

Review

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Martina Duca , [Nadia Malagolini](#) , [Fabio Dall'Olio](#) *

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Review

The Sd^a Carbohydrate Antigen and its Biosynthetic Enzyme B4GALNT2 in Health and Disease

Martina Duca, Nadia Malagolini and Fabio Dall'Olio *

Department of Medical and Surgical Sciences (DIMEC), University of Bologna, General Pathology Building,
Via San Giacomo 14, 40126, Bologna, Italy

* Correspondence: fabio.dallolio@unibo.it; Tel.: +390512094704

Abstract: The carbohydrate antigen Sd^a is expressed on cells and secretions of the vast majority of Caucasians. The epitope is formed by a terminal GalNAc residue β 4-linked to a α 3-sialylated galactose. Different carbohydrate chains *N*- or *O*-linked to glycoproteins can be terminated by this epitope. The final step of Sd^a biosynthesis is catalyzed by the GalNAc transferase B4GALNT2. In this review, we will discuss the multifaceted aspects of B4GALNT2/Sd^a biology. In particular, we will see its implications in fertility and pregnancy, in susceptibility to infectious diseases, in chronic kidney diseases and in Duchene muscular dystrophy. In particular, we will show its regulation and prognostic implications in cancer.

Keywords: Sd^a antigen; B4GALNT2; uromodulin; Tamm-Horsfall glycoproteins; influenza virus; Duchene muscular dystrophy

1. Introduction

About 96% of Caucasians display the carbohydrate antigen Sd^a on the erythrocytes, secretions [1] and a few organs [2]. This antigen, which behaves as a dominant genetic character, was discovered independently by two groups [3,4]. Among the 4% Sd^a-negative individual, only a few contains “natural” anti Sd^a antibodies in serum. This is in sharp contrast with the AB0 blood group system in which all individuals form “natural” antibodies against the antigens they don't express. A stronger version of the Sd^a antigen, known as the Cad antigen, reacts more strongly with anti-Sd^a antibodies [5]. The structural and molecular differences between Cad and Sd^a are still not clear [6].

Basically, the structure of the Sd^a epitope is composed by a α 2,3-sialylated galactose substituted by a β 1,4-linked GalNAc [7]. However, the epitope can be found at the end of different sugar structures, including type 1 and type 2 lactosaminic chains, as well as core 1, core 2 and core 3 *O*-linked structures [8] and the glycolipid sialosylparagloboside [9] (Figure 1).

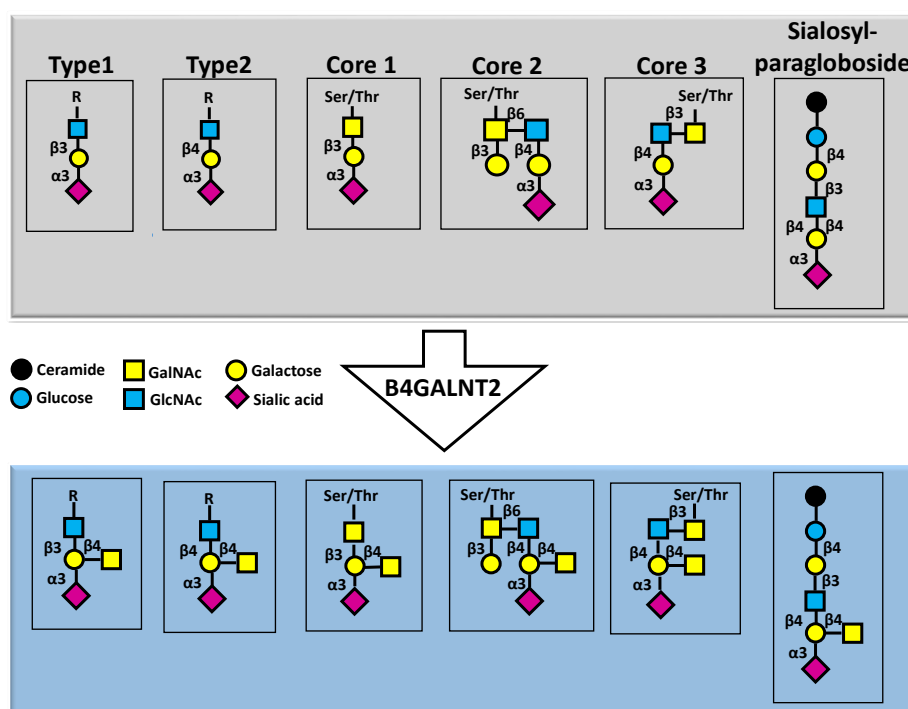


Figure 1. Structure of the Sd^a antigen. Different carbohydrate structures of glycoproteins (first four structure) as well as the glycolipid sialosylparagloboside, all terminated by a sialic acid α 3-linked to galactose (upper grey panel), can be substrates of B4GALNT2, which synthesizes the Sd^a antigen on these chains (lower light blue panel).

The UDP-GalNAc β 1,4-N-acetylgalactosaminyltransferase 2 (B4GALNT2), encoded by the *B4GALNT2* gene is the only enzyme able to catalyze the final step of Sd^a biosynthesis. The enzymatic activity was first detected in Guinea-pig kidney [10] and found to require the presence of a sialic acid α 2,3-linked to galactose in the acceptor. The mouse *B4galnt2* cDNA [11] and the human *B4GALNT2* cDNA were successively cloned [12,13]. The human *B4GALNT2* maps on 17q21.33, it is formed by at least 12 coding exons and generates transcripts diverging in their 5'- and 3'-UTR (Figure 2). The presence of two alternative 5'-UTR is particularly relevant. In fact, the alternative use of two distinct first exons, both provided with a translation start codon, results in two polypeptides with different amino-terminal portions [12,13]. The mRNA forms containing either exon 1 long (1L) or exon 1 short (1S) encode for 566 aa or 506 aa long B4GALNT2 proteins. The long form is characterized by a cytoplasmic tail of 67 aa, which is among the longest among glycosyltransferases. Both isoforms are mainly localized in the Golgi. However, the long form displays also post-Golgi vesicles and plasma membrane localization [14]. B4GALNT2 protein contains two unconventional N-glycosylation sites. The first is occupied by a complex type chain, which is necessary for stability, proper intracellular localization and homodimer formation [15].

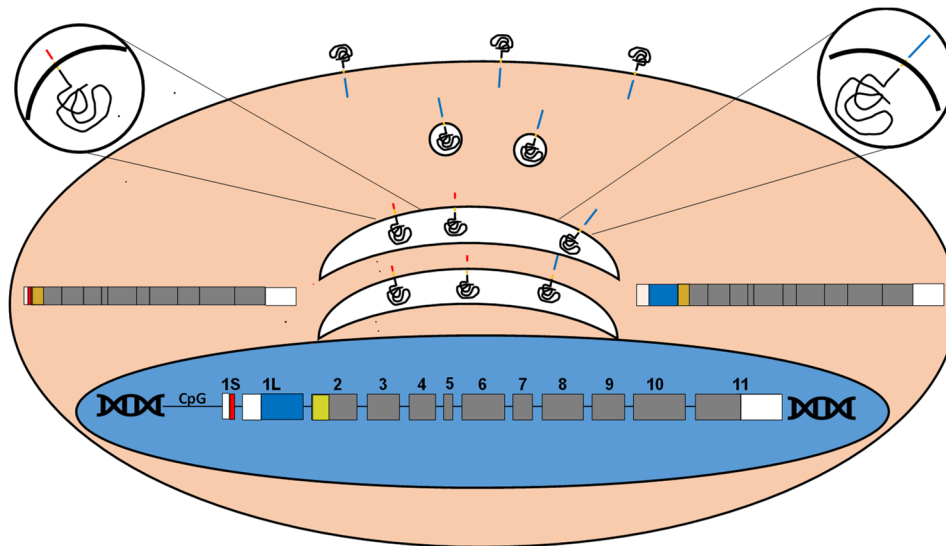


Figure 2. The *B4GALNT2* gene, transcripts and proteins. The *B4GALNT2* genomic organization, spanning at least 12 exons is depicted. The coding regions of the short and long forms of exon 1 are depicted in red and blue, respectively, while the 5'- and 3'UTRs are in white. The transmembrane domain is in yellow. Transcription of the two exons generates two polypeptides with different aminoterminal (cytoplasmic) domains, depicted in red and blue, respectively. Both localize mainly in the Golgi apparatus (white), while a portion of the long form localize also in post-Golgi vesicles (white) and the plasma membrane. The insets represent enlarged sections of the Golgi with a short form (left) and a long form (right). The cytoplasmic portions in red and blue are not drawn to scale.

The regulation of *B4GALNT2* expression is a complex issue. The genomic sequences upstream of the transcription start site(s) display the features of a CpG island and CpG shores. Methylation of these sequences can block *B4GALNT2* expression [16,17]. However, promoter methylation does not appear to be an all-or-none switch. In fact, data from The Cancer Genome Atlas (TCGA) reveal that the methylation status of specific sites in the promoter and inside the gene is more predictive of the expression level than methylation as a whole [18,19]. TCGA data have revealed also a potentially regulatory role of miR-204-5p [19]. Another crucial role in *B4GALNT2* regulation can be played by transcription factors. The transcription factors ETS1 and, to a lesser extent, SP1 are necessary for gene transcription, although their different expression does not appear to be responsible for *B4GALNT2* modulation in pathological conditions, such as colon cancer (see below) [20].

Despite the fact that a small but significant percentage of population is *Sd^a/B4GALNT2* negative without overt phenotypic consequences, the *Sd^a* antigen is involved in a broad variety of physio-pathological phenomena in humans and animals. The present review aims to focus on these physio-pathological phenomena, while a comprehensive history of the *Sd^a* antigen discovery has been published previously [8,21].

2. Why Some People are *Sd^a*?

Genomic sequencing of the *B4GALNT2* gene of nine *Sd^a* individuals revealed in six of them a homozygous p.Cys466Arg substitution in the enzymatically active domain. In two other persons, the p.Gln436Arg was associated either with a p.Arg523Trp substitution or with a splice site mutation [22]. Transfection of the p.Cys466Arg mutant form failed to turn a *Sd^a* cell line into a *Sd^{a+}* status. Unexpectedly, both p.Gln436Arg and p.Arg523Trp mutant forms induced a *Sd^a* expression level comparable with wild type [23]. Another important question regards the origin of the *Cad* status. Reasonably, it is possible that differences in the regulatory regions of the *B4GALNT2* gene allow a stronger expression in these individuals. However, neither the coding sequence nor the 2000 bp upstream genomic region of the *B4GALNT2* gene in 5 *Cad^{a+}* individuals revealed common alterations

potentially accounting for their Cad status [23]. In conclusion, some of the Sd^a phenotypes are due to mutations in the coding sequence of B4GALNT2, but the origin of other cases remains obscure [6].

3. Which are the Evolutionary Forces Which Led to the Selection of the Sd^a Phenotypes?

The answer to this question involves at least two systems: 1) resistance to microorganism infection and 2) regulation of proliferacy.

3.1. How B4GALNT2/Sd^a Regulate Microbe Infections

3.1.1. Viral Infections

Viruses often employ sialylated carbohydrate structures present on the cell surface of host cells as receptors for their sugar binding proteins. A very good example of these mechanisms is represented by the influenza viruses which are provided with a viral lectin called hemagglutinin (H) which binds to sialic acid residues on host cell glycoconjugates. The various influenza virus strains and their tropism for species and tissues are distinguished according to their different H molecules (H1, H2....) with different specificities for α 2,3- or α 2,6-linked sialic acids. The CRISPR synergistic activation mediator (CRISPR SAM) technology, is an adaptation of the CRISPR/Cas9 technology which, by recruiting transcriptional activators, allows to perform genome-wide gene overexpression screens. This approach has identified B4GALNT2 as the major factor whose overexpression inhibits binding of avian influenza viruses, including the α 2,3 sialic acid-specific H5, H9, and H7 strains, previously found to cause disease in humans [24]. However, owing to the strict specificity of B4GALNT2 for α 2,3 sialylated chains, this inhibitory activity is not expected to regard hemagglutinins specific for α 2,6 sialylated glycans. This notion has been experimentally confirmed by the reduced entry and infection of influenza virus strains expressing hemagglutinins specific for α 2,3-sialylated chains in MDCK cells forced to express B4GALNT2 by gene transfer [25]. In chicken fibroblasts genetically modified with human B4GALNT2, the entry and infectivity of avian influenza virus and Newcastle disease virus was reduced. Both virus types express mainly receptors for α 2,3-linked sialic acid [26].

3.1.2. Bacterial Infections

The role of B4GALNT2/Sd^a in bacterial interactions is complex (for an extensive review see [27]). A study reports the differences in intestinal microbiota composition between wt and *B4galnt2* KO mice [28]. The distribution of phyla and individual bacterial species varies among gastrointestinal mucosa tracts. The most remarkable differences between wt and *B4galnt2* KO mice include a reduced proportion of Proteobacteria, a parallel increase of Bacteroidetes and the virtual absence of the *Helicobacter* spp. species in the intestine of *B4galnt2* KO mice [28]. These data are consistent with a view in which the absence of Sd^a provides a fitness advantage towards bacteria requiring terminal β 4-linked GalNAc for their adhesion. This view was confirmed by a study showing reduced effects of *Salmonella typhimurium* infection in mice deficient in intestinal *B4galnt2* expression because of a reduced production of inflammatory cytokines and immune cell infiltration. The presence of intestinal B4galnt2 is also associated to an increased susceptibility to *Morganella morganii* [29], but decreased susceptibility to *Citrobacter rodentium* infection, a murine model pathogen for human enteropathogenic *Escherichia coli* [30]. B4galnt2/Sd^a can be naturally absent from intestinal epithelium in mice laboratory strains and wild mice populations. In fact, B4galnt2 can be alternatively expressed in the intestine or in endothelial cells as determined by a polymorphic region located 30 kb upstream the *B4galnt2* gene, which is present in two allelic forms [31]. While the C57 allele (present in the C57BL6/J mouse strain) is responsible for the intestinal expression, the RIII allele (present in the RIIS/J mouse strain) determines the endothelial expression. Both alleles can be present also in wild mice populations [32], together with a third allele dictating neither the endothelial nor the epithelial localization [33]. When endothelial cells express B4galnt2, a plasma glycoprotein promoting hemostasis, called the Von Willebrand factor (vWf), becomes decorated by the Sd^a antigen. This leads to a quicker uptake of vWf from blood mediated by the asialo glycoprotein receptor, a liver lectin

which removes glycoproteins terminating with galactose or GalNAc from blood circulation, thus resulting in a bleeding disorder. Concomitantly, B4galnt2/Sd^a are not expressed in the intestine. The fact that this obviously detrimental condition is observed also in wild mice, suggests a putative advantage of the B4galnt2 loss in intestinal tissues, which may be due to reduced pathogen susceptibility. In kidney, the Sd^a antigen is carried by the N-linked chains of uromodulin (also known as Tamm-Horsfall glycoprotein). It has been proposed that the presence of the β 4-linked GalNAc hinders the binding of pyelonephritogenic *E. coli* strains through their S-fimbriae specific for Sia α 3Gal β 4GlcNAc [34]. The opposite effects that B4galnt2 expression exerts in mice on the susceptibility towards different bacterial species might be explained at least in part by the specificity of microbial adhesion molecules. If those specific for terminal galactose are prevalent on those specific for Sia α 2,3Gal chains, then the Sd^a antigen could help infection. Otherwise, it may be protective. The crucial role of B4GALNT2/Sd^a in protection against bacteria is supported by the finding that they are expressed only after birth upon bacterial colonization in both rat [35] and humans [36].

3.1.3. Worm Infections

The gastrointestinal round worm *Nippostrongylus brasiliensis* infects rodents. During its infection, mucin glycosylation is altered by the induction of *B4galnt2*, leading to the biosynthesis of O-linked oligosaccharides carrying the Sd^a epitope [37–39]. This provides an example of how, besides bacteria, also metazoan pathogens can shape intestinal glycosylation.

3.2. How B4GALNT2/Sd^a Antigen Regulates Reproduction

In animals, B4GALNT2/Sd^a play multiple roles associated with both pre- and post-fertilization steps of reproduction. Here, we will discuss the role in gamete formation, implantation and regulation of the mother's immune response.

3.2.1. Gametes

The Sd^a antigen is expressed by both male and female gametes of various animals [40] and in particular in the murine zona pellucida of the oocyte [41]. The involvement of B4GALNT2 in sheep prolificacy of some breeds is indicated by the following findings. In some breeds, ovulation rates and fertility are associated with the genetic locus *FecL* in sheep chromosome 11 [42,43]. *FecL*(L) is the high prolificacy allele of the *FecL* locus [42]. Of the genes contained in the *FecL* locus, *B4GALNT2* is the one responsible for increased prolificacy because it is ectopically overexpressed (about 1000 fold) in the ovarian follicles and particularly in granulosa cells of the *FecL*(L) carrier animals only [42]. As a consequence, the Sd^a antigen becomes expressed by some glycoproteins including inhibin, an important hormone regulating ovarian function. However, in breeds of Chinese origin B4GALNT2 affects prolificacy through quite different mechanisms [44,45].

3.2.2. Implantation

In mouse uterine tissues, progesterone and estrogens modulate *B4galnt2* gene expression, which reaches a maximum in parallel with the level of placental progesterone production [46]. *B4galnt2* down-regulation by siRNA treatment results in a reduced number of implanted embryos [46]. Moreover, the attachment of blastocyst to endometrial cells *in vitro* can be inhibited either by antibodies against *B4galnt2* or by lectins recognizing the Sd^a epitope [47]. A recent paper has investigated the relationship between B4galnt2 expression, implantation and preeclampsia. Preeclampsia, a major cause of maternal and neonatal morbidity and mortality, is defined as a complication of pregnancy developing after the 20th week of gestation, characterized by hypertension with or without edema and proteinuria. Although the etiopathogenesis of preeclampsia remains still unclear, a crucial role is played by an abnormally implanted placenta, which results in poor uterine and placental perfusion. Proper placenta implantation requires adequate trophoblast invasion of uterine epithelium, a process associated with spiral artery remodeling. In murine models, the lack of maternal galectin-1 causes preeclampsia because of an insufficient trophoblast invasion

ability [48]. Galectin-1 stimulates the expression of *B4galnt2* and consequently of Sd^a-capped N-glycans by trophoblast cells, which are necessary for invasion [48].

3.2.3. Regulation of the Mother's Immune Response

Glycodelin is a glycoprotein present in four differentially glycosylated forms. Three are from the female genital tract and one from sperm. A portion of the N-linked chains of the three female glycodelins is terminated by the Sd^a antigen [49]. Glycodelin A present in the amniotic fluid, contribute to protect fetus from rejection by the mother's immune system by skewing T-cell differentiation toward the Th-2 phenotype and inhibition of NK activity. The immunosuppressive activity is largely due α 2,6-sialylated glycodelin A. However, if α 2,6-sialylation decreases in favor of increased Sd^a expression, as occurs in gestational diabetes mellitus, the immunosuppressive activity of glycodelin A is reduced [50].

Data from sheep and mouse animals indicate a positive role of B4GALNT2 in promoting proliferacy, while data from different mice strains indicate either a positive or a negative effect of intestinal B4GALNT2 expression. In the vast majority of humans B4GALNT2 is expressed in blood and large intestine, suggesting a possible positive role.

4. How B4GALNT2/Sd^a Play a Role in Cancer

B4GALNT2/Sd^a display a strong dependence on the neoplastic transformation. The dramatic down-regulation of B4GALNT2 in colorectal cancer (CRC) [51] is due to a marked mRNA reduction [52]. The Sd^a antigen is also down-regulated in CRC [53,54]. The availability of large public databases, such as TCGA, reporting clinical and molecular data of hundreds of patients, has confirmed the marked reduction of B4GALNT2 mRNA in CRC samples. In most of patients, B4GALNT2 is completely switched off. However, those patients retaining a nearly normal expression level, display a much longer overall survival [19,55]. These patients display high levels of genes related to normal colon functions, such as mucus secretion, ion transport and proper glycosylation, while genes associated with tumor progression, such as *WIF1* and *TSIX* were poorly expressed [19]. In ulcerative colitis, a pre-neoplastic intestinal inflammatory condition, *B4GALNT2* is also transcriptionally modulated [56]. In colon, the Sd^a biosynthesis is strictly connected with that of the sialyl Lewis x (sLex) [Sia α 2,3Gal β 1,4(Fuc α 1,3)GlcNAc] antigen [57] (Figure 3), which act as ligands for the cell adhesion molecules of the selectin family, playing a physiological role in leukocyte extravasation and a pathological role in metastasis formation [58].

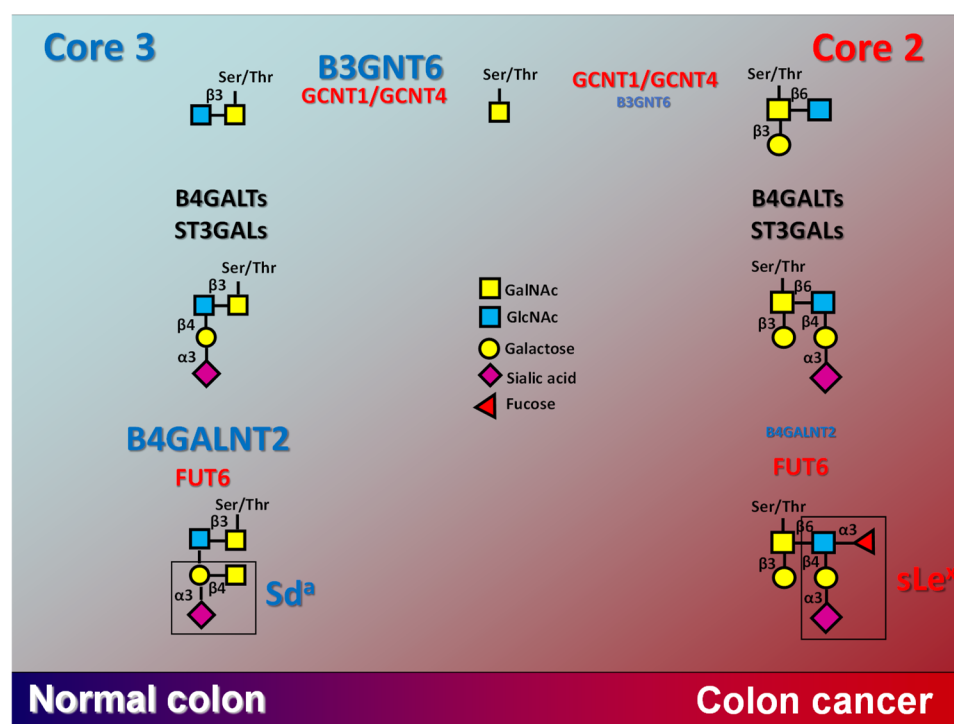


Figure 3. Alternative biosynthesis of Sd^a and sLe^x in normal and colon cancer. In normal colon, the biosynthesis of core 3 structure is largely predominant over that of core 2, because of the high level of B3GNT6, while the high level of B4GALNT2 prevents the biosynthesis of sLe^x. In colon cancer, the down-regulation of B3GNT6 leads to the replacement of core 3 with core 2 structures, while the down-regulation of B4GALNT2 leads to sLe^x expression. GCNT1 and GCNT4 are core 2 synthases. The size of the lettering grossly represents the expression level of the enzyme.

Due to steric hindrance, the biosynthesis of sLe^x and of Sd^a antigens are mutually exclusive. In fact, GalNAc (Sd^a) or Fuc (sLe^x) are added by the respective glycosyltransferases on two adjacent sugars (Figure 3). The Sd^a epitope, but not sLe^x, is expressed by mucins from normal colon, while structures in which the β 4-linked GalNAc to galactose and Fuc α 3-linked to GlcNAc are in adjacent positions have never been detected [59]. The transition from normal colon to CRC is accompanied by a switch from O-linked chains with core 3 structures, which are often decorated by Sd^a, to those with core 2 structures, which are often terminated by sLe^x antigens [60]. The core 3 to core 2 transition is mainly due the down-regulation of core 3 biosynthesis rather than to the up-regulation of core 2 biosynthesis [60] (Figure 3). In addition, the B4GALNT2 level plays a role in keeping low sLe^x biosynthesis in normal colon by competing with FUT6 for the same substrate acceptor(s) [61]. Thus, the switch from core 3/Sd^a in normal colon from core 2/sLe^x in CRC is due to down-regulation of B3GNT6 and B4GALNT2, rather than to the up-regulation of core 2/sLe^x synthases and FUT6 in cancer (Figure 3). Interestingly, the core-3 synthase B3GNT6 and B4GALNT2 display a grossly proportional positive relationship with B4GALNT2 in the colon cancer (COAD) TCGA cohort [19].

In the stomach, the level of B4GALNT2 expression level is about 50-fold lower than in colon while in the near totality of gastric cancer samples it is virtually switched off [18]. Consistently, the Sd^a antigen which is carried in normal gastric mucosa by the glycolipid sialylparagloboside, is lost in gastric cancer [62].

Even if the kidney is a major site of B4GALNT2/Sd^a expression, no data have been reported so far on its modulation in malignancy. TCGA reports two kidney cancer cohorts: the kidney renal clear cell carcinoma (KIRC), which accounts for 70-80% of the cases, and the kidney renal papillary carcinoma (KIRP), accounting for 10-15%. As shown in Figure 4, the B4GALNT2 expression was markedly reduced in cancer samples of both cohorts, although at a variable degree. In KIRC patients, the B4GALNT2 relationship with survival is complex, and non-significant. In fact, in the first 1500 days, high B4GALNT2 expressers display better survival, while after 1500 days the opposite occurs.

Interestingly, the putative tumor suppressor gene *HEPACAM2* is expressed only by high *B4GALNT2* expressers. Like in colon cancer (see above) *TSIX* was strongly down-regulated in high *B4GALNT2* expressers. In KIRP, the association of high *B4GALNT2* expression and longer survival is highly significant (Figure 4).

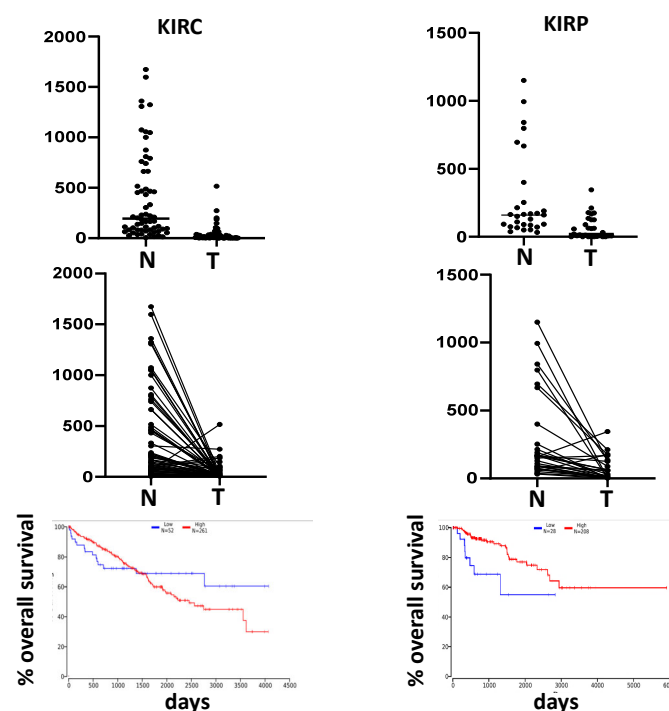


Figure 4. B4GALNT2 in kidney cancers. In both KIRC and KIRP cohorts, *B4GALNT2* is strongly down regulated in cancer, compared with normal. Kaplan Mayer survival curves indicate that in KIRP high expressers (red line) display better prognosis ($p=0.005$), while in KIRC cohort the relationship is more complex. Data have been downloaded from TCGA as described [55]. Kaplan Meier curves have been obtained as described [63]. For KIRC, the low (blue line) and high (red line) expressers percentiles were 10 and 50, while for KIRP they were 10 and 73%, respectively. .

The involvement of *B4GALNT2*/Sd^a in breast cancer is suggested by TCGA data, showing that in normal breast tissue *B4GALNT2* is virtually not expressed. However, in a minority of breast cancer cases it reaches a relevant level, suggesting an ectopic expression of the gene. In contrast with colon cancer, patients expressing high *B4GALNT2* display shorter overall survival and a gene expression profile closely associated with malignancy [18]. The overexpression of the *KRT20* gene, encoding cytokeratin 20, which is about 500-fold higher in high- than in low expressers is particularly relevant.

An association does not necessarily imply a causal relationship. To establish the existence of a causal nexus between *B4GALNT2* and malignancy relationship, the ability of the Sd^a antigen to modulate the behavior of cancer cells was investigated by gene transfer experiments. Previous experiments in colon cancer have shown that forced *B4GALNT2* expression by gene transfer results in a strong inhibition of sLe^x antigen [53,64] and in a reduction of the metastatic potential of transfected cells [64,65]. This finding raises a key question: is this effect attributable to inhibition of sLe^x antigen or is it independent? To answer this question, the two colon cancer cell lines SW480 and SW620 (the former derived from a primary tumor and the latter from a metastasis of the same patient), originally devoid of both sLe^x and Sd^a antigens, were forced to express either Sd^a by *B4GALNT2* transfection or sLe^x by transfection with the main sLe^x synthase *FUT6* [66,67]. Consistent with previous data obtained with the LS174T cell line [55], in SW480 and SW620 *B4GALNT2* expression reduced stemness [67]. Importantly, this model showed that the phenotypic effects were oriented toward reduced malignancy independently on sLe^x inhibition [67]. Consistent with TCGA data, experimental studies with triple negative breast cancer cell lines have shown a positive

correlation between high B4GALNT2 and malignancy [68,69]. In particular, it has been shown that the B4GALNT2 protein is able to interact with proteins of the major histocompatibility complex (HLA-B) [69]. Thus, in both colon- and breast cancer, experimental data show that the level of B4GALNT2 expression strongly affects the behavior of cancer cells.

5. How B4GALNT2/Sd^a Could Cure Duchene Muscular Dystrophy

Duchenne muscular dystrophy (DMD) is an extremely severe condition caused by the absence of the cytoplasmic protein dystrophin. The biological role of dystrophin is to connect the cytoskeleton of muscle cells with membrane β -dystroglycan, which in turn is connected to α -dystroglycan and laminin in the extracellular matrix. *B4galnt2* overexpression protects skeletal muscle cells from injury in mouse models of DMD [70–73]. The mechanisms underlying this phenomenon are very complex and only partially elucidated [74–76]. *B4galnt2* gene delivery through a viral vector in a dog model of DMD has revealed to be safe and able to induce B4galnt2 expression, although with little or no improvement of the pathology [77]. The administration of the viral vector in two DMD-affected boys resulted in functional improvements only in the younger patient treated with a higher dose of B4GALNT2 vector [78].

6. How B4GALNT2/Sd^a May Play a Role in Kidney Disease

Uromodulin/ Tamm-Horsfall glycoprotein (see above), product of the *UMOD* gene, represents the major urinary protein of healthy individuals and is a major carrier of the Sd^a antigen [34]. Genome wide association studies (GWAS) have identified some polymorphisms in the *UMOD* gene as risk factors for chronic kidney disease. These genetic variants are associated with increased transcription of *UMOD* gene in kidney and of uromodulin level in urine and serum [79]. In search for other genetic loci whose polymorphisms affect uromodulin levels, Li et al. [79] have found that the p.Cys466Arg inactive variant of *B4GALNT2* (see above) is also associated with increased uromodulin levels in serum. It is conceivable that the lack of terminal GalNAc in uromodulin of p.Cys466Arg individuals reduces its uptake by the asialo glycoprotein receptor (see above). However, the demonstration of a higher susceptibility to chronic kidney disease of Sd^a individuals is still lacking.

7. How B4GALNT2/Sd^a Affects Xenotransplantation

The availability of organs for transplantation from deceased persons represents a strongly limiting factor in modern surgery. The use of pig organs may be a possible solution but is strongly limited by the occurrence of hyperacute rejection. This reaction, takes place when the recipient's contains preformed antibodies against surface antigens of the donor organ, and leads to complement-mediated rejection in a very short time. In pig organs, there are at least three carbohydrate antigens toward which human blood can trigger the hyperacute rejection. One of them is the so called "Galili antigen", consisting in a terminal α 1,3-linked galactose residue whose addition is mediated by the α -galactosyltransferase encoded by the *GGTA1* gene. During evolution, *GGTA1* has been inactivated in a precursor of humans and Old World primates (gorillas, bonobos, chimpanzees). Consequently, these species do not express the Galili antigen on their cells, while a significant percentage of their circulating antibodies is able to react with it. This is probably due to cross-reactivity with microorganism antigens and lack of tolerance by the human immune system against this antigen. A second type of non-human carbohydrate is represented by glycoconjugates terminating with N-glycolylneuraminic acid. This type of sialic acid is present in animals but not in human glycoconjugates because of the inactivation of the *CMAH* gene, which encodes CMP-N-acetylneuraminic acid hydroxylase. This event occurred after divergence of humans from the other Old World apes. Surprisingly, the third is the Sd^a antigen encoded by porcine *B4GALNT2* [80,81]. In fact, human cells forced to express porcine *B4GALNT2* display increased complement-mediated lysis with serum from primates pre-immunized with pig organs [82]. Blood cells from *CMAH/GGTA1/B4GALNT2* triple KO pigs display reduced reactivity with human plasma than cells from *CMAH/GGTA1* double KO pigs [83–86]. It is not clear why the human immune system is not

tolerant toward a self antigen when it is synthesized by pig B4GALNT2. Whatever the reason, the presence of the Sd^a antigens on pig cells is an obstacle that must be removed before a pig-to-human transplantation is considered.

8. Conclusions

Even if a small but significant fraction of human population lacks the Sd^a antigen from their cells and secretions without obvious pathological consequences, many lines of evidence indicate that it may be involved in determining the fertility, the susceptibility to microbial pathogens or to kidney diseases. In addition, Sd^a absence or presence may be crucial for some malignancies outcome. However, large epidemiological studies aimed to elucidate the real impact of the Sd^a antigen on human physiopathology have never been carried out. We strongly believe these intriguing issues deserve to be investigated and we are confident that they will disclose a much stronger impact of this antigen and its biosynthetic enzyme on human health.

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References

1. Morton, J.A.; Pickles, M.M.; Terry, A.M. The Sd^a blood group antigen in tissues and body fluids. *Vox Sang.* **1970**, *19*, 472-482
2. Morton, J.A.; Pickles, M.M.; Vanhegan, R.I. The Sda antigen in the human kidney and colon. *Immunol. Invest* **1988**, *17*, 217-224
3. Renton, P.H.; Howell, P.; Ikin, E.W.; Giles, C.M.; Goldsmith, K.L. Anti Sd^a: a new blood group antibody. *Vox Sang.* **1967**, *13*, 493-501
4. Macvie, S.I.; Morton, J.A.; Pickles, M.M. The reactions and inheritance of a new blood group antigen. *Vox Sang.* **1967**, *13*, 485-492
5. Sanger, R.; Gavin, J.; Tippett, P.; Teesdale, P.; Eldon, K. Plant agglutinin for another human blood-group. *Lancet* **1971**, *1*, 1130
6. Stenfelt, L.; Hellberg, A.; Olsson, M.L. SID: a new carbohydrate blood group system based on a well-characterized but still mysterious antigen of great pathophysiologic interest. *Immunohematology*. **2023**, *39*, 1-10. immunohematology-2023-002 [pii];10.21307/immunohematology-2023-002 [doi].
7. Donald, A.S.; Yates, A.D.; Soh, C.P.; Morgan, W.T.; Watkins, W.M. A blood group Sd^a-active pentasaccharide isolated from Tamm-Horsfall urinary glycoprotein. *Biochem. Biophys. Res. Commun.* **1983**, *115*, 625-631
8. Dall'Olio, F.; Malagolini, N.; Chiricolo, M.; Trinchera, M.; Harduin-Lepers, A. The expanding roles of the Sd^a/Cad carbohydrate antigen and its cognate glycosyltransferase B4GALNT2. *Biochim. Biophys. Acta* **2014**, *1840*, 443-453
9. Blanchard, D.; Piller, F.; Gillard, B.; Marcus, D.; Cartron, J.P. Identification of a novel ganglioside on erythrocytes with blood group Cad specificity. *J. Biol. Chem.* **1985**, *260*, 7813-7816
10. Serafini-Cessi, F.; Dall'Olio, F. Guinea-pig kidney β -N-acetylgalactosaminyltransferase towards Tamm-Horsfall glycoprotein. Requirement of sialic acid in the acceptor for transferase activity. *Biochem. J.* **1983**, *215*, 483-489
11. Smith, P.L.; Lowe, J.B. Molecular cloning of a murine N-acetylgalactosamine transferase cDNA that determines expression of the T lymphocyte-specific CT oligosaccharide differentiation antigen. *J. Biol. Chem.* **1994**, *269*, 15162-15171
12. Lo Presti L.; Cabuy, E.; Chiricolo, M.; Dall'Olio, F. Molecular Cloning of the Human β 1,4 N-Acetylgalactosaminyltransferase Responsible for the Biosynthesis of the Sd^a Histo-Blood Group Antigen: The Sequence Predicts a Very Long Cytoplasmic Domain. *J. Biochem. (Tokyo)* **2003**, *134*, 675-682

13. Montiel, M.D.; Krzewinski-Recchi, M.A.; Delannoy, P.; Harduin-Lepers, A. Molecular cloning, gene organization and expression of the human UDP-GalNAc:Neu5Ac α 2-3Gal β -R β 1,4-N-acetylgalactosaminyltransferase responsible for the biosynthesis of the blood group Sda/Cad antigen: evidence for an unusual extended cytoplasmic domain. *Biochem. J.* **2003**, *373*, 369-379
14. Groux-Degroote, S.; Schulz, C.; Cogez, V.; Noel, M.; Portier, L.; Vicogne, D.; Solorzano, C.; Dall'Olio, F.; Steenackers, A.; Mortuaire, M.; Gonzalez-Pisfil, M.; Henry, M.; Foulquier, F.; Heliot, L.; Harduin-Lepers, A. The extended cytoplasmic tail of the human B4GALNT2 is critical for its Golgi targeting and post-Golgi sorting. *FEBS J.* **2018**, *285*, 3442-3463. 10.1111/febs.14621 [doi].
15. Cogez, V.; Vicogne, D.; Schulz, C.; Portier, L.; Venturi, G.; de, R.J.; Decloquement, M.; Lensink, M.F.; Brysbaert, G.; Dall'Olio, F.; Groux-Degroote, S.; Harduin-Lepers, A. N-Glycan on the Non-Consensus N-X-C Glycosylation Site Impacts Activity, Stability, and Localization of the Sd^a Synthase B4GALNT2. *Int. J. Mol. Sci.* **2023**, *24*, 4139. ijms24044139 [pii];ijms-24-04139 [pii];10.3390/ijms24044139 [doi].
16. Kawamura, Y.I.; Toyota, M.; Kawashima, R.; Hagiwara, T.; Suzuki, H.; Imai, K.; Shinomura, Y.; Tokino, T.; Kannagi, R.; Dohi, T. DNA hypermethylation contributes to incomplete synthesis of carbohydrate determinants in gastrointestinal cancer. *Gastroenterology* **2008**, *135*, 142-151
17. Wang, H.R.; Hsieh, C.Y.; Twu, Y.C.; Yu, L.C. Expression of the human Sd^a β -1,4-N-acetylgalactosaminyltransferase II gene is dependent on the promoter methylation status. *Glycobiology* **2008**, *18*, 104-113
18. Dall'Olio, F.; Pucci, M.; Malagolini, N. The Cancer-Associated Antigens Sialyl Lewis^{a/x} and Sd^a: Two Opposite Faces of Terminal Glycosylation. *Cancers. (Basel)* **2021**, *13*, 5273. cancers13215273 [pii];10.3390/cancers13215273 [doi].
19. Pucci, M.; Malagolini, N.; Dall'Olio, F. Glycosyltransferase B4GALNT2 as a Predictor of Good Prognosis in Colon Cancer: Lessons from Databases. *Int. J. Mol. Sci.* **2021**, *22*. ijms22094331 [pii];10.3390/ijms22094331 [doi].
20. Wavelet-Vermuse, C.; Groux-Degroote, S.; Vicogne, D.; Cogez, V.; Venturi, G.; Trinchera, M.; Brysbaert, G.; Krzewinski-Recchi, M.A.; Bachir, E.H.; Schulz, C.; Vincent, A.; van, S., I.; Harduin-Lepers, A. Analysis of the proximal promoter of the human colon-specific B4GALNT2 (Sd^a synthase) gene: B4GALNT2 is transcriptionally regulated by ETS1. *Biochim. Biophys. Acta Gene Regul. Mech.* **2021**, *1864*, 194747. S1874-9399(21)00065-1 [pii];10.1016/j.bbagr.2021.194747 [doi].
21. Duca, M.; Malagolini, N.; Dall'Olio, F. The story of the Sd^a antigen and of its cognate enzyme B4GALNT2: What is new? *Glycoconj. J.* **2023**, *40*, 123-133. 10.1007/s10719-022-10089-1 [doi];10.1007/s10719-022-10089-1 [pii].
22. Stenfelt, L.; Hellberg, A.; Moller, M.; Thornton, N.; Larson, G.; Olsson, M.L. Missense mutations in the C-terminal portion of the B4GALNT2-encoded glycosyltransferase underlying the Sd^a phenotype. *Biochem. Biophys. Rep.* **2019**, *19*, 100659. 10.1016/j.bbrep.2019.100659 [doi];S2405-5808(19)30121-9 [pii];100659 [pii].
23. Stenfelt, L.; Nilsson, J.; Hellberg, A.; Liew, Y.W.; Morrison, J.; Larson, G.; Olsson, M.L. Glycoproteomic and Phenotypic Elucidation of B4GALNT2 Expression Variants in the SID Histo-Blood Group System. *Int. J. Mol. Sci.* **2022**, *23*, 3936. ijms23073936 [pii];10.3390/ijms23073936 [doi].
24. Heaton, B.E.; Kennedy, E.M.; Dumm, R.E.; Harding, A.T.; Sacco, M.T.; Sachs, D.; Heaton, N.S. A CRISPR Activation Screen Identifies a Pan-avian Influenza Virus Inhibitory Host Factor. *Cell Rep.* **2017**, *20*, 1503-1512. S2211-1247(17)31039-2 [pii];10.1016/j.celrep.2017.07.060 [doi].
25. Wong, H.H.; Fung, K.; Nicholls, J.M. MDCK-B4GalNT2 cells disclose a α 2,3-sialic acid requirement for the 2009 pandemic H1N1 A/California/04/2009 and NA aid entry of A/WSN/33. *Emerg. Microbes. Infect.* **2019**, *8*, 1428-1437. 10.1080/22221751.2019.1665971 [doi].
26. Park, J.S.; Woo, S.J.; Song, C.S.; Han, J.Y. Modification of surface glycan by expression of β -1,4-N-acetyl-galactosaminyltransferase (B4GALNT2) confers resistance to multiple viruses infection in chicken fibroblast cell. *Front Vet. Sci.* **2023**, *10*, 1160600. 10.3389/fvets.2023.1160600 [doi].
27. Galeev, A.; Suwandi, A.; Cepic, A.; Basu, M.; Baines, J.F.; Grassl, G.A. The role of the blood group-related glycosyltransferases FUT2 and B4GALNT2 in susceptibility to infectious disease. *Int. J. Med. Microbiol.* **2021**, *311*, 151487. S1438-4221(21)00016-3 [pii];10.1016/j.ijmm.2021.151487 [doi].
28. Staubach, F.; Kunzel, S.; Baines, A.C.; Yee, A.; McGee, B.M.; Backhed, F.; Baines, J.F.; Johnsen, J.M. Expression of the blood-group-related glycosyltransferase B4galnt2 influences the intestinal microbiota in mice. *ISME. J.* **2012**, *6*, 1345-1355

29. Vallier, M.; Suwandi, A.; Ehrhardt, K.; Belheouane, M.; Berry, D.; Cepic, A.; Galeev, A.; Johnsen, J.M.; Grassl, G.A.; Baines, J.F. Pathometagenomics reveals susceptibility to intestinal infection by *Morganella* to be mediated by the blood group-related B4galnt2 gene in wild mice. *Gut Microbes*. **2023**, *15*, 2164448. 2164448 [pii];10.1080/19490976.2022.2164448 [doi].
30. Suwandi, A.; Alvarez, K.G.; Galeev, A.; Steck, N.; Riedel, C.U.; Puente, J.L.; Baines, J.F.; Grassl, G.A. B4galnt2-mediated host glycosylation influences the susceptibility to *Citrobacter rodentium* infection. *Front Microbiol*. **2022**, *13*, 980495. 10.3389/fmicb.2022.980495 [doi].
31. Johnsen, J.M.; Levy, G.G.; Westrick, R.J.; Tucker, P.K.; Ginsburg, D. The endothelial-specific regulatory mutation, Mvzf1, is a common mouse founder allele. *Mamm. Genome* **2008**, *19*, 32-40
32. Johnsen, J.M.; Teschke, M.; Pavlidis, P.; McGee, B.M.; Tautz, D.; Ginsburg, D.; Baines, J.F. Selection on cis-regulatory variation at B4galnt2 and its influence on von Willebrand factor in house mice. *Mol. Biol. Evol.* **2009**, *26*, 567-578
33. Linnenbrink, M.; Johnsen, J.M.; Montero, I.; Brzezinski, C.R.; Harr, B.; Baines, J.F. Long-term balancing selection at the blood group-related gene B4galnt2 in the genus *Mus* (Rodentia; Muridae). *Mol. Biol. Evol.* **2011**, *28*, 2999-3003
34. Serafini-Cessi, F.; Monti, A.; Cavallone, D. N-Glycans carried by Tamm-Horsfall glycoprotein have a crucial role in the defense against urinary tract diseases. *Glycoconj. J.* **2005**, *22*, 383-394
35. Dall'Olio, F.; Malagolini, N.; Di Stefano, G.; Ciambella, M.; Serafini-Cessi, F. Postnatal development of rat colon epithelial cells is associated with changes in the expression of the β 1,4-N-acetylgalactosaminyltransferase involved in the synthesis of Sda antigen and of α 2,6-sialyltransferase activity towards N-acetyllactosamine. *Biochem. J.* **1990**, *270*, 519-524
36. Robbe-Masselot, C.; Maes, E.; Rousset, M.; Michalski, J.C.; Capon, C. Glycosylation of human fetal mucins: a similar repertoire of O-glycans along the intestinal tract. *Glycoconj. J.* **2009**, *26*, 397-413
37. Karlsson, N.G.; Olson, F.J.; Jovall, P.A.; Andersch, Y.; Enerback, L.; Hansson, G.C. Identification of transient glycosylation alterations of sialylated mucin oligosaccharides during infection by the rat intestinal parasite *Nippostrongylus brasiliensis*. *Biochem. J.* **2000**, *350*, 805-814
38. Holmen, J.M.; Olson, F.J.; Karlsson, H.; Hansson, G.C. Two glycosylation alterations of mouse intestinal mucins due to infection caused by the parasite *Nippostrongylus brasiliensis*. *Glycoconj. J.* **2002**, *19*, 67-75
39. Tsubokawa, D.; Goso, Y.; Kawashima, R.; Ota, H.; Nakamura, T.; Nakamura, K.; Sato, N.; Kurihara, M.; Dohi, T.; Kawamura, Y.I.; Ichikawa, T.; Ishihara, K. The monoclonal antibody HCM31 specifically recognises the Sd^a tetrasaccharide in goblet cell mucin. *FEBS Open. Bio* **2012**, *2*, 223-233. 10.1016/j.fob.2012.07.006 [doi];FOB36 [pii].
40. Easton, R.L.; Patankar, M.S.; Lattanzio, F.A.; Leaven, T.H.; Morris, H.R.; Clark, G.F.; Dell, A. Structural Analysis of Murine Zona Pellucida Glycans. Evidence for the expression of core 2-type o-glycans and the sd(a) antigen. *J. Biol. Chem.* **2000**, *275*, 7731-7742
41. Klisch, K.; Contreras, D.A.; Sun, X.; Brehm, R.; Bergmann, M.; Alberio, R. The Sda/GM2-glycan is a carbohydrate marker of porcine primordial germ cells and of a subpopulation of spermatogonia in cattle, pigs, horses and llama. *Reproduction*. **2011**, *142*, 667-674
42. Drouilhet, L.; Mansanet, C.; Sarry, J.; Tabet, K.; Bardou, P.; Woloszyn, F.; Lluch, J.; Harichaux, G.; Viguie, C.; Monniaux, D.; Bodin, L.; Mulsant, P.; Fabre, S. The highly prolific phenotype of Lacaupe sheep is associated with an ectopic expression of the B4GALNT2 gene within the ovary. *PLoS. Genet.* **2013**, *9*, e1003809
43. Ben Jemaa, S.; Ruesche, J.; Sarry, J.; Woloszyn, F.; Lassoued, N.; Fabre, S. The high prolificacy of D'man sheep is associated with the segregation of the FecL(L) mutation in the B4GALNT2 gene. *Reprod. Domest. Anim* **2019**, *54*, 531-537. 10.1111/rda.13391 [doi].
44. Guo, X.; Wang, X.; Liang, B.; Di, R.; Liu, Q.; Hu, W.; He, X.; Zhang, J.; Zhang, X.; Chu, M. Molecular Cloning of the B4GALNT2 Gene and Its Single Nucleotide Polymorphisms Association with Litter Size in Small Tail Han Sheep. *Animals. (Basel)* **2018**, *8*, 160. ani8100160 [pii];10.3390/ani8100160 [doi].
45. Ji, X.; Cao, Z.; Hao, Q.; He, M.; Cang, M.; Yu, H.; Ma, Q.; Li, X.; Bao, S.; Wang, J.; Tong, B. Effects of New Mutations in BMPRII, GDF9, BMP15, LEPR, and B4GALNT2 Genes on Litter Size in Sheep. *Vet. Sci.* **2023**, *10*, 258. vetsci10040258 [pii];vetsci-10-00258 [pii];10.3390/vetsci10040258 [doi].
46. Li, P.T.; Liao, C.J.; Wu, W.G.; Yu, L.C.; Chu, S.T. Progesterone-regulated B4galnt2 expression is a requirement for embryo implantation in mice. *Fertil. Steril.* **2011**, *95*, 2404-9, 2409

47. Li, P.T.; Liao, C.J.; Yu, L.C.; Wu, W.G.; Chu, S.T. Localization of B4GALNT2 and its role in mouse embryo attachment. *Fertil. Steril.* **2012**, *97*, 1206-1212
48. Xie, Y.; Zhao, F.; Freitag, N.; Borowski, S.; Wang, Y.; Harms, C.; Pang, P.C.; Desforges, J.; Wen, T.; Schwedhelm, E.; Singh, M.; Dechend, R.; Dell, A.; Haslam, S.M.; Dveksler, G.; Garcia, M.G.; Blois, S.M. Maternal-derived galectin-1 shapes the placenta niche through Sd^a terminal glycosylation: Implication for preeclampsia. *PNAS. Nexus.* **2023**, *2*, ad247. pgad247 [pii];10.1093/pnasnexus/pgad247 [doi].
49. Lee, C.L.; Pang, P.C.; Yeung, W.S.; Tissot, B.; Panico, M.; Lao, T.T.; Chu, I.K.; Lee, K.F.; Chung, M.K.; Lam, K.K.; Koistinen, R.; Koistinen, H.; Seppala, M.; Morris, H.R.; Dell, A.; Chiu, P.C. Effects of differential glycosylation of glycodepins on lymphocyte survival. *J. Biol. Chem.* **2009**, *284*, 15084-15096
50. Lee, C.L.; Chiu, P.C.; Pang, P.C.; Chu, I.K.; Lee, K.F.; Koistinen, R.; Koistinen, H.; Seppala, M.; Morris, H.R.; Tissot, B.; Panico, M.; Dell, A.; Yeung, W.S. Glycosylation failure extends to glycoproteins in gestational diabetes mellitus: evidence from reduced α 2-6 sialylation and impaired immunomodulatory activities of pregnancy-related glycodepin-A. *Diabetes* **2011**, *60*, 909-917
51. Malagolini, N.; Dall'Olio, F.; Di Stefano, G.; Minni, F.; Marrano, D.; Serafini-Cessi, F. Expression of UDP-GalNAc:NeuAc α 2,3Gal β -R beta 1,4(GalNAc to Gal) N-acetylgalactosaminyltransferase involved in the synthesis of Sd^a antigen in human large intestine and colorectal carcinomas. *Cancer Res.* **1989**, *49*, 6466-6470
52. Dohi, T.; Yuyama, Y.; Natori, Y.; Smith, P.L.; Lowe, J.B.; Oshima, M. Detection of N-acetylgalactosaminyltransferase mRNA which determines expression of Sda blood group carbohydrate structure in human gastrointestinal mucosa and cancer. *Int. J. Cancer* **1996**, *67*, 626-631
53. Malagolini, N.; Santini, D.; Chiricolo, M.; Dall'Olio, F. Biosynthesis and expression of the Sd^a and sialyl Lewis x antigens in normal and cancer colon. *Glycobiology* **2007**, *17*, 688-697
54. Robbe-Masselot, C.; Herrmann, A.; Maes, E.; Carlstedt, I.; Michalski, J.C.; Capon, C. Expression of a core 3 disialyl-Le^x hexasaccharide in human colorectal cancers: a potential marker of malignant transformation in colon. *J. Proteome. Res.* **2009**, *8*, 702-711
55. Pucci, M.; Gomes Ferreira, I.; Orlandani, M.; Malagolini, N.; Ferracin, M.; Dall'Olio, F. High Expression of the Sd^a Synthase B4GALNT2 Associates with Good Prognosis and Attenuates Stemness in Colon Cancer. *Cells* **2020**, *9*, 948. cells9040948 [pii];10.3390/cells9040948 [doi].
56. Low, E.N.D.; Mokhtar, N.M.; Wong, Z.; Raja Ali, R.A. Colonic Mucosal Transcriptomic Changes in Patients with Long-Duration Ulcerative Colitis Revealed Colitis-Associated Cancer Pathways. *J. Crohns. Colitis.* **2019**, *13*, 755-763. 5430265 [pii];10.1093/ecco-jcc/jjz002 [doi].
57. Groux-Degroote, S.; Vicogne, D.; Cogez, V.; Schulz, C.; Harduin-Lepers, A. B4GALNT2 Controls Sd^a and SLe^x Antigen Biosynthesis in Healthy and Cancer Human Colon. *Chembiochem.* **2021**, *22*, 3381-3390. 10.1002/cbic.202100363 [doi].
58. Trinchera, M.; Aronica, A.; Dall'Olio, F. Selectin Ligands Sialyl-Lewis a and Sialyl-Lewis x in Gastrointestinal Cancers. *Biology (Basel)* **2017**, *6*, 16. biology6010016 [pii];10.3390/biology6010016 [doi].
59. Capon, C.; Maes, E.; Michalski, J.C.; Leffler, H.; Kim, Y.S. Sd^a-antigen-like structures carried on core 3 are prominent features of glycans from the mucin of normal human descending colon. *Biochem. J* **2001**, *358*, 657-664
60. Madunic, K.; Mayboroda, O.A.; Zhang, T.; Weber, J.; Boons, G.J.; Morreau, H.; van, V.R.; van, W.T.; Lageveen-Kammeijer, G.S.M.; Wuhler, M. Specific (sialyl-)Lewis core 2 O-glycans differentiate colorectal cancer from healthy colon epithelium. *Theranostics.* **2022**, *12*, 4498-4512. 10.7150/thno.72818 [doi];thnov12p4498 [pii].
61. Groux-Degroote, S.; Wavelet, C.; Krzewinski-Recchi, M.A.; Portier, L.; Mortuaire, M.; Mihalache, A.; Trinchera, M.; Delannoy, P.; Malagolini, N.; Chiricolo, M.; Dall'Olio, F.; Harduin-Lepers, A. B4GALNT2 gene expression controls the biosynthesis of Sd^a and sialyl Lewis X antigens in healthy and cancer human gastrointestinal tract. *Int. J. Biochem. Cell Biol.* **2014**, *53*, 442-449
62. Dohi, T.; Ohta, S.; Hanai, N.; Yamaguchi, K.; Oshima, M. Sialylpentaosylceramide detected with anti-GM2 monoclonal antibody. Structural characterization and complementary expression with GM2 in gastric cancer and normal gastric mucosa. *J. Biol. Chem.* **1990**, *265*, 7880-7885
63. Pucci, M.; Duca, M.; Malagolini, N.; Dall'Olio, F. Glycosyltransferases in Cancer: Prognostic Biomarkers of Survival in Patient Cohorts and Impact on Malignancy in Experimental Models. *Cancers. (Basel)* **2022**, *14*, 2128. cancers14092128 [pii];10.3390/cancers14092128 [doi].

64. Kawamura, Y.I.; Kawashima, R.; Fukunaga, R.; Hirai, K.; Toyama-Sorimachi, N.; Tokuhara, M.; Shimizu, T.; Dohi, T. Introduction of Sd^a carbohydrate antigen in gastrointestinal cancer cells eliminates selectin ligands and inhibits metastasis. *Cancer Res.* **2005**, *65*, 6220-6227
65. Kawamura, Y.I.; Adachi, Y.; Curiel, D.T.; Kawashima, R.; Kannagi, R.; Nishimoto, N.; Dohi, T. Therapeutic adenoviral gene transfer of a glycosyltransferase for prevention of peritoneal dissemination and metastasis of gastric cancer. *Cancer Gene Ther.* **2014**, *21*, 427-433
66. Trinchera, M.; Malagolini, N.; Chiricolo, M.; Santini, D.; Minni, F.; Caretti, A.; Dall'Olio, F. The biosynthesis of the selectin-ligand sialyl Lewis x in colorectal cancer tissues is regulated by fucosyltransferase VI and can be inhibited by an RNA interference-based approach. *Int. J. Biochem. Cell Biol.* **2011**, *43*, 130-139
67. Pucci, M.; Gomes, F., I.; Malagolini, N.; Ferracin, M.; Dall'Olio, F. The Sd^a Synthase B4GALNT2 Reduces Malignancy and Stemness in Colon Cancer Cell Lines Independently of Sialyl Lewis X Inhibition. *Int. J. Mol. Sci.* **2020**, *21*, 6558. [ijms21186558 \[pii\];10.3390/ijms21186558 \[doi\]](#).
68. Qusa, M.H.; Abdelwahed, K.S.; Siddique, A.B.; El Sayed, K.A. Comparative Gene Signature of (-)-Oleocanthol Formulation Treatments in Heterogeneous Triple Negative Breast Tumor Models: Oncological Therapeutic Target Insights. *Nutrients.* **2021**, *13*, 1706. [nu13051706 \[pii\];10.3390/nu13051706 \[doi\]](#).
69. Yu, P.; Zhu, L.; Cui, K.; Du, Y.; Zhang, C.; Ma, W.; Guo, J. B4GALNT2 Gene Promotes Proliferation, and Invasiveness and Migration Abilities of Model Triple Negative Breast Cancer (TNBC) Cells by Interacting With HLA-B Protein. *Front Oncol.* **2021**, *11*, 722828. [10.3389/fonc.2021.722828 \[doi\]](#).
70. Nguyen, H.H.; Jayasinha, V.; Xia, B.; Hoyte, K.; Martin, P.T. Overexpression of the cytotoxic T cell GalNAc transferase in skeletal muscle inhibits muscular dystrophy in mdx mice. *Proc. Natl. Acad. Sci. U. S. A* **2002**, *99*, 5616-5621
71. Xu, R.; Chandrasekharan, K.; Yoon, J.H.; Camboni, M.; Martin, P.T. Overexpression of the cytotoxic T cell (CT) carbohydrate inhibits muscular dystrophy in the dyW mouse model of congenital muscular dystrophy 1A. *Am. J. Pathol.* **2007**, *171*, 181-199
72. Xu, R.; Camboni, M.; Martin, P.T. Postnatal overexpression of the CT GalNAc transferase inhibits muscular dystrophy in mdx mice without altering muscle growth or neuromuscular development: evidence for a utrophin-independent mechanism. *Neuromuscul. Disord.* **2007**, *17*, 209-220
73. Xu, R.; Devries, S.; Camboni, M.; Martin, P.T. Overexpression of Galgt2 reduces dystrophic pathology in the skeletal muscles of alpha sarcoglycan-deficient mice. *Am. J. Pathol.* **2009**, *175*, 235-247
74. Thomas, P.J.; Xu, R.; Martin, P.T. B4GALNT2 (GALGT2) Gene Therapy Reduces Skeletal Muscle Pathology in the FKRP P448L Mouse Model of Limb Girdle Muscular Dystrophy 2I. *Am. J. Pathol.* **2016**, *186*, 2429-2448. [S0002-9440\(16\)30218-8 \[pii\];10.1016/j.ajpath.2016.05.021 \[doi\]](#).
75. Jayasinha, V.; Hoyte, K.; Xia, B.; Martin, P.T. Overexpression of the CT GalNAc transferase inhibits muscular dystrophy in a cleavage-resistant dystroglycan mutant mouse. *Biochem. Biophys. Res. Commun.* **2003**, *302*, 831-836
76. Martin, P.T.; Xu, R.; Rodino-Klapac, L.R.; Oglesbay, E.; Camboni, M.; Montgomery, C.L.; Shontz, K.; Chicoine, L.G.; Clark, K.R.; Sahenk, Z.; Mendell, J.R.; Janssen, P.M. Overexpression of Galgt2 in skeletal muscle prevents injury resulting from eccentric contractions in both mdx and wild-type mice. *Am. J. Physiol Cell Physiol* **2009**, *296*, C476-C488
77. Martin, P.T.; Zygmunt, D.A.; Ashbrook, A.; Hamilton, S.; Packer, D.; Birch, S.M.; Bettis, A.K.; Balog-Alvarez, C.J.; Guo, L.J.; Nghiem, P.P.; Kornegay, J.N. Short-term treatment of golden retriever muscular dystrophy (GRMD) dogs with rAAVrh74.MHCK7.GALGT2 induces muscle glycosylation and utrophin expression but has no significant effect on muscle strength. *PLoS. One.* **2021**, *16*, e0248721. [10.1371/journal.pone.0248721 \[doi\];PONE-D-20-33809 \[pii\]](#).
78. Flanigan, K.M.; Vetter, T.A.; Simmons, T.R.; Iammarino, M.; Frair, E.C.; Rinaldi, F.; Chicoine, L.G.; Harris, J.; Cheatham, J.P.; Cheatham, S.L.; Boe, B.; Waldrop, M.A.; Zygmunt, D.A.; Packer, D.; Martin, P.T. A first-in-human phase I/IIa gene transfer clinical trial for Duchenne muscular dystrophy using rAAVrh74.MCK.GALGT2. *Mol. Ther. Methods Clin. Dev.* **2022**, *27*, 47-60. [10.1016/j.omtm.2022.08.009 \[doi\];S2329-0501\(22\)00119-X \[pii\]](#).
79. Li, Y.; Cheng, Y.; Consolato, F.; Schiano, G.; Chong, M.R.; Pietzner, M.; Nguyen, N.Q.H.; Scherer, N.; Biggs, M.L.; Kleber, M.E.; Haug, S.; Gocmen, B.; Pigeyre, M.; Sekula, P.; Steinbrenner, I.; Schlosser, P.; Joseph, C.B.; Brody, J.A.; Grams, M.E.; Hayward, C.; Schultheiss, U.T.; Kramer, B.K.; Kronenberg, F.; Peters, A.; Seissler, J.; Steubl, D.; Then, C.; Wuttke, M.; Marz, W.; Eckardt, K.U.; Gieger, C.; Boerwinkle, E.; Psaty, B.M.; Coresh, J.; Oefner, P.J.; Pare, G.; Langenberg, C.; Scherberich, J.E.; Yu, B.; Akilesh, S.; Devuyst, O.; Rampoldi, L.;

- Kottgen, A. Genome-wide studies reveal factors associated with circulating uromodulin and its relations with complex diseases. *JCI. Insight*. **2022**, 7, e157035. 157035 [pii];10.1172/jci.insight.157035 [doi].
80. Byrne, G.; Ahmad-Villiers, S.; Du, Z.; McGregor, C. B4GALNT2 and xenotransplantation: A newly appreciated xenogeneic antigen. *Xenotransplantation*. **2018**, 25, e12394. 10.1111/xen.12394 [doi].
 81. Zhao, C.; Cooper, D.K.C.; Dai, Y.; Hara, H.; Cai, Z.; Mou, L. The Sda and Cad glycan antigens and their glycosyltransferase, beta1,4GalNAcT-II, in xenotransplantation. *Xenotransplantation*. **2018**, 25, e12386. 10.1111/xen.12386 [doi].
 82. Byrne, G.W.; Du, Z.; Stalboerger, P.; Kogelberg, H.; McGregor, C.G. Cloning and expression of porcine β 1,4 N-acetylgalactosaminyl transferase encoding a new xenoreactive antigen. *Xenotransplantation*. **2014**, 21, 543-554
 83. Estrada, J.L.; Martens, G.; Li, P.; Adams, A.; Newell, K.A.; Ford, M.L.; Butler, J.R.; Sidner, R.; Tector, M.; Tector, J. Evaluation of human and non-human primate antibody binding to pig cells lacking GGTA1/CMAH/beta4GalNT2 genes. *Xenotransplantation*. **2015**, 22, 194-202
 84. Feng, H.; Li, T.; Du, J.; Xia, Q.; Wang, L.; Chen, S.; Zhu, L.; Pan, D.; Wang, Y.; Chen, G. Both Natural and Induced Anti-Sda Antibodies Play Important Roles in GTKO Pig-to-Rhesus Monkey Xenotransplantation. *Front Immunol*. **2022**, 13, 849711. 10.3389/fimmu.2022.849711 [doi].
 85. Fischer, K.; Rieblinger, B.; Hein, R.; Sfriso, R.; Zuber, J.; Fischer, A.; Klinger, B.; Liang, W.; Flisikowski, K.; Kurome, M.; Zakhartchenko, V.; Kessler, B.; Wolf, E.; Rieben, R.; Schwinzer, R.; Kind, A.; Schnieke, A. Viable pigs after simultaneous inactivation of porcine MHC class I and three xenoreactive antigen genes GGTA1, CMAH and B4GALNT2. *Xenotransplantation*. **2020**, 27, e12560. 10.1111/xen.12560 [doi].
 86. Wang, Z.Y.; Li, P.; Butler, J.R.; Blankenship, R.L.; Downey, S.M.; Montgomery, J.B.; Nagai, S.; Estrada, J.L.; Tector, M.F.; Tector, A.J. Immunogenicity of Renal Microvascular Endothelial Cells From Genetically Modified Pigs. *Transplantation* **2016**, 100, 533-537. 10.1097/TP.0000000000001070 [doi];00007890-201603000-00017 [pii].

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