [7,10], we explored the effect of genetic disruption of GnT-Va on the expression of other glycosyltransferase and galectin transcripts. We report here, for the first time, that the genetically targeted deletion of GnT-Va causes the altered gene expression of several glycosyltransferases and galectins in MEFs using a newly developed qRT-PCR transcript analysis platform [11]. We speculate that some of the inhibited invasiveness-related phenotypes in MEFs caused by deletion of GnT-Va may result, at least in part, from the combination of altered expression of these glycosyltransferases and/or galectins.

2. Materials and methods

2.1. Antibodies and chemicals

Antibodies against galectin-1 and galectin-3, ERK1/2, and HRP-labeled anti-rabbit IgG and anti-mouse IgG were from Santa Cruz Biotechnology. Monoclonal antibody against β 1 integrin was a product of BD Biosciences. OL.28 anti-polysialic acid antibody was kind gift from Dr. Karen Colley. Streptavidin–HRP and streptavidin–agarose were obtained from Rockland. WGA–agarose and biotinylated lectins were products of Vector Laboratories.

2.2. Isolation of MEFs

Mouse embryo fibroblasts (MEFs) were isolated from E13.5 embryos from heterozygous GnT-Va knock-out mice mating, and the genotype of MEFs was identified by PCR analysis using genomic DNA as described earlier [6].

2.3. GnT-V activity assay

The assay of GnT-V activity was performed using a synthetic trisaccharide acceptor as previously described [2].

2.4. Assay of cell growth and survival

MEFs were harvested, re-suspended in DMEM growth medium and seeded into six-well plates with $2\text{--}3 \times 10^5$ cells/well. Assay of cell growth and survival was performed as previously described [12].

2.5. N-Glycan analysis by HPLC

About 100 mg liver tissue were homogenized and *N*-glycans were released from glycoproteins by digestion of the sample with 10 U PN-Gase-F at 37 °C overnight. Released glycans were fluorescently labeled with 2-aminopyridine (2-AP) as previously reported [13]. Excess 2- AP was removed by gel filtration using a Sephadex G-15 column (1 \times 50 cm) and the 2-AP labeled glycans were fractionated on a 4.6 \times 250 mm TSK Amide-80 column as described previously [14].

2.6. Immuno- and lectin-blotting and lectin pull-down

Cells were harvested, lysed, and 20 μ g of protein was used for immuno- and lectin-blotting [10]. For some experiments, cell lysates were boiled in denaturing buffer containing 0.1% SDS and 1 mM DTT for 5 min and treated with PNGase-F at 37 °C overnight before they

were subjected to lectin-blotting. For lectin pull-down experiments, 500 μg of protein was incubated with 5 μg of a biotinylated lectin or lectin–agarose at 4 °C overnight, followed by addition of agarose–streptavidin.

2.7. Quantitative RT-PCR analysis

Primer pairs for investigated genes and control genes were designed within a single exon using conditions described in Nairn et al. [11] and listed in Table 1. Primers were validated with respect to primer efficiency and single product detection [11].

The RNeasy kit (Qiagen) was used to isolate total RNA from MEFs. One microgram of total RNA was used for a 20 μl cDNA synthesis reaction using the SuperScript III First-Strand Synthesis System (Invitrogen). Reactions were assembled as described [11]. The control gene, Ribosomal Protein L4 (RPL4, NM_024212) was included on each plate to control for run variation and to normalize individual gene expression.

Triplicate cycle threshold (Ct) values for each gene were averaged and the standard deviation was calculated. Samples that resulted in a standard deviation of >0.5 Ct units were re-run until values with standard deviations within an acceptable range were acquired. The logarithmic average Ct value for each gene and the control gene was converted to a linear value using the conversion: $2^{-\text{Ct}}$. Converted values were normalized to RPL4 by dividing the individual gene value by the control gene value. Normalized values were scaled so that genes that were below the level of detection were given a value of 1×10^{-6} and this value was used as the lower limit on histograms. Following normalization and scaling, expression data is reported as the relative transcript abundance for each gene assayed. Error bars represent one standard deviation from the mean value.

3. Results

3.1. Characterization of MEFs from embryos

Compared with wild-type, GnT-Va activity was absent in major tissues from GnT-Va null mice (Fig. 1A). Analysis of 2-AP labeled *N*-linked glycans by HPLC indicated a complete disappearance of the peak representing the asialo tetra-antennary *N*-glycan in liver (Fig. 1B), indicating the disappearance of *N*-linked $\beta(1,6)$ branching caused by the absence of GnT-Va. We have previously observed that GnT-Va null MEFs displayed undetectable GnT-Va activity and lost the typical morphology of a fibroblast when compared to wild-type cells [10]. GnT-Va deficient (KO) and wild-type (WT) MEFs were isolated from 13.5 days embryos. Cell growth in serum-containing medium (Fig. 1C, top) and cell viability in serum-free medium (Fig. 1C, bottom) were measured. GnT-Va null MEFs showed significant decreases in both cell growth and cell survival indicating that the rate of proliferation of MEFs was affected by deletion of GnT-Va.

3.2. Aberrant *N*-glycan expressions in GnT-Va deficient MEFs

To further study *N*-glycans changes caused by deletion of GnT-Va in MEFs, lectin-blotting and -precipitation experiments were performed. The binding of not only leucoagglutinating phytohemagglutinin (L-PHA), but also DSA was extensively suppressed in GnT-Va null MEFs (Fig. 2A), indicating the suppression of both $\beta(1,6)$ branched and poly-*N*-acetylglucosamine structures, respectively. This result confirmed our observation that poly-*N*-acetylglucosamine is synthesized preferentially on *N*-glycans expressing the $\beta(1,6)$ branch [6]. After treatment of cell lysates with PNGase-F, most binding of L-PHA and DSA were abolished, confirming these bound *N*-linked glycans. The expression of either high mannose or biantennary *N*-linked oligosaccharides was not significantly affected by deletion of GnT-Va, as detected by ConA binding (Fig. 2A). Increased E-PHA binding was observed for a

few, but not all proteins from GnT-Va knockout cells (indicated as arrows, Fig. 2A), indicating selective increased expression of bisecting *N*-linked GlcNAc. $\alpha(1,3)$ fucosylation did not appear to be affected, as detected by *Lotus* agglutinin (LTA) binding (data not shown). To study the effect of GnT-Va knockout on sialylation, we used WGA–agarose first to pull-down sialic acid-containing glycoproteins, followed by blotting with MAA, SNA and anti-polysialic acid antibody (OL.28), respectively. Surprisingly, $\alpha(2,6)$ -sialylation detected by *Sambucus nigra* agglutinin (SNA) was increased, while $\alpha(2,3)$ -sialylation and $\alpha(2,8)$ -polysialylation, detected by *Maackia ameurinsin* agglutinin (MAA) binding and OL.28, respectively, were dramatically decreased in GnT-Va null MEFs (Fig. 2B). Consistent with these findings, $\beta 1$ integrin, an abundant surface proteins on MEFs, showed undetectable expression of $\beta(1,6)$ branching in GnT-Va null MEFs, but a significant increase of $\alpha(2,6)$ -sialylation, compared to wild-type cells (Fig. 2C). These results indicate that deletion of GnT-Va caused significant changes in not only $\beta(1,6)$ branching, but also other *N*-glycan expression in MEFs.

3.3. Altered gene expression of glycosyltransferases in GnT-Va null MEFs

To begin a study of the mechanisms by which the different *N*-glycan structures were changed in GnT-Va deficient MEFs, transcript levels of relevant groups of glycosyltransferases, listed in Table 2, were detected by qRT-PCR using total RNA isolated from MEFs. As shown in Fig. 3 and Table 2, altered gene expression patterns were observed for several groups of glycosyltransferases after deletion of GnT-Va. The expression of 7*N*-acetylglucosaminyltransferase (GnT) transcripts was determined (Fig. 3A and Table 2). The expression of GnT-I, GnT-III and GnT-IVb was significantly increased in GnT-Va null cells. GnT-Va was detected in wild-type but not in GnT-Va null cells. GnT-Vb was not detected in either wild-type or GnT-Va null cells. Two other groups of glycosyltransferases that regulate the initiation and extension of *N*-acetylglucosamine chains on glycoproteins and glycolipids, $\beta(1,3)$ -*N*-acetylglucosaminyltransferases ($\beta 3$ GnT) and $\beta(1,4)$ galactosyltransferases ($\beta 4$ GalT), were affected by either up- or down-regulation after deletion of GnT-Va. Among them, $\beta 3$ GnT-III, V and VII were extremely reduced in GnT-Va null MEFs, while $\beta 3$ GnT-I and II and $\beta 4$ GalT-I, II, VI, were increased (Fig. 3B and Table 2).

An expression pattern of either up- or down-regulation was observed within another group of glycosyltransferases, the sialyltransferases (ST), which consist of three specific subfamilies based on their substrate specificity and type of sialyl linkages of the products ($\alpha(2,3)$, $\alpha(2,6)$ and $\alpha(2,8)$). The $\alpha(2,3)$ sialyltransferases (ST3Gals) catalyze the formation of $\alpha(2,3)$ sialylated structures and play an essential role in performing the sialylation of Le^X structure (sLe^X). As shown in Fig. 3C, the expression of ST3Gal-I, III and V were increased, while ST3Gal-IV and VI were reduced after deletion of GnT-Va. Decreased expression of ST3Gal-IV and VI could result in the observed significant inhibition of the biosynthesis of $\alpha(2,3)$ sialylated structures (Fig. 2B). The $\alpha(2,6)$ sialyltransferases (ST6Gals) are another group of the sialyltransferase family and generate $\alpha(2,6)$ -linked sialylated structures. A significant increase in ST6Gal-I transcripts was found in GnT-Va null cells, which was consistent with *N*-glycan changes detected by SNA binding (Fig. 2B). A terminal linear homopolymer of $\alpha(2,8)$ linked sialic acids is another important form of sialylation catalyzed by $\alpha(2,8)$ sialyltransferase (ST8sia), which participates in neural development [15]. Two major ST8sia which are responsible for the biosynthesis of polysialylation (PSA) are ST8sia-II/STX and ST8sia-IV/PST [16,17]. Interestingly, the expression of ST8sia-II/STX was extremely decreased in GnT-Va null MEFs, which was consistent with reduced polysialylation determined by anti-polysialylation antibody binding, whereas the level of ST8sia-IV/PST was undetectable (Fig. 3C). A large decrease of ST8sia-V was also observed in null MEFs, although its expression level was, however, very low.

In addition, deletion of GnT-Va also caused changes in some glycosyltransferase transcripts responsible for the biosynthesis of *O*-glycans. The expression of core2GnT-I, which catalyzes the formation of the branched core2 and core4 *O*-glycans [18] was reduced (Fig. 3D), while core2GnT-II and III expression were very low and undetectable, respectively, in MEFs.

3.4. Altered gene expression of galectins in GnT-Va null MEFs

Galectins (Gal) are a family of carbohydrate-binding proteins with an affinity for β -galactosides [19]. To date, 15 mammalian galectins have been identified. To investigate if deletion of GnT-Va causes altered expression of galectins in MEFs, the transcript expression pattern of galectins was studied. The transcript abundance of Gal-1, Gal-2, Gal-3 and Gal-9 was increased to different degrees, while transcript levels of Gal-4, Gal-6 and Gal-8 were reduced in GnT-Va null MEFs (Fig. 4A and Table 3). Consistent with the transcript expression, Western blotting showed increased protein expression of both Gal-1 and Gal-3 (Fig. 4B), the two most abundant galectins in mouse, in GnT-Va null MEFs.

4. Discussion

In the present study, we isolated MEFs from GnT-Va knockout mice and explored the effects of genetic disruption of one glycosyltransferase, GnT-Va, on the gene expressions of other glycosyltransferases and galectins. We showed that deletion of GnT-Va had marked effects on MEFs growth on plastic and cell survival. Supporting these observation, the phosphorylation of both ERK and PKB was significantly inhibited in GnT-Va null mammary tumor cells and MEFs [5,7], suggesting a possible role of GnT-Va in regulating cell growth and apoptosis.

Consistent with undetectable activity of GnT-Va in the null MEFs, we found that the expression of $\beta(1,6)$ branched oligosaccharides and poly-*N*-acetylglucosamine, determined by L-PHA and DSA binding, respectively, were almost entirely inhibited after deletion of GnT-Va in MEFs. Interestingly, “bisected” *N*-linked glycans produced by GnT-III, and recognized by E-PHA binding, were increased in GnT-Va null MEFs, consistent with the possible competition between GnT-III and GnT-Va for biantennary substrates during glycoprotein biosynthesis in the Golgi [20]. Of note, significant decreases in $\alpha(2,3)$ sialylation and $\alpha(2,8)$ polysialylation were observed after deletion of GnT-Va, reflecting changes in enzymatic activity of both $\alpha(2,3)$ sialyltransferases and $\alpha(2,8)$ polysialyltransferases. A significant increase in $\alpha(2,6)$ sialylation, detected by SNA binding was observed in GnT-Va null cells, indicating increased activity of ST6Gal resulting from deletion of GnT-Va. This result was consistent with our recent finding that knockdown of GnT-Va by siRNA led to increased $\alpha(2,6)$ sialylation expression on cell surface EGFR in human mammary tumor cells [8]. Supporting these findings, a recent report [21] demonstrated that targeted deletion of *Mgat4a* also caused absence of *N*-linked $\beta(1,6)$ branching on *Glut-2* of pancreatic islet β cells. These results suggested that genetic disruption of one glycosyltransferase could affect others changes in glycan expression.

To further investigate if altered *N*-glycan structures resulted from changes in gene expression of different glycosyltransferases, transcript levels of different families of glycosyltransferases were evaluated using qRT-PCR. In the family of GnTs, several transcripts were up-regulated due to the deletion of GnT-Va. Increased gene expression of GnT-III may account for the enhanced E-PHA binding in GnT-Va null MEFs, indicating for the first time that the opposing expression pattern of GnT-Va and GnT-III glycan products may be due to regulation of these enzymes at the level of transcription. This result is further supported by our recent observation that increased gene expression of GnT-Va was accompanied by reduced GnT-III gene expression in Her-2/neu-induced mouse mammary

tumor tissue (unpublished data), indicating that these two genes might be regulated by an opposing manner.

Poly-*N*-acetylglucosamine is synthesized preferentially on *N*-glycans expressing the $\beta(1,6)$ branch. In our study, we found that poly-*N*-acetylglucosamine showed a concomitant decreased expression when *N*-linked $\beta(1,6)$ branches were eliminated by knockout of GnT-Va. Although some of the transcript levels of glycosyltransferases which are involved in the biosynthesis of poly-*N*-acetylglucosamine were extremely down-regulated, enhanced gene expression within this family was also observed. Reduced expression of poly-*N*-acetylglucosamine, detected by DSA binding likely resulted, at least in part, from altered gene expression of relevant glycosyltransferases.

Sialylation is another important modification of glycoproteins and aberrant sialylation has been reported to relate to the progression of several types of human tumors [22,23]. In mammalian cells, six $\alpha(2,3)$ sialyltransferases (ST3Gal I–VI) have been identified. Down-regulation of ST3Gal IV and VI was observed in GnT-Va knockout MEFs, which likely accounts for the significant decrease in expression of $\alpha(2,3)$ sialylation detected by MAA binding.

The transcripts for ST6GalTs, forming $\alpha(2,6)$ sialylation structures, were significantly up-regulated in GnT-Va deficient MEFs, which is consistent with the observed increased expression of $\alpha(2,6)$ sialylation, detected by SNA binding, suggesting that elimination of GnT-Va expression negatively regulate the formation of $\alpha(2,6)$ sialylation. This hypothesis was further supported by our recent observations that knockdown of GnT-Va caused increased $\alpha(2,6)$ sialylation on EGFR [8], and enhanced gene expression of GnT-Va in Her-2/neu induced mammary tumor tissue was accompanied by down-regulation of ST6GalT (unpublished data). Indeed, $\alpha(2,6)$ sialylation of cell surface *N*-glycans was reported to inhibit tumor formation in vivo [24] and attenuate the neoplastic phenotype of human colon cancer cells [22], indicating an opposing role of increased ST6GalT, compared to increased GnT-Va, in mediating tumorigenesis.

Polysialic acid (PSA) is another post-translational modification consisting of a homopolymer of $\alpha(2,8)$ linked sialic acid, which plays a critical role in regulating neural development and tumor progression [15]. Dramatically, concomitant with a marked reduction of polysialylation detected by anti-poly-sialylation antibody, we found that the transcript levels of ST8Sia II/STX were significantly reduced after deletion of GnT-Va, while the level of ST8Sia IV/PST was very low. N-CAM is a major carrier of PSA [25], and the presence of PSA attenuates the adhesive property of N-CAM and increases cell motility. Decreased expression of PSA caused by down-regulation of ST8Sia II/STX transcripts after deletion of GnT-Va may, therefore, lead to a phenotype with increased cell adhesive properties and decreased cell motility. This hypothesis is consistent with inhibited cell growth and supportive of previous findings that GnT-Va null MEFs and tumor cells showed decreased cell motility [7,10].

Intriguingly, deletion of GnT-Va also affected the transcript expression level of core2GnT-I, a Golgi enzyme that catalyzes the formation of $\beta(1,6)$ branched structure attached to *O*-GalNAc [18]. This enzyme was found to be expressed in the developing mouse embryo and was closely correlated with vessel invasion and depth of invasion of several human cancers.

Among 15 identified mammalian galectins, Gal-1 and -3 have been intensively studied for their roles in regulating cell proliferation, apoptosis and tumor progression [26,27]. In our study, we found that knockout of GnT-Va also resulted in increased transcript levels of Gal-1 and Gal-3, accompanied by increased protein expression detected by Western blotting.

Gal-9 was originally identified as a tumor antigen and is associated with suppression of cell growth and induces apoptosis. Increased Gal-1, -3 and -9 may be related to the inhibited cell growth and survival of GnT-Va null MEFs observed in our study. Of note, gene expression of Gal-1 was inhibited in Her-2/neu-induced mouse mammary tumor tissue and cells [28], suggesting that up-regulated gene expression of Gal-1 after deletion of GnT-Va may function in GnT-Va-mediated mammary tumor progression[5]. Among the down-regulated galectins, Gal-8 showed relatively higher expression in GnT-Va null MEFs. This galectin has been reported to be a matrix protein that can positively or negatively modulate cell adhesion [29] and its down-regulation may, therefore, contribute to some of the altered cell adhesion properties caused by deletion of GnT-Va in MEFs [10].

The altered gene expression of several groups of glycosyltransferases and galectins caused by genetic disruption of one specific glycosyltransferase has not as yet been reported. Studies have shown that knockout or knockdown of GnT-Va caused aberrant *N*-glycosylation of surface growth factor receptors and are associated with altered signaling pathway mediated by these receptors [7,8,10,30]. These aberrant signals might up- or down-regulate gene expression patterns of some glycosyltransferases and glycan-recognition proteins. We did find that knockout of GnT-Va in MEFs caused increased gene expression of β 1 integrin [10]. Furthermore, increased gene expression of some integrins was also observed in SH-SY5Y cells after GnT-Vb knockdown by siRNA [31]. These findings suggest that the expression level of GnT-Va indeed played a role in regulating expression of other proteins. In addition, it is believed that the galectin binding increases if galactose is attached to an *N*-acetylglucosamine of glycoproteins on the cell surface forming *N*-acetyllactosamine [32]. A recent paper shows that galectins cross-link glycoproteins on the cell surface with avidities dependent on *N*-glycan structure and number [30]. Knockout of GnT-Va caused reduced expression of poly-*N*-acetyllactosamine structures, preferred ligands of the galectins, which could possibly render feedback regulation on the expression of some galectins, causing increased gene expression of Gal-1 and Gal-3. In conclusion, our results suggest possible coordination between GnT-Va expression and the expression of several glycosyltransferases and lectins, although the mechanisms that result in this coordination are, as yet, unknown.

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Abbreviations

MEFs	mouse embryo fibroblasts
GnT-Va	<i>N</i> -acetylglucosaminyltransferase Va
GlcNAc	<i>N</i> -acetylglucosamine
<i>N</i>-glycan	asparagine-linked glycans
L-PHA	leucoagglutinating phytohemagglutinin
qRT-PCR	quantitative reverse transcriptase-PCR

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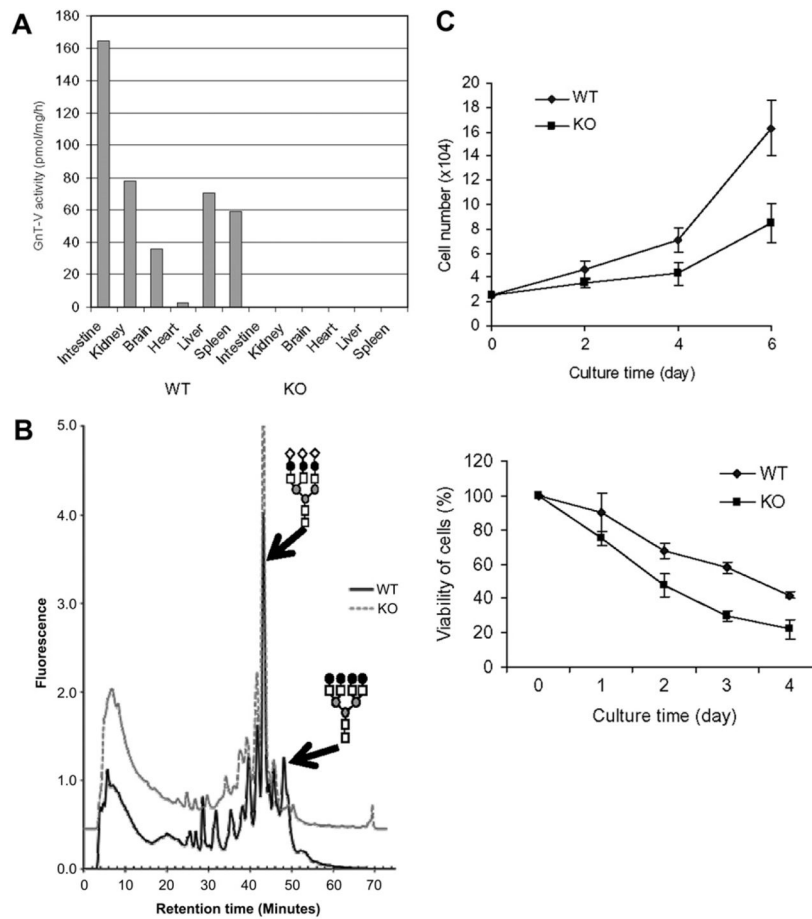


Fig. 1. Isolation of mouse embryonic fibroblasts (MEFs) from GnT-Va null mice. (A) Mouse tissues were collected, homogenized, and GnT-Va activities determined. (B) *N*-Glycans were released from liver tissue and fluorescently labeled with 2-PA, *N*-glycan structures were characterized by HPLC. *N*-glycan structural symbol: tri-antennary *N*-glycan (top) and tetra-antennary *N*-glycan (bottom). (C) (top) growth curve of MEFs cultured in serum-containing medium; (bottom) viability of cells cultured in serum-free medium. Each point represents the mean value of cell number from three independent experiments. WB: Western blotting.

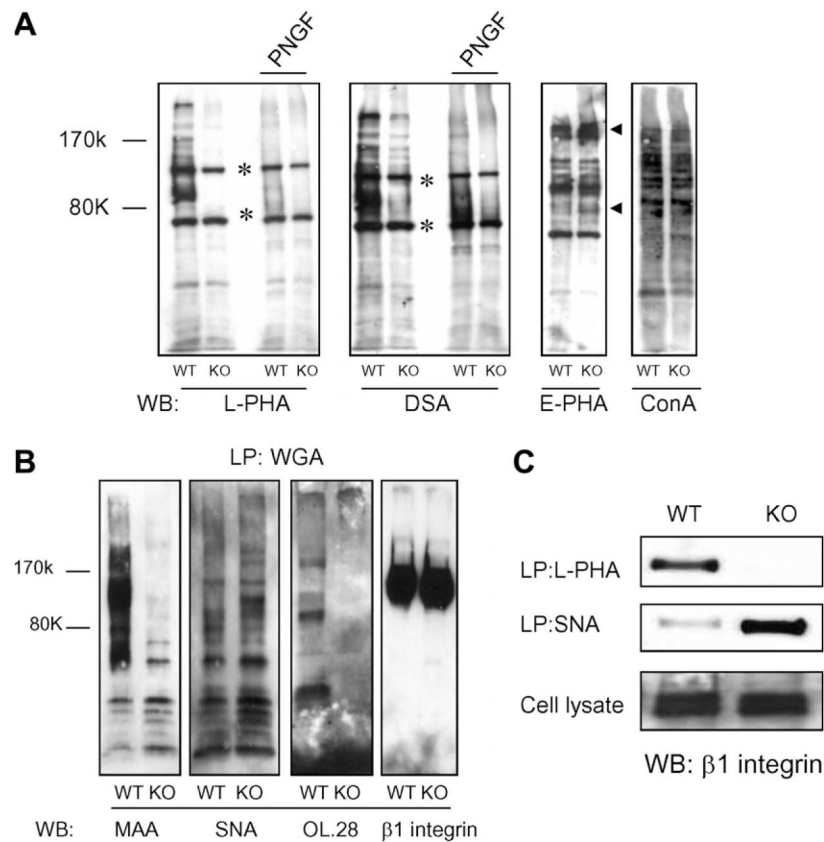


Fig. 2. Deletion of GnT-Va caused altered *N*-glycan expression. (A) *N*-Glycan structures were detected using cell lysates (with or without PNGase-F pre-treatment) by lectin-blotting. *Non-specific binding. The small arrows indicated increased binding proteins. L-PHA, leucoagglutinating phytohemagglutinin; DSA, *D. stramonium* agglutinin; E-PHA, erythroagglutinating phytohemagglutinin; ConA, concanavalin A; PNGF, PNGase-F; WB:Western blotting. (B) Glycoproteins were lectin precipitated (LP) from cell lysates using WGA-agarose, followed by blotting with lectins and antibodies. Anti- β 1 integrin was used for loading control. WGA, wheat germ agglutinin; MAA, *M. amurensis* agglutinin; SNA, *S. nigra* agglutinin; OL.28, anti-polysialic acid antibody. (C) Cell lysates were subjected to L-PHA and SNA precipitation (LP), respectively, followed by SDS-PAGE, blotting, and detection using antibody against β 1 integrin (top two panels). Immunoblots of cell lysates with anti- β 1 integrin were performed as control to confirm amount of precipitated β 1 integrin in both cell lines (bottom panel).

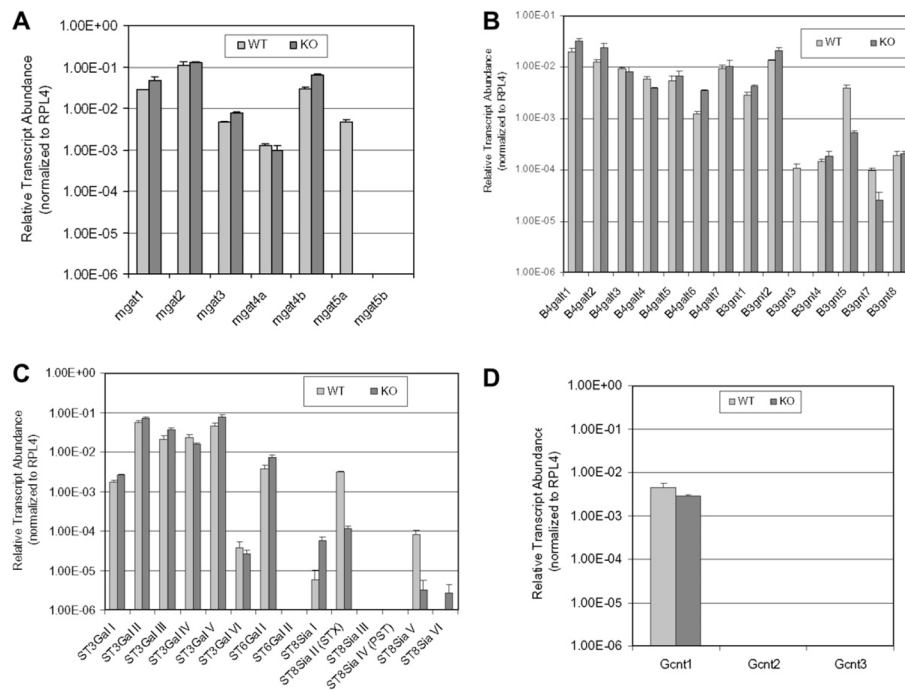


Fig. 3. Deletion of GnT-Va resulted in altered gene expression of glycosyltransferases. (A) Expression of several *N*-acetylglucosaminyltransferases (*gnt*). (B) Relative transcript levels of β 4-galactosyltransferases (B4galt 1–7) and β 3-*N*-acetylglucosaminyltransferases (B3gnt 1–8). (C) Relative transcript levels of α 2,3-sialyltransferases (ST3Gal I–VI), α 2,6-sialyltransferases (ST6Gal I–II) and α 2,8-sialyltransferases (ST8Sia I–VI). (D) Relative transcript levels of core2*N*-acetylglucosaminyltransferase (Gcnt1), I-branching *N*-acetylglucosaminyltransferase (Gcnt2), mucin-type *N*-acetylglucosaminyltransferase (Gcnt3). Data expressed as “relative transcript abundance” are plotted on a log scale for each gene assayed. Error bars represent one standard deviation from the mean of triplicate values.

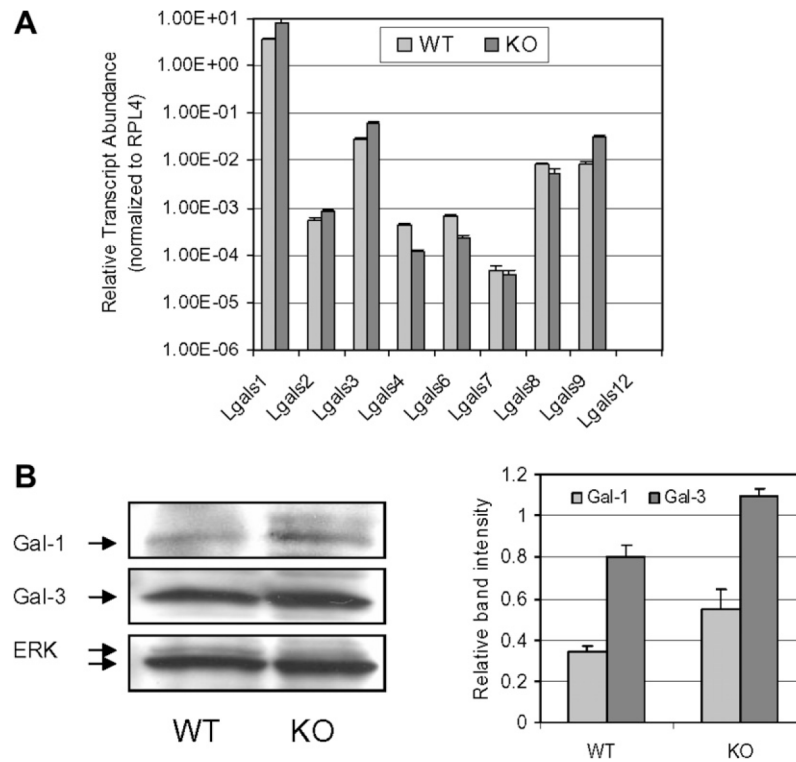


Fig. 4. Deletion of GnT-Va resulted in altered galectins gene expression. (A) Relative transcript levels for galectins: galectin 1 (Lgals1), galectin 2 (Lgals2), galectin 3 (Lgals3), galectin 4 (Lgals4), galectin 6 (Lgals6), galectin 7 (Lgals7), galectin 8 (Lgals8), galectin 9 (Lgals9) and galectin 12 (Lgals12). (B) The expression levels of galectins-1 and -3 and ERK in MEFs were detected by immunoblotting using the respective antibodies (left panel). Relative levels of gal-1 and -3 were calculated by band intensity of gal-1 or gal-3/intensity of ERK, and data represent the mean (\pm S.D.) of three independent experiments (right panel).

Table 1

Sequences of primers used in qRT-PCR

Gene	Abbreviation	Accession number	Forward primer (5'-3')	Reverse primer (5'-3')
Mannoside acetylglucosaminyltransferase 1	Mgat1	NM_010794	CCCTTCACCCAGTTGGACCTG	GCACATAGACCTGGGCGGAG
Mannoside acetylglucosaminyltransferase 2	Mgat2	NM_146035	TGCTGGAGACTGTGGTATGC	ACTCAATTTGGGCACTCTGG
Mannoside acetylglucosaminyltransferase	Mgat3	NM_010795	GCGTGATGGTGTGTGTTC	ACAGGGACTTCCGATGTGG
Mannoside acetylglucosaminyltransferase 4, isoenzyme A	Mgat4a	NM_173870	GCGACAGACAGAAAGGCAAAACC	CCGACAGACAGAGTGTAGGC
Mannoside acetylglucosaminyltransferase 4, isoenzyme B	Mgat4b	NM_145926	AGGTGACCTGGTGGACATTT	GCITCAGGCTCTCTTGGCTCA
Mannoside N-acetylglucosaminyltransferase Va	Mgat5a	NM_145128	CCCTGGAAAGTTGTCTCTCA	TCCTCTGCCAGTGCCTTAAT
Mannoside acetylglucosaminyltransferase 5, isoenzyme B	Mgat5b	NM_172948	TGCCCTGTGACAGCACTGAG	GTAGGTAGCACTCTTGGCCGG
Glucosaminyl (N-acetyl) transferase 1, core2	Gent1	NM_173442	CGAAGGCCATGTTTCCAAACGG	TCCGAAAGACGCACACAGAGC
Glucosaminyltransferase, I-branching enzyme	Gent2	NM_008105	CGGTTGATTTGCTCCATTG	TATTGAGCGTCACCCAGAAAT
Glucosaminyl (N-acetyl) transferase 3, mucin type	Gent3	NM_028087	ACCCAAGGCTCTGCTGAAATAA	AGGTAGTCGGCCCTCTGTGAA
Beta-1,3-glucuronyltransferase 1 (glucuronosyltransferase P)	B3gat1	NM_029792	ACCGTGACATCGTGGAAAGTGG	GGATGGTGGCAGCGGTGC
Beta-1,3-glucuronyltransferase 2 (glucuronosyltransferase S)	B3gat2	NM_172124	CACACTGGACAGAGAAGGTC	CCTCAATGTTCACTGTGTCCA
UDP-Gal:betaGlcNAc-beta-1,4-galactosyltransferase, polypeptide 1	B4gal1	NM_022305	GGCGTCACCCTCGTCTATTA	GCCCTGCAGTGTAGAGGAGA
UDP-Gal:betaGlcNAc-beta-1,4-galactosyltransferase, polypeptide 2	B4gal2	NM_017377	GCCGCTAITCTCCACCCGAC	GGTGCTCCCGGTGCTAAAAGG
UDP-Gal:betaGlcNAc-beta-1,4-galactosyltransferase, polypeptide 3	B4gal3	NM_020579	TGAAGAATGGGACTGCTTGT	TCCGACACATACAGGTTATGG
UDP-Gal:betaGlcNAc-beta-1,4-galactosyltransferase, polypeptide 4	B4gal4	NM_019804	GACGTGGACCTGGTGCCTG	GTTCGCGCCCAACCCCAAG
UDP-Gal:betaGlcNAc-beta-1,4-galactosyltransferase, polypeptide 5	B4gal5	NM_019835	CGGTCTCTCTGAGACACCT	CACATAGAAGGGCAACTGCA
UDP-Gal:betaGlcNAc-beta-1,4-galactosyltransferase, polypeptide 6	B4gal6	NM_019737	CGGCACACAACCAATCAAT	ATCACACAGTCCCAGGCTCT
Xylosylprotein beta1,4-galactosyltransferase, polypeptide 7	B4gal7	NM_146045	CGGGCAGCACTCATCAATGTG	GAGGGAGCAGGTCCACATCG
UDP-GlcNAc:betaGal-beta-1,3-N-acetylglucosaminyltransferase 1	B3gnt1	NM_175383	GGAGAGCTTGTGAGACCTG	CCTTTCCTCCAGCCACATAA
UDP-GlcNAc:betaGal-beta-1,3-N-acetylglucosaminyltransferase 2	B3gnt2	NM_016888	TTAGGTGGGTGAGCACTTCC	GATGGGTGTTACAAAACACG
UDP-GlcNAc:betaGal-beta-1,3-N-acetylglucosaminyltransferase 3	B3gnt3	NM_028189	TCCTTCTTGTGGGCTCAGAC	GTAITTTCTGCGCTTCCAGCT
Beta-1,3-N-acetylglucosaminyltransferase-4	B3gnt4	NM_198611	CCAGCCGTCATCGCCTTTC	CACACTCCGAAGGCTCCAGC
UDP-GlcNAc:betaGal-beta-1,3-N-acetylglucosaminyltransferase 5	B3gnt5	NM_052993	GCAAGGCATTCAGATGACAA	ATGTTGGCTGGAAATCTGCAT
UDP-GlcNAc:betaGal-beta-1,3-N-acetylglucosaminyltransferase 7	B3gnt7	NM_145222	GGGCCACGAAITGGGAATCAG	TCCTCTTGTGTGGAGGCTGTG
UDP-GlcNAc:betaGal-beta-1,3-N-acetylglucosaminyltransferase 8	B3gnt8	NM_146184	CGGGCTATGGTGAACCTACTG	TCAGCAGCAGCAGGCTCTTG
Sialyltransferase 4A (beta-galactosidase alpha-2,3-sialyltransferase)	St3gal1	NM_009177	TCCTACAACATGCACAGCGTCCG	TGTTTCGCCCTGGTGCCTGG
Sialyltransferase 5 (N-acetylglucosaminide alpha 2,3-sialyltransferase)	St3gal2	NM_178048	GCTCTCTTCGCGGTGTGTTC	ATGCTGTGTGCGGAGTAGGTG
Sialyltransferase 6 (N-acetylglucosaminide alpha 2,3-sialyltransferase)	St3gal3	NM_009176	CAGCAAGAAACCCAGACCCAT	ATGAATGGCTCCCTCCATAG

Gene	Abbreviation	Accession number	Forward primer (5'-3')	Reverse primer (5'-3')
Sialyltransferase 4C (beta-galactosidase alpha-2,3-sialyltransferase)	St3gal4	NM_009178	GCTCCTGTGGCTGGCTACG	GGGTCAAAAGTGGGCCGACTC
Sialyltransferase 9 (CMP-NeuAc:lactosylceramide alpha-2,3-sialyltransferase)	St3gal5	NM_011375	AGCCTCTTGGGATATGCTGCC	CGTTCCCAACAAACACACACAGC
Sialyltransferase 10 (alpha-2,3-sialyltransferase VI)	St3gal6	NM_018784	ATGGTGGCATTCCCGTAGTA	AAGTGCACCTCGCTGGTTT
Sialyltransferase I (beta-galactoside alpha-2,6-sialyltransferase)	St6gal1	NM_145933	TGCGTGTGGAAGAAAGGGAGC	CTCCTGGCTCTTCGGCATCTG
Beta-galactoside alpha-2,6-sialyltransferase II (ST6GalII-pending)	St6gal2	NM_172829	AGCAATCCTCGGGCACCTATG	CCGCTGCTTGGCCCTGTAGAG
Sialyltransferase 8A (alpha-2,8-sialyltransferase)	St8sia1	NM_011374	TTTTGGTGAAGTGCAGCCTTGGG	TGCATGTTTCAACCGAGAAAGGGC
Sialyltransferase 8B (alpha-2,8-sialyltransferase)	St8sia2, STX	NM_009181	CGGCCTCAATGGGAGCATCC	AGCATTGACCCCACTCCACACG
Sialyltransferase 8C (alpha-2,8-sialyltransferase)	St8sia3	NM_009182	TCCAACACAGCACAACACAT	AATAAATTCCTCCGGTCCCTGCT
Sialyltransferase 8D (alpha-2,8-sialyltransferase)	St8sia4, PST	NM_009183	CCTAGCACAGGTCTCCTCATG	GGAAATGGCCAGAATCCATA
ST8 alpha-N-acetylneuraminidase alpha-2,8-sialyltransferase 5	St8sia5	NM_153124	CCTGGTATCCTTCCGAGTCA	TGGGGTGGAAAGAGTAGAC
Alpha-2,8-sialyltransferase VI	St8sia6	NM_145838	GTTGATGGAGGGAAAGCAGAG	GGGCTCCAGAGTGTCTTTCAG
Lectin, galactose binding, soluble 1	Lgals1	NM_008495	CAGCAACAACCTGTGCCTAC	ACAATGGTGTGGCGTCTC
Lectin, galactose binding, soluble 2	Lgals2	NM_025622	AAGAAACCCCTCAACCTGCATT	GGCCACCTTCACTGGTGTTA
Lectin, galactose binding, soluble 3	Lgals3	NM_010705	CCTTGCCTGGAGGAGTCAT	TGTTTGGCTGGGTTTTCAC
Lectin, galactose binding, soluble 4	Lgals4	NM_010706	TCGTCCGGAGACATAGCTTT	CATAAAGCTGTTGCCAAACA
Lectin, galactose binding, soluble 6	Lgals6	NM_010707	CAACTTCTCCCATCGGTTCC	TGAGGTCACCATTGATCTCCA
Lectin, galactose binding, soluble 7	Lgals7	NM_008496	CTATGCGGTGAGGAGCAAG	ACAACCTCGGAAGTGTCCAG
Lectin, galactose binding, soluble 8	Lgals8	NM_018886	GACTTTCAGCTGGGCAACA	TGAAACCGAGGGTTAAAGTGG
Lectin, galactose binding, soluble 9	Lgals9	NM_010708	CCGTTTCAATGAGAAATGCTG	AGCAGACTTCGCTCTTCTCTG
Lectin, galactose binding, soluble 12	Lgals12	NM_019516	TCAGCCAGATGTTGCCTTC	GTGGGTGTTGCAGATGACAT
Ribosomal Protein L4 ^a	RPL4	NM_024212	GACAGCCCTATGCCCGTCAAGT	GCCACAGCTCTGCCCACTACC

^aNormalization control gene.

Table 2

Altered gene expression profile of glycosyltransferases in GnT-Va null MEFs

Gene	WT (mean) ^a	KO (mean) ^a	Ratio (WT/KO)
Mgat1	0.027673	0.046369	0.596791
Mgat2	0.108606	0.124820	0.870100
Mgat3	0.004548	0.007684	0.591858
Mgat4a	0.001230	0.000953	1.290909
Mgat4b	0.029364	0.063232	0.464377
Mgat5a	0.004605	0.000001	4605.313126
Mgat5b	0.000001	0.000001	1.000000
Gcnt1	0.004553	0.002759	1.650467
Gcnt2	0.000001	0.000001	1.000000
Gcnt3	0.000001	0.000001	1.000000
B4galt1	0.019953	0.032838	0.607618
B4galt2	0.012594	0.024487	0.514288
B4galt3	0.009568	0.008144	1.174870
B4galt4	0.005826	0.003895	1.495915
B4galt5	0.005516	0.006892	0.800403
B4galt6	0.001240	0.003591	0.345244
B4galt7	0.009342	0.010353	0.902375
B3gnt1	0.002855	0.004278	0.667305
B3gnt2	0.013662	0.020811	0.656500
B3gnt3	0.000110	0.000001	110.138458
B3gnt4	0.000144	0.000184	0.781529
B3gnt5	0.003916	0.000536	7.311063
B3gnt7	0.000098	0.000027	3.681326
B3gnt8	0.000195	0.000205	0.948217
St3gal1	0.001698	0.002569	0.660952
St3gal2	0.057057	0.070288	0.811768
St3gal3	0.021313	0.036801	0.579139
St3gal4	0.023461	0.015987	1.467521
St3gal5	0.044011	0.077479	0.568035
St3gal6	0.000038	0.000026	1.446651
St6gal1	0.003774	0.007324	0.515373
St6gal2	0.000001	0.000001	1.000000
St8sia1	0.000006	0.000056	0.103812
St8sia2, STX	0.003064	0.000116	26.359330
St8sia3	0.000001	0.000001	1.000000
St8sia4, PST	0.000001	0.000001	1.000000
St8sia5	0.000078	0.000003	23.826403
St8sia6	0.000001	0.000003	0.376690

0.000001 = below the level of detection.

The ratio is a simple division of the data for WT and KO for a specific gene.

^aRelative transcript abundance (as described in Section 2).

Table 3

Altered gene expression profile of galectins in GnT-Va null MEFs

Gene	WT ^a	KO ^a	Ratio (WT/KO)
Lgals1	3.569301	7.843389	0.455071
Lgals2	0.000560	0.000838	0.668573
Lgals3	0.027261	0.062521	0.436029
Lgals4	0.000433	0.000118	3.661759
Lgals6	0.000673	0.000227	2.963587
Lgals7	0.000048	0.000038	1.282823
Lgals8	0.008210	0.005495	1.494213
Lgals9	0.008108	0.031355	0.258580
Lgals12	0.000001	0.000001	1.000000

0.000001 = below the level of detection.

The ratio is a simple division of the data for WT and KO for a specific gene.

^aRelative transcript abundance (as described in Section 2).