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Expression of MUC1 mucin in full-term pregnancy human placenta

Paszkiewicz-Gadek A, Porowska H*, Średzińska K

Department of Medical Chemistry, Medical University of Bialystok, Bialystok, Poland

* CORRESPONDING AUTHOR:
Department of Medical Chemistry,
Medical University of Bialystok,
Mickiewicza 2A str,
15-239 Bialystok, Poland
telephone: +48 85 748 56 73; fax: +48 85 748 54 16.
e-mail address: zachemog@umwb.edu.pl (Halina Porowska)

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ABSTRACT

Purpose: MUC1 mucin is a component of glycocalyx in human endometrium and may play an important role in generation of "receptive window" at embryo implantation. Considering that MUC1 expression in human placenta is changed during pregnancy, and that MUC1 structure and function are not completely known in this organ, we have undertaken isolation of this mucin and detection of glycan epitopes, since they are crucial for its properties.
Material and methods: Samples of human placenta were homogenized and MUC1 was extracted in different conditions with the use of ionic or non-ionic detergents. Identification of this glycoprotein was performed by Western and lectin blotting, after its purification on Sepharose 4B column.
Results: The best extraction of MUC1 glycoprotein was achieved with a non-ionic detergent, Triton X-100. Reactions with anti-MUC1 antibody showed a few glycoforms with molecular weights between 116 and 205 kDa, with the most visible glycoform approximating 205 kDa. Reactions with lectins enabled detection of carbohydrate antigens, such as T and Tn with sialic acid linked by α2, 3 and to a lesser extent by α2, 6 bond.
Conclusion: MUC1 mucin is present in several glycoforms on the maternal side of human placenta after term delivery. They contain short glycan structures, similar to some tumor carbohydrate antigens.

Key words: Human placenta, MUC1 mucin, carbohydrate antigens

INTRODUCTION

MUC1 is a large transmembrane glycoprotein, which is expressed on the apical surface of most simple epithelia and overexpressed by some carcinomas [1,2]. The core protein of this glycoprotein is translated as a single polypeptide that is cleaved into 2 subunits in endoplasmic reticulum [3,4]. The two fragments form a stable but noncovalent heterodimeric complex that is heavily O-glycosylated during transit of the Golgi complex. After exposure on the plasma membrane, MUC1 is internalized via endocytosis and further sialylated before it is recycled to the cell surface [5,6]. A portion of the membrane-associated MUC1 can be shed, presumably by the second proteolytic cleavage of the ectodomain. In this way, MUC1 enters body fluids or layers on epithelial surfaces, and in effect can modulate cell adhesion to extracellular matrix (ECM) components and contribute to the growth and metastatic properties of tumor [7]. However, its influence varies among different tumor types and its precise function is not completely known [8].

The transmembrane MUC1 mucin is supposed to play a fundamental role in the embryo implantation. Studies of some mammals, such as baboons, pigs, mice and rats have shown a decrease in MUC1 expression throughout the apical surface of uterine epithelium at the time of blastocyst implantation [9, 10]. However, in humans MUC1 glycoprotein is expressed in endometrium in both the proliferative and secretory phase, and MUC1 is lost from epithelial cells only beneath and close to the attached embryo, while normal expression persists in neighbouring cells [11,12]. This loss of MUC1 on the epithelium has been proposed to contribute to successful blastocyst attachment to the uterine epithelium since the antiadhesive effect of mucin molecule is reduced [13].

In addition, MUC1 expression was detected in the human placental syncytiotrophoblast during all stages of pregnancy, and in cells of the decidua in the first and the second trimester of pregnancy [14]. Other investigations have shown that in the mouse placenta, Muc1 protein was located exclusively in the apical surface of the labyrinthine trophoblast around maternal blood sinuses, resembling its luminal location on secretory epithelia [15,16]. In the amnion of normal human placenta, which exhibits a unique physiological barrier between the fetal and external environment, MUC1 gene expression has also been found [17]. Supposedly, MUC1 mucin as transmembrane glycoprotein may occur on all other surfaces of the placenta, although MUC1 glycoprotein structure and its function are unknown in this organ. The aim of our study was to isolate MUC1 membrane glycoprotein from the full-term placenta, and to identify its glycan antigens, since they affect the protective and adhesive properties of mucin.

MATERIAL AND METHODS

The study was approved by the Committee for Ethics and Supervision on Human and Animal Research, Medical University of Bialystok, with informed consent from the patients.

Tissue material

Placental tissue samples were provided by the Department of Obstetrics and Gynecology of J. Śniadecki District Hospital, Bialystok. The tissues were obtained from 10 healthy mothers, aged 20-30, after term vaginal delivery between 37 and 40 weeks of gestation. A thin layer (about 2mm) from the maternal part of each placenta was excised using microsurgical technique and collected in ice. The tissue samples were washed with 0.9% NaCl and submitted to isolation of MUC1 mucin; additionally, the sections were subjected to histopathological examination, which confirmed the presence of decidual transformation.

Extraction and purification of MUC1 mucin

The isolation and extraction procedures were carried out at 4°C. Preliminary homogenates (25% w/v) were prepared in phosphate-buffered saline (PBS, 0.01M Na_2HPO_4, 0.01M NaH_2PO_4, and 0.14M NaCl, pH 7.4) containing 1mM EDTA and protease inhibitors cocktail (Sigma), with the use of a knife homogenizer. Mucin extraction was performed by mixing (on a magnetic stirrer, at 4°C for 1h) these homogenates with a solution of detergents: SDS or Triton X-100, at final concentration of 0.5% and 1% in PBS. Some homogenates were sonificated (3 times for 15s; 0°C, 50 Hz) and then extracted with 1% Triton X-100. The other extraction procedure included long-time mixing (12h) of crushed tissue without detergents. All the extracts were centrifuged at 16,000 rpm for 30min and a protein concentration was determined in the supernatants, with the use of BCA Protein Assay Reagent Kit (PIERCE, USA). The supernatants were used for further experiments as "homogenates".

In order to partly purify MUC1 mucin, the homogenate with the highest glycoprotein contents (estimated after electrophoresis and reaction with Schiff's reagent) was passed through Sepharose 4B column (1.3 × 20.0cm) [18], which was equilibrated and eluted with PBS, containing 0.1M NaCl and 0.1% Triton X-100. Each protein fraction was examined in a dot-blot test with anti-MUC1 mucin antibody [19]. Briefly, 20µl of each fraction were dotted onto an Immobilon-P membrane, dried at room temperature; then the membrane was submitted to the procedure analogous to Western blotting. MUC1–positive fractions were concentrated in dialyzing tube against solid polyethylene glycol, then dialyzed with PBS containing 0.1M NaCl and 0.1% Triton X-100.

Sodium dodecyl sulphate - polyacrylamide gel electrophoresis (SDS –PAGE), Western and lectin blotting

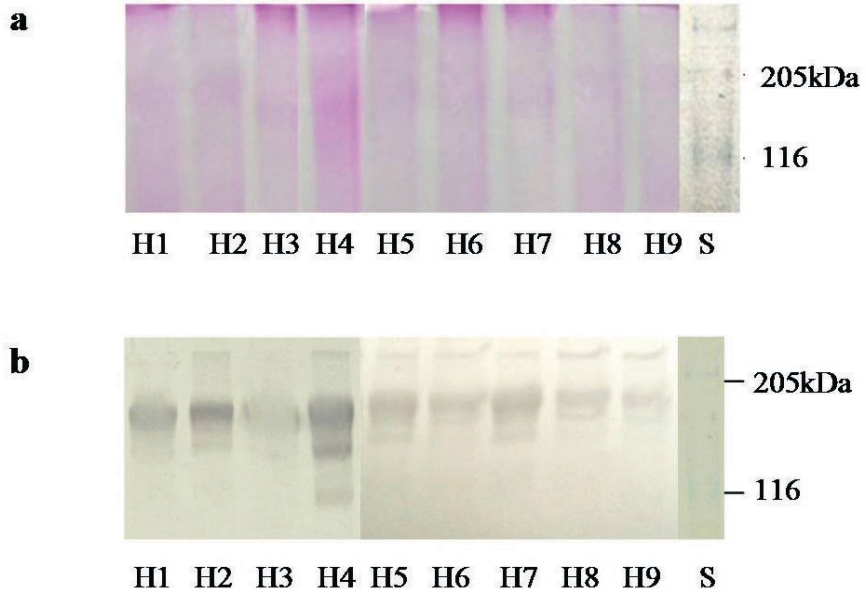
Equal amounts of protein (30-50µg) from homogenates or MUC1-positive fractions (after Sepharose) were submitted to electrophoresis on 4% stacking and 7.5% running SDS-polyacrylamide gels, according to Laemmli method [20]. Pre-stained high molecular weight protein markers (range 29,000-205,000, Sigma) were used as standards. Some gels were stained for glycoproteins with periodic acid/Schiff's reagent [21] and other gels were used for electro-transferring onto Immobilon-P membranes (Millipore, USA) [18]. MUC1 protein was detected on the membrane by the use of anti-MUC1 monoclonal antibody (MAb 4058, Chemicon International, USA; 1:500 dilutions, 2h) and anti-mouse IgG as secondary antibody, which was conjugated with peroxidase.

The sialic acid residues and other carbohydrate epitopes were detected on the membrane by the reaction with peroxidase-labelled lectins: from *Maackia amurensis* (MAA) to recognize α2,3 linked sialic acid, from *Sambucus nigra* (SNA) to recognize α2,6 linked SA, from *Arachis hypogaea* - to detect T antigen and from *Vicia villosa* - to detect Tn antigen. Epitopes: T and Tn were identified after the removal of terminal sialic acid residues by membrane incubation with *Vibrio cholerae* neuraminidase (15mU/ml, 1.5h). All the membranes were submitted to the reaction with 4-chloro-1-naphtol/H_2O_2, as a specific reagent for peroxidase.

RESULTS

Extraction of MUC1 was performed with the use of SDS or Triton X-100, ionic and non-ionic detergents, at a final concentration of 0.5% and 1%, to find the best isolation conditions. Additionally, some preliminary homogenates were sonificated before extraction with the most effective detergent; the other procedure included long-time mixing of crushed tissue without detergents. The samples of each

Figure 1. **Electrophoresis and detection of MUC1 glycoprotein in homogenates by reaction with Schiff's reagent (a) and by Western blotting with anti-MUC1 antibody (b).**

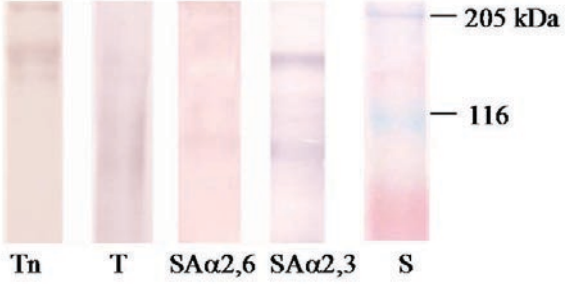


H1- homogenate mixed with 0.5% SDS (1h)
H2 – homogenate mixed with 0.5% TritonX-100 (1h)
H3 - homogenate mixed with 1.0% SDS (1h)
H4 - homogenate mixed with 1.0% TritonX-100 (1h)
H5 - sonificated homogenate mixed with 0.5% Triton X-100 (1h)
H6 - sonificated homogenate mixed with 1% Triton X-100 (1h)
H7 - sonificated homogenate without detergent
H8 – tissue homogenate without detergent (12h)
H9 – crushed tissue mixed with PBS (12h)

homogenate were electrophoresed and subjected to detection of glycoproteins, in reaction with Schiff's reagent, and with anti-MUC1 antibody. As could be seen in Figure 1A, the glycoproteins were located on the top of gels and additionally in the position occupied by high molecular weight proteins (116 - 205 kDa) (Fig. 1A, Schiff's reaction). The reaction with anti-MUC1 antibody (Western blotting) confirmed the presence of MUC1 in all homogenates and this protein was represented during electrophoresis as several distinct bands, corresponding to the proteins with molecular weights between 116 and 205 kDa, with the most visible protein approximating 205 kDa (Fig.1B). The highest efficiency of MUC1 extraction was obtained with 1% of Triton X-100, similarly to the highest concentration of carbohydrates (homogenate H4).

In order to separate MUC1 protein from other higher molecular weight glycoproteins, the extract with the highest concentration of MUC1 (H4) was used for gel filtration on Sepharose 4B column. The fractions containing MUC1 protein were concentrated and submitted to Western and lectin blotting. Reactions with lectins showed the presence of glycan epitopes, namely Tn (N-acetylgalactosamine-α1-O-Ser/Thr) and T (galactose-β1-3-N-acetylgalactosamine-α1-O-Ser/Thr) antigens. Some of these epitopes were terminated with sialic acid residues, linked by α2,3 bond and, to a lesser degree, by α2,6 bond, which was shown in reactions with

Figure 2. **Detection of glycan epitopes using lectins (PNA, VVA, MAA and SNA), in partially purified MUC1 from H4 homogenate.**



Maackia amurensis and *Sambucus nigra* lectins (Fig. 2). Since the glycan bands on the membrane were found in the same position as MUC1, it could be supposed that these antigens were carried by MUC1 protein.

DISCUSSION

The blastocyst attachment to endometrial epithelium is followed by trophoblast invasion of the endometrium and endometrial vasculature. The interaction between trophoblast and

endothelial cells is important for normal placental development and for establishing a blood supply to the developing fetus. Several adhesion molecule systems have been proposed to play a role in this interaction [22, 23]. Some authors suggest that MUC1 is expressed by early pregnancy macaque trophoblast and plays a role in the blastocyst attachment to endothelium and migration across it [24]. Although the placenta after delivery is among the most easily accessible human tissues, only a few studies have been concerned with detection of MUC1 transmembrane glycoprotein in this organ [25, 26]. To sum up, there are no available data related to the role of MUC1 and to its terminal saccharide epitopes in the placenta in a later stage of pregnancy.

Short carbohydrate epitopes, such as T antigen, occur in only limited amounts in normal adult human tissues [27]. Their expression is restricted to specific carrier proteins, for example MUC1 glycoprotein, and the expression of T antigen is also owned as an oncodevelopmental property [28]. Recently, it has been shown that the T oncofetal carbohydrate antigen, associated with MUC1, is a natural ligand for galectin-3, a member of the family of naturally occurring galactoside-binding lectins. This interaction may play a critical role in cancer cell adhesion to endothelium and hence in cancer progression and metastasis [29]. The expression of T antigen was also evaluated in the syncytiotrophoblast layer of placenta in all three trimesters of normal pregnancy and abort placentas by immunohistochemical methods, confirming the phenotypic similarities between the trophoblast cells and carcinoma cells [15, 26]. Moreover, bacteria have been shown to interact with specific saccharides and oligosaccharides expressed on mucins in both the respiratory [30] and gastric organs [31]. Several studies have confirmed that mucins can inhibit *Helicobacter pylori* binding to the epithelium [31, 32]. If cell surface mucins (MUC1) expose ligands for *H pylori* adhesions, then a reduction in mucin synthesis could decrease binding of bacteria to epithelial cells. Similarly, it was indicated that the addition of sialic acid residues to mucin (mouse Muc1) significantly inhibited bacterial adhesion to the epithelium [31].

CONCLUSIONS

In our study, MUC1 protein with such glycan epitopes as T and Tn, terminated with sialic acid residues, occurs in full-term pregnancy human placenta. Although the function of this protein on the surface of the full-term placental tissue remains to be determined, it is known that glycans resembling some tumor carbohydrate antigens linked to MUC1 protein can be the ligands for lectins or other proteins and mediate in physiological and pathological interactions.

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