The International Journal of Biochemistry & Cell Biology 53 (2014) 442–449

Contents lists available at ScienceDirect

The International Journal of Biochemistry & Cell Biology

journal homepage: www.elsevier.com/locate/biocel

B4GALNT2 gene expression controls the biosynthesis of Sd^a and sialyl Lewis X antigens in healthy and cancer human gastrointestinal tract

Sophie Groux-Degroote[a,1], Cindy Wavelet[a,1], Marie-Ange Krzewinski-Recchi[a], Lucie Portier[a], Marlène Mortuaire[a], Adriana Mihalache[a], Marco Trinchera[b], Philippe Delannoy[a], Nadia Malagolini[c], Mariella Chiricolo[c], Fabio Dall'Olio[c], Anne Harduin-Lepers[a,*]

[a] Structural and Functional Glycobiology Unit, UMR CNRS 8576, University Lille Nord de France, Lille1, 59655 Villeneuve d'Ascq, France
[b] Department of Medicine Clinical and Experimental (DMCS), University of Insubria Medical School, Varese, Italy
[c] Department of Experimental, Diagnostic and Specialty Medicine (DIMES), University of Bologna, Bologna, Italy

ARTICLE INFO

Article history:
Received 14 April 2014
Received in revised form 6 June 2014
Accepted 13 June 2014
Available online 19 June 2014

Keywords:
Glycosylation
Sd^a antigen
Colon cancer
B4GALNT2
Glycosyltransferases

ABSTRACT

The histo blood group carbohydrate Sd^a antigen and its cognate biosynthetic enzyme B4GALNT2 show the highest level of expression in normal colon. Their dramatic down regulation previously observed in colon cancer tissues could play a role in the concomitant elevation of the selectin ligand sLe^x, involved in metastasis. However, down regulation of sLe^x expression by B4GALNT2 has been so far demonstrated *in vitro*, but not in tissues. The human *B4GALNT2* gene specifies at least two transcripts, diverging in the first exon, never studied in normal and cancer tissues. The long form contains a 253 nt exon 1L; the short form contains a 38 nt exon 1S. Using qPCR, we showed that cell lines and normal or cancerous colon, expressed almost exclusively the short form, while the long form was mainly expressed by the embryonic colon fibroblast cell line CCD112CoN. Immunochemistry approaches using colon cancer cells permanently expressing either *B4GALNT2* cDNAs as controls, led to the observation of several protein isoforms in human normal and cancerous colon, and cell lines. We showed that tissues expressing B4GALNT2 protein isoforms were able to induce Sd^a and to inhibit sLe^x expression; both of which are expressed mainly on PNGase F-insensitive carbohydrate chains. Concomitant expression of *B4GALNT2* and siRNA-mediated inhibition of *FUT6*, the major fucosyltransferase involved in sLe^x synthesis in colon, resulted in a cumulative inhibition of sLe^x. In normal colon samples a significant relationship between sLe^x expression and the ratio between FUT6/B4GALNT2 activities exists, demonstrating for the first time a role for B4GALNT2 in sLe^x inhibition *in vivo*.

1. Introduction

The histo-blood group carbohydrate antigen Sd^a (GalNAcβ1-4[Neu5Acα2-3]Galβ) (Morton et al., 1970) occurs in several human tissues with high tissue and cell type specificity (Dall'Olio et al., 2014). In particular, it has been primarily described along the gastrointestinal tract on the brush borders of epithelial cells and in the goblet cells of the large intestine. Structural analyses have shown that the Sd^a antigen is expressed on core 3 *O*-glycans of mucins in the human healthy descending colon (Capon et al., 2001) with an increasing gradient from ileum to descending colon (Robbe et al., 2003). In addition, it is not detected in human fetal colonic mucins (Robbe-Masselot et al., 2009b), consistent with the observation that Sd^a is developmentally regulated and its expression increases with age (Dall'Olio et al., 1990, Macvie et al., 1967). The use of various monoclonal antibodies specific for Sd^a antigen and/or G_{M2} ganglioside allowed the detection on immunostained thin-layer chromatography of an Sd^a containing glycolipid different from G_{M2} mostly expressed in chief cells enriched fractions of the healthy stomach (Dohi et al., 1991, Dohi and Kawamura, 2008, Kawamura et al., 2005) and confirmed immunohistologically the presence of Sd^a onto glycoproteins in healthy colon (Dohi et al., 1991, Kawamura et al., 2005). Interestingly, although the biological significance of Sd^a in healthy colon remains largely unknown, Sd^a carbohydrate shows an altered expression patterns

* Corresponding author. Unité de Glycobiologie Structurale et Fonctionnelle, UMR CNRS 8576, Université Lille Nord de France, Lille1, 59655 Villeneuve d'Ascq, France. Tel.: +33 320 33 62 46; fax: +33 320 43 65 55.
E-mail address: anne.harduin@univ-lille1.fr (A. Harduin-Lepers).
[1] Equal contribution.

http://dx.doi.org/10.1016/j.biocel.2014.06.009
1357-2725/

Table 1
Oligonucleotide sequences of primers used for qPCR. Table shows the oligonucleotide sequences (Eurogentec, Seraing, Belgium) used as primers for the PCR reactions. Primer pairs were designed using Primer3 software (http://frodo.wi.mit.edu/primer3/). Primer concentration, length of amplified fragment and efficiency of amplification checked by serial dilutions of cDNA from CCD 841 CoN cells, CCD 112 CoN or a plasmid containing the MF transcript and was found to be between 99.4 ± 1.13 and 101 ± 0.14 are also indicated.

Primer/exon location	Sequence	Primer Concentration (nM)	Amplified fragment	Efficiency (%)
HPRT	5′-GCCAGACTTTGTTGGATTTG-3′	300 nM	141 bp	99.6 ± 1.55
HPRT	5′-CTCTCATCTTAGGCTTTGTATTTTG-3′			
B4GALNT2 SF/E1s	5′-AGTCTGAGGGCGGCGGGATTC-3′	100 nM	143 bp	100.3 ± 1.06
B4GALNT2 SF/E2	5′-GGGCTGCTGAACACTGCTTG-3′			
B4GALNT2 LF/E1L	5′-GAACTCAGAGGCGCTGACC-3′	200 nM	97 bp	100.1 ± 0.14
B4GALNT2 LF/E2	5′-CCAATATCTTGAGGAGCCACAG-3′			
B4GALNT2 MF/E1M	5′-GGACACAGCATAGCGAGGAA-3′	300 nM	150 bp	98.9 ± 0.92
B4GALNT2 MF/E2	5′-ACTTGGGAGTTCTGGCTTGG-3′			

during oncodevelopmental processes with reduced expression in human colon carcinoma cells (Malagolini et al., 2007). It is strikingly absent from mucin *O*-glycans in cancerous gastrointestinal tract (Robbe-Masselot et al., 2009a), whereas the selectin ligand sialyl Lewis x (sLe^x, Neu5Acα2-3Galβ1-4[Fucα1-3]GlcNAc) is up-regulated. Owing to the common structure of the oligosaccharide(s) carrying either the Sd^a or sLe^x antigens, it has been demonstrated *in vitro* that the biosynthesis of the two structures is mutually exclusive (Kawamura et al., 2005, Malagolini et al., 2007). However, the molecular basis of sLe^x over-expression at the expense of Sd^a antigen is still largely unknown.

The coordinated action of galactosyltransferases (B4GALT1 or B3GALT5) and α2,3-sialyltransferases (ST3GAL3, ST3GAL4 or ST3GAL6) results in a carbohydrate precursor, which is the common substrate of either α1,3/4-fucosyltransferases (FUT3, FUT4, FUT5, FUT6, FUT7) leading to sLe^x antigen, or of a unique B4GALNT2 leading to Sd^a synthesis. Sialyltransferases have received much attention since they are up regulated in cancer (Dall'Olio and Chiricolo, 2001, Gomes and Reis, 2013, Harduin-Lepers et al., 2012, Harduin-Lepers et al., 2001). Although a major role in generating sLe^x in colon was attributed to FUT6 (Trinchera et al., 2011), no up-regulation of one or another of these glycosyltransferases could be demonstrated in colorectal cancer that could account for increased sLe^x expression (Ito et al., 1997, Kudo et al., 1998, Misonou et al., 2009). *B4GALNT2* gene expression levels in cancer cells are partially controlled by DNA hypermethylation (Kawamura et al., 2008, Wang et al., 2008); this could play a role in regulating sLe$^{x/a}$ expression (Malagolini et al., 2007, Trinchera et al., 2011). Of particular interest is the observation that forced expression of *B4GALNT2* cDNA in various gastrointestinal cell lines caused, besides Sd^a expression (Malagolini et al., 2007), a remarkable elimination of carbohydrate ligands for selectins and reduced metastatic potential (Kawamura et al., 2005). Another intriguing issue is the observation that there is no close quantitative relation between *B4GALNT2* mRNA level and enzyme activity, which appears to be lower in colorectal carcinoma compared to the healthy mucosa surrounding the tumor (Dohi and Kawamura, 2008, Malagolini et al., 1989) even though they could be detected in 30–40% of gastric and colonic cancer tissues (Dohi et al., 1996). Interestingly, the human *B4GALNT2* gene specifies at least two sets of mRNA differing in their 5′-end in differentiated Caco-2 cells resulting from the use of two different start sites located in exon 1S (short) and in exon 1L (long), 200 bp apart in the human genome (Lo Presti et al., 2003, Montiel et al., 2003). Beside these two transcript variants, a third one was predicted *in silico*, but never experimentally reported. It is devoid of any putative transmembrane sequence and would result from the use of a third alternative first exon called 1 M (middle) (Dall'Olio et al., 2014). Consequently, the human *B4GALNT2* gene can potentially give rise to two different transmembrane and one soluble polypeptide isoforms.

In this work, we aimed to clarify the molecular basis of Sd^a expression in healthy human gastrointestinal tract and its down regulated expression in colon cancer to the benefit of sLe^x. In an attempt to understand the differential contribution of the previously identified B4GALNT2 isoforms in Sd^a biosynthesis, we firstly conducted a complete study focusing on mRNA, protein and enzymatic activity of B4GALNT2 as well as on the expression of the Sd^a and sLe^x antigens in various colonic cell lines and in human gastrointestinal samples. We showed differential spatio-temporal *B4GALNT2* gene expression and expression of several B4GALNT2 protein isoforms, highlighting differences between colonic cell lines and human normal/cancer paired-biopsies. Most of the changes in glycosylation pattern that occur during colorectal oncogenesis are associated with down regulated expression of the short *B4GALNT2* transcript variant and protein isoform. We also addressed the issue of the downregulated expression of sLe^x in healthy colon samples, showing that concomitant B4GALNT2 expression and FUT6 silencing cooperate in the inhibition of sLe^x expression.

2. Materials and methods

2.1. Commercial and surgical human specimens

Total RNA from normal human stomach pooled from 3 female donors aged 39, 52 and 70 years (Batch 0006056559), fetal colon from a single 20 weeks old female donor (Batch 0006071667), and fetal stomach pooled from 10 male donors gestation 18 (2 donors), 19 (3 donors), 20 (4 donors) and 21 weeks (Batch 0006049059) were from Stratagene/Agilent Technologies Inc. (Les Ulis, Cedex, France). Total RNA from human colon adenocarcinoma isolated from a 60 year-old male (Batch 1106273A), stomach adenocarcinoma isolated from a 69-year-old male Caucasian (Batch 1012237A), and normal colon with mucosal lining pooled from 5 suddenly dead male Asians, aged 20-44 (Batch 1005014) were from Clontech/Ozyme (St Quentin en Yvelines Cedex). Proteins from fetal colon from a single female 37 weeks old donor (Batch B410228), healthy adult stomach from a single 60 years old male donor (Batch A310005), fetal stomach from a single 20 weeks old female donor (Batch A605258) and tumor stomach from a single 52 years old male donor (Batch A808231) were from Biochain/Clinisciences (Nanterre, France). Colonic paired samples (tumor and adjacent normal colonic mucosa of the same patient) were collected after colorectal surgery according to a protocol approved by the French Ministry of Higher Education and Research, agreement number DC-2008-242 and from the S. Orsola University Hospital of Bologna, approved by the Senior Committee Board regulating noninterventional studies. The fresh specimens were stored at −80 °C until use.

2.2. Cell lines and culture

Cell lines were from ATCC (LGC Standards SARL, Molsheim, France), and transfected LS 174T cells were previously described (Malagolini et al., 2007). The human colon carcinoma cells HT-29