The International Journal of Biochemistry & Cell Biology 45 (2013) 792–797

Contents lists available at SciVerse ScienceDirect

The International Journal of Biochemistry & Cell Biology

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

CA19.9 antigen circulating in the serum of colon cancer patients: Where is it from?

Lydia Mare[a], Anna Caretti[b], Riccardo Albertini[c], Marco Trinchera[a,*]

[a] Department of Medicine Clinical and Experimental (DMCS), University of Insubria Medical School, 21100 Varese, Italy
[b] Department of Health Sciences, San Paolo Hospital, Università degli Studi di Milano, 20142 Milan, Italy
[c] Laboratory of Clinical Chemistry, IRCCS Policlinico San Matteo, P.le Golgi 5, 27100 Pavia, Italy

ARTICLE INFO

Article history:
Received 30 October 2012
Received in revised form 3 December 2012
Accepted 8 January 2013
Available online 17 January 2013

Keywords:
Tumor marker
Colon cancer
Carbohydrate antigen
Hepatobiliary system
Gastrointestinal tract

ABSTRACT

CA19.9 antigen is a glycoprotein present in human serum and found elevated in various diseases. It is intensively studied since long time as a potential marker for managing cancers of the gastrointestinal tract, but its reliability is widely accepted only for pancreatic cancers. Here, we focused on the tetrasaccharide epitope (NeuAcα2-3Galβ1-3[Fucα1-4]GlcNAc) sialyl-Lewis a studying the biosynthesis, expression, and secretion in colon cancers and related cancer cell lines. We found that the β1,3 galactosyltransferase β3Gal-T5, responsible for sialyl-Lewis a synthesis, is dramatically reduced in colon adenocarcinomas, in terms of both transcript and enzyme activity levels. Moreover, no or very faint antigen is detectable in colon cancer homogenates, by dot-blot or enzyme immunoassay, while it is commonly evident in sera from different patients. In cancer cell lines synthesizing CA19.9, the amount of antigen secreted is proportional to that expressed on the cell surface, and depends on appreciable levels of β3Gal-T5, which appear much higher than those measured in colon cancer specimens. Since colon cancers appear unable to synthesize relevant amount of CA19.9, we suggest that the antigen circulating in the serum of colon cancer patients may have a different and more complex origin than expected so far.

1. Introduction

Sialyl-Lewis a is the tetrasaccharide epitope (NeuAcα2-3Galβ1-3[Fucα1-4]GlcNAc)[1] characterizing the CA19.9 antigen (Magnani et al., 1983; Yue et al., 2011a) present in human serum and found elevated in various diseases (Mann et al., 2000) including cancers of the digestive tract, as pancreas (Ballehaninna and Chamberlain, 2012), bile ducts (Kikkawa et al., 2012), stomach (Kim et al., 2011), and colon (Yamashita and Watanabe, 2009). At present, CA19.9 is recommended by medical societies and study groups for managing cancers of the pancreas but not of the colon (Duffy et al., 2003, 2007, 2010; Locker et al., 2006), but is still widely used in clinical practice, trials, and studies concerning colorectal cancer diagnosis, prognosis, surveillance, and response to therapy (de Haas et al., 2010; Byström et al., 2012; Lin et al., 2012). The existence of such controversial issues and the finding of high CA19.9 in the serum of patients suffering non-malignant diseases (van der Veek et al., 2011), open questions about the biology of such molecule and the rationale of the use as a reliable marker. Little is known about CA19.9 structure, synthesis and secretion in the different tissues, and nothing about the origin of that circulating in health individuals. Recently, it was found that multiple proteins carry the epitope in pancreatic cancer and none appears responsible for cancer up-regulation (Yue et al., 2011b). This may suggest that the synthesis of the carbohydrate epitope is limiting in determining the serum levels. In fact, Narimatsu et al. (1998) reported that *FUT3* (Lewis gene) dosage affects CA19.9 positively, while *FUT2* (Secretor type α1,2 fucosyltransferase, competing for the synthesis) negatively. In colon cancer, however, among specific glycosyltransferases required for epitope biosynthesis (fucosyltransferase Fuc-TIII, α2,3 sialyltransferases ST3GalIV or ST3GalIII, and β1,3 galactosyltransferase β3Gal-T5) none is up regulated (Ito et al., 1997; Kudo et al., 1998; Misonou et al., 2009; Dall'Olio et al., 2012). In particular, β3Gal-T5 transcript is expressed in normal colon mucosa but dramatically down-regulated in adenocarcinomas (Salvini et al., 2001; Isshiki et al., 2003; Caretti et al., 2012), confirming the observation that β1,3Gal-T activity is impaired in colon cancers (Seko et al., 1996; Misonou et al., 2009), where type 2 chain oligosaccharides (based on Galβ1-4GlcNAc sequence) predominate over type 1 chain (based on Galβ1-3GlcNAc sequence) in various glycoconjugates (Misonou et al., 2009). On this light, the

* Corresponding author at: DMCS, via JH Dunant 5, 21100, Varese, Italy. Tel.: +39 0332 397 160; fax: +39 0332 397 119.
E-mail address: marco.trinchera@uninsubria.it (M. Trinchera).

[1] The abbreviations used are: sLea, sialyl-Lewis a, NeuAcα2-3Galβ1-3[Fucα1-4]GlcNAc; sLex, sialyl-Lewis x, NeuAcα2-3Galβ1-4[Fucα1-3]GlcNAc; Gal-T, galactosyltransferase; Fuc-TIII, α1,3/1,4fucosyltransferase type III (product of *FUT3* gene); EIA, enzyme immunoassay; CEA, carcinoembryonic antigen.

1357-2725/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biocel.2013.01.004

concept that CA19.9 is a cancer-associated antigen in human colon appears paradoxical and deserves more investigations.

To address this issue we measured the levels of β3Gal-T5 transcript and β1,3Gal-T activity in bioptic samples pairs from colon adenocarcinomas and surrounding normal mucosa, and determined the amount of sLea antigen expressed in the tissues. We also evaluated in cultured cell lines expressing the antigen their ability to secrete it in the medium. We then compared the obtained data with the amount of antigen detected in control and patient sera.

2. Materials and methods

2.1. Cell cultures

COLO-205, HCT-15, CACO-2, HT-29 (from human colon adenocarcinomas), and MKN-45 (from human gastric cancer) cells were cultured as previously described (Valli et al., 1998; Salvini et al., 2001). MKN-45 cells expressing Fuc-TIII, named MKN-45-FT, and human pancreatic adenocarcinoma cells BxPC3 and Panc-1, were obtained and cultured as reported (Mare and Trinchera, 2004). CACO-2 cells differentiated in culture were obtained according to the procedure described (Isshiki et al., 2003).

2.2. Preparation of human colon samples and cultured cells for analytical procedures

Human bioptic specimens were collected at surgery, immediately frozen in dry ice and placed in liquid nitrogen until used, as previously reported (Salvini et al., 2001; Caretti et al., 2012). They originated from different parts of the large intestine, including the rectum (samples 3 and 6). Cancers were staged from B2 to C2 (Dukes') and graded from moderately to poorly differentiated. The health mucosa surrounding the cancer lesion in the same surgical resection was referred to as the normal mucosa. For RNA extraction, 1–2 mm^3 of frozen material was cut, placed in 0.3 ml of lysis buffer (Ambion RNAqueous minikit, Invitrogen), homogenated with a rotary homogenizer, and processed for total RNA extraction and DNA removal according to the manufacturer's recommendations. For biochemical analysis, 2–3 mm^3 of frozen material was cut, carefully rinsed twice in 0.5 ml of ice-cold phosphate buffered saline, placed in 0.5 ml of 0.1 M Tris/HCl buffer, pH 7.5, and homogenated with a rotary homogenizer. An aliquot of the homogenates was diluted to different protein concentrations and used for dot-blots, another aliquot was made 0.5 mg/ml Triton X-100 and used as the enzyme source for β1,3Gal-T *in vitro* assay, and a third aliquot was lysated for EIA according to a published procedure (Baeckström et al., 1991). Cultured cells were harvested and freshly processed for flow cytometry as reported (Bardoni et al., 1999; Salvini et al., 2001). For dot-blot, EIA, and enzyme assay, cell pellets were treated as described for bioptic samples but vortexing instead of using the rotary homogenizer.

2.3. RNA analysis

For transcript quantification, competitive RT-PCR was performed essentially as previously reported (Trinchera et al., 2011; Caretti et al., 2012). First strand cDNA was prepared for samples and controls in the presence, or omitting, the reverse transcriptase, respectively, and reactions incubated under reported conditions. It was amplified (25 μl reaction volume) in the presence of 10 fg of competitor for 35 cycles (β3Gal-T5), or 10 pg of competitor for 25 cycles (β-actin), under reported conditions. No amplification was detected when the control reactions were used as template. Human β-actin and β3Gal-T5 competitors, and oligonucleotide primers, were those already described (Salvini et al., 2001). Fuc-TIII transcript was quantitated exactly as reported (Trinchera et al., 2011).

2.4. Enzyme assay

β1,3Gal-T activity was determined in the reported reaction mixture (Salvini et al., 2001), using 0.6 M GlcNAc as acceptor, in the presence of cell or tissue homogenates at various protein concentrations: 0.5–4.0 mg/ml for cell lines and clones, 0.5–2.0 mg/ml for normal colon mucosa, and 5.0–10 mg/ml for colon cancers. Incubations were done at 37 °C for 120 (cancers) or 60 min (all others). At the end of incubation, reaction products were assayed by Dowex chromatography and characterized according to previously reported protocols (Valli et al., 1998). In all cases the reaction product was found to be a disaccharide sensitive to β1,3galactosidase, as expected. In fact, GlcNAc is not used as acceptor by β1,4galactosyltransferases under the reported assay conditions (Valli et al., 1998; Bardoni et al., 1999). One unit of β1,3Gal-T activity corresponds to one nanomole of transferred Gal per mg of protein homogenate per hour.

2.5. sLea detection

Detection of sLea by immunofluorescence and flow cytometry was performed as previously reported (Salvini et al., 2001). For dot-blots, aliquots from homogenates, sera, bile, or culture media were applied to the membrane by vacuum aspiration. Serial dilutions of samples were performed in preliminary experiments to set the optimal protein concentrations and amounts needed for detection. Blotting membranes were washed, blocked, stained with primary and peroxidase-labeled secondary antibodies, and visualized by enhanced chemiluminescence as reported for western-blot (Caretti et al., 2012). Monoclonal anti-sLea (from hybridoma 1116-NS-19-9) antibody was prepared as reported (Bardoni et al., 1999). Quantification of CA19.9 in serum and bile samples, in culture media, or in tissue and cell lysates was performed by EIA with an automated analytical system (Cobas Core II, Roche Diagnostics) equipped with dedicated reagents, according to the instructions of the manufacturer.

3. Results

3.1. β1,3Gal-T activity, β3Gal-T5 transcript levels, and sLea expression in normal colon mucosa and adenocarcinomas.

To evaluate the actual ability of colon tissues to synthesize large amounts of sLea, we determined β3Gal-T5 transcript and activity in 9 sample pairs from patient biopsies, each representing colon adenocarcinomas and surrounding normal mucosa. Serum levels of CA19.9 were 115, 84.6, and 19.2 U/ml in patients 9, 8, and 4, respectively (normal range <37 U/ml), and not available in the others. Fuc-TIII transcript was found to be heterogeneously expressed in all samples (not shown), as expected (Ito et al., 1997; Salvini et al., 2001; Trinchera et al., 2011).

The amount of β3Gal-T5 transcript expressed in normal colon mucosa is high but variable, ranging from 3 to 20 fg/pg of β-actin, and the enzyme activity ranges from 22 to 120 U. In cancer samples, β1,3Gal-T activity closely follows transcript down-regulation (Fig. 1, panel A, note the different scales used). The calculated reduction is 36.2-folds on average, ranging from a minimum of 6.2-folds in sample 6 to a maximum of 68-folds in sample 9. Interestingly, in a single adenocarcinoma case (sample 5) where the transcript level is relatively maintained (0.9 fg/pg of β-actin) the enzyme activity reaches the value of 13.2 U, while it is lower than 4 U in all other samples. This data indicate that β3Gal-T5 down-regulation in colon cancers leads to extremely low values of enzyme activity.