Biochimica et Biophysica Acta xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

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

Unexpected distribution of CA19.9 and other type 1 chain Lewis antigens in normal and cancer tissues of colon and pancreas: Importance of the detection method and role of glycosyltransferase regulation

Adele Aronica [a], Laura Avagliano [a], Anna Caretti [a], Delfina Tosi [a], Gaetano Pietro Bulfamante [a,b], Marco Trinchera [c,*]

[a] Department of Health Sciences, San Paolo Hospital Medical School, University of Milan, 20142 Milano, Italy
[b] Unit of Human Pathology, Cytogenetics and Molecular Biology, ASST Santi Paolo e Carlo, 20142 Milano, Italy
[c] Department of Medicine Clinical and Experimental (DMCS), University of Insubria Medical School, 21100 Varese, Italy

ARTICLE INFO

Article history:
Received 22 April 2016
Received in revised form 29 July 2016
Accepted 12 August 2016
Available online xxxx

Keywords:
Carbohydrate antigen
Colon cancer
Glycosyltransferase
Pancreatic cancer
Tumor marker, DNA methylation

ABSTRACT

Background: CA19.9 antigen has been assumed as an abundant product of cancer cells, due to the reactivity found by immunohistochemical staining of cancer tissues with *anti*-CA19.9 antibody.
Methods: Expression and biosynthesis of type 1 chain Lewis antigens in the colon and the pancreas were studied by immunodetection in tissue sections and lysates, quantification of glycosyltransferase transcripts, bisulfite sequencing, and chromatin immunoprecipitation assays.
Results: CA19.9 was poorly detectable in normal colon mucosa and almost undetectable in colon cancer, while it was easily detected in the pancreatic ducts, together with Lewis b antigen, under both normal and cancer conditions. B3GALT5 transcripts were down-regulated in colon cancer, while they remained expressed in pancreatic cancer. Even ST3GAL3 transcript appeared well expressed in the pancreas but poorly in the colon, irrespective of normal or cancer conditions. CpG islands flanking B3GALT5 native promoter presented an extremely low degree of methylation in pancreatic cancer with respect to colon cancer. In a DNA region about 1 kb away from the B3GALT5 retroviral promoter, a stretch of CG dinucleotides presented a methylation pattern potentially associated with transcription. Such a DNA region and the transcription factor binding site provided overlapping results by chromatin immunoprecipitation assays, corroborating the hypothesis.
Conclusions: CA19.9 appears as a physiological product whose synthesis strongly depends on the tissue specific and epigenetically-regulated expression of B3GALT5 and ST3GAL3.
General significance: CA19.9 and other Lewis antigens acquire tumor marker properties in the pancreas due to mechanisms giving rise to reabsorption into vessels and elevation in circulating levels.

1. Introduction

Expression of type 1 chain Lewis antigens in the gastrointestinal tract requires the concurrent expression of a set of specific glycosyltransferases. β1,3 galactosyltransferase isoenzyme 5 (B3GALT5) and α1,3/4 fucosyltransferase isoenzyme 3 (FUT3) give rise to the minimal trisaccharide structure of Lewis a antigen (Lea), while α1,2 fucosyltransferase isoenzyme 2 (FUT2) or galactose α2,3 sialyltransferase isoenzyme 3 (ST3GAL3) may complete the tetrasaccharide structures of Lewis b (Leb) and sialyl-Lewis a (sLea), respectively, the latter being the epitope of CA19.9 antigen (Fig. 1). The role of FUT2 and FUT3 is known through in vitro studies [1,2] and well established in vivo by the relevant effects of their polymorphisms in the human population [3–5]. The role of B3GALT5 and ST3GAL3 is currently supported by in vitro studies dealing with acceptor specificity [6,7], over-expression [7,8], and silencing in cancer cell lines [9], while data in vivo are scarce and restricted to B3GALT5 in the colon [7,10]. Since the development of specific *anti*-Lewis antibodies several years ago [11,12] Lea and Leb have been considered present in the membranes and secretions of normal epithelia of the gastrointestinal tract [13] while sLea and cognate CA19.9 antigen have been assumed as a rather specific product of cancer cells, due to the abundant reactivity found by immunohistochemical staining of cancer tissues with *anti*-

Abbreviations: 5AZA, 5'aza-2'deoxycitidine; B3GALT5, β1,3 galactosyltransferase isoenzyme 5; ChIP, chromatin immunoprecipitation; DAPI, 4,6-diamidino-2- phenylindole; FUT2, α1,2 fucosyltransferase isoenzyme 2; FUT3, α1,3/4 fucosyltransferase isoenzyme 3; H&E, hematoxylin/eosin; IF, immunofluorescence; IH, immunohistochemistry; Lea, Lewis a; Leb, Lewis b; LTR, long terminal repeat; qPCR, quantitative real-time polymerase chain reaction; RT, reverse transcription; sLea, sialyl-Lewis a; ST3GAL3, galactose α2,3 sialyltransferase isoenzyme 3; ST6GALNAC6, *N*-acetylgalactosamine α2,6 sialyltransferase isoenzyme 6; UTR, untranslated region.

* Corresponding author at: DMCS, via JH Dunant 5, 21100 Varese, Italy.
E-mail address: marco.trinchera@uninsubria.it (M. Trinchera).

http://dx.doi.org/10.1016/j.bbagen.2016.08.005
0304-4165/

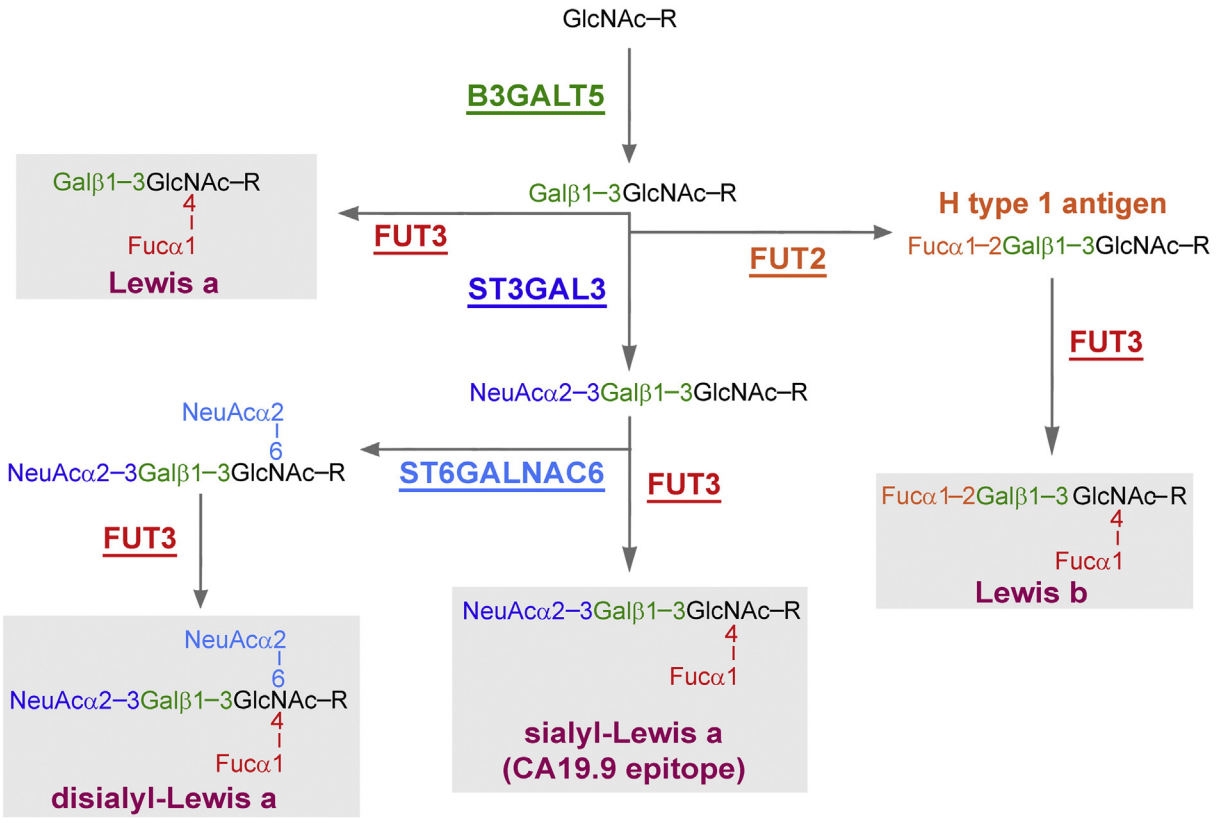

Fig. 1. Biosynthesis of type 1 chain Lewis antigens in epithelial cells of the gastrointestinal tract. Enzymes (underlined) are named according to the HUGO gene nomenclature committee.

CA19.9 antibody [14–18]. Discovery of another glycosyltransferase, *N*-acetylgalactosamine α2,6 sialyltransferase isoenzyme 6 (ST6GALNAC6), able to form disialyl-Lewis a and silenced in colon cancer [17,19], corroborated the concept that CA19.9 is indeed a tumor marker expressed in colon cancer due to the lack of the second sialylation. In addition to the clinical relevance of circulating CA19.9 as a putative indicator of tumor growth, the potential expression of the epitope on the membrane of cancer cells assumed further importance because sLea was found to be an *E*-selectin ligand acting as an adhesion molecule and involved in tumor malignancy [20]. However, detection of CA19.9 in non-malignant conditions has been constantly reported [21–24] and disagrees with the above picture.

Expression of the B3GALT5 gene is driven by two main promoters [25]. One is named the native promoter, since it is conserved through mammalian evolution: it is sensitive to NF-Y transcription factor [26] and is epigenetically regulated through the methylation of two flanking CpG islands [27]. The other is named the LTR promoter because it represents a long terminal repeat of retroviral origin inserted late in evolution, being present in primates following the division of Old World monkeys [28]. It is sensitive to HNF1α/β transcription factors [29] and inhibited by 5'aza-2'deoxycitidine (5AZA) [10], a DNA methyltransferase inhibitor more frequently used to reactivate gene silenced through hypermethylation of their promoters [30,31]. Paradoxically for the biosynthesis of a tumor marker, both B3GALT5 transcripts appeared strongly down-regulated, although through different epigenetic mechanisms, in colon cancer [7,10,27,29]. Moreover, CA19.9 was scarcely detectable by dot-blot staining of colon cancer lysates [32]. More importantly, we recently found a false reactivity of mouse tissues by immunohistochemistry (IH) with *anti*-CA19.9 antibody, while the correct antibody specificity was attained by immunofluorescence (IF) [33]. If confirmed in human tissues, such results may have important consequences for the current concept of CA19.9 as a tumor marker. Consequently, we wanted to revisit the histological, biochemical and molecular aspects of Lewis antigen expression in vivo to assess their actual relevance in different cancers.

In this paper, we stained tissue sections from two colon and pancreas specimens by IF with antibodies against type 1 chain Lewis antigens, comparing IF versus IH in the relevant case of *anti*-CA19.9 antibody. Then, we determined the expression levels of ST3GAL3 and both B3GALT5 transcripts in normal and cancer tissues derived from the colon and the pancreas, and compared the amounts with those of each Lewis antigen present in the corresponding samples, by dot-blot staining of tissue lysates obtained from some of the same matched pairs. Due to the emerging role played by B3GALT5, we also investigated the DNA methylation status of the promoters. In particular, we analyzed the two CpG islands flanking the native promoter and studied some stretches of CG dinucleotides upstream and non-adjacent to the LTR promoter, which appeared to be candidates to act as potential epigenetic regulators of transcription. Chromatin immunoprecipitation (ChIP) assays were also performed in this case to assess the relevance of specific DNA sequences.

2. Materials and methods

2.1. Cell and tissue processing

COLO-205 (colon cancer), MKN-45 (gastric cancer), Huh-7 (hepatocarcinoma), MDA-MB-231 (breast cancer), MDA-MB-231/HNF1α (clone α-1, [10]), BxPC-3 and Capan-2 (both pancreatic cancer) cell lines were grown and treated with 5AZA as previously reported [9,10]. Six human colon and two human pancreas samples were collected at surgery as reported [26]. A matched pair of RNAs from cancer and adjacent normal pancreas (Ambion) and two RNAs from normal pancreas (Clontech and Stratagene, respectively) were of commercial origin. Specimens of tumor and adjacent normal tissue (about 80 mm^3 each) were immediately frozen in dry ice and kept at −80 °C. Frozen

Please cite this article as: A. Aronica, et al., Unexpected distribution of CA19.9 and other type 1 chain Lewis antigens in normal and cancer tissues of colon and pancreas: Import..., Biochim. Biophys. Acta (2016), http://dx.doi.org/10.1016/j.bbagen.2016.08.005