Expression of tumor associated antigens CA 15-3 and CA 19-9 in trophoblast of the normal human placenta

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Summary

Mucin 1 (MUC1) is abundantly expressed by various organs, including human placenta and endometrium. Since glycan modifications of MUC1 are potentially relevant for physiological as well as pathological processes, this study was aimed at establishing an expression profile of two MUC1 glycoepitopes, CA 15-3 and CA 19-9, in trophoblast throughout pregnancy. Immunohistochemical analysis of normal placenta demonstrated that trophoblast cells express both mucin antigens throughout gestation with a distinct staining pattern. The staining of villous trophoblast was non-uniform for both antigens, and stronger for CA 15-3. Only a proportion of extravillous trophoblast of the cell column, in decidual stroma or lining blood vessels was also stained. Whether the studied MUC1 glycoforms can be linked to trophoblast cells invasion remains to be established.

Key words: CA 15-3, CA 19-9; Glycosylation; Trophoblast.

Introduction

Mucins are large, heavily glycosylated proteins produced by secretory epithelia, lining the luminal surfaces of gastrointestinal, respiratory and reproductive tracts [1]. One of the mucin glycoproteins, mucin 1 (MUC1), is abundantly expressed by various organs, including acini and ducts of salivary and mammary glands, and prostate gland epithelium [2]. In the reproductive tract of women, MUC1 is expressed at the apical surface of the uterine epithelium, where it has been proposed to regulate embryo attachment [3-5], and in the placenta throughout pregnancy [6, 7]. Studies on human placenta showed that MUC1 is mainly expressed at the fetomaternal interface, by syncytiotrophoblast, fetal epithelium in contact with maternal blood, and by extravillous trophoblast cells invading the decidualized endometrium [6, 7].

Altered glycosylation is significant for the beginning, development and outcome of different human diseases [8, 9]. Remarkable diversity in the carbohydrate composition of MUC1 molecules between normal and tumor tissues has been shown [10]. Glycan modifications of MUC1 in normal and cancer cells are potentially relevant for physiological as well as pathological processes and could be significant for clinical practice.

MUC1 is a carrier of two differently glycosylated antigens, CA 15-3 and CA 19-9, both known as effective serum markers currently used in clinical practice for breast (CA 15-3), colon, and pancreas (CA 19-9) carcinoma [11, 12]. In contrast to the investigation of MUC1 molecules in human trophoblast, expression of different MUC1 glycoforms throughout pregnancy remains poorly understood so far. Previous investigation of CA 19-9 expression in placental tissue and amniotic fluid, revealed presence of this glycoprotein in decidual and amnion epithelial cells, but not in trophoblast subpopulations [13, 14]. Therefore, the aim of this work was to investigate expression of CA 15-3 and CA 19-9 antigens in normal placenta during gestation.

Materials and Methods

Tissue samples and immunohistochemical analysis

For this study material from the first (11 cases) and second trimester (5 cases) of pregnancy and at term (5 cases) was used. Tissue samples were obtained from the Institute of Obstetrics and Gynecology, Clinical Centre of Serbia, Belgrade, in accordance with ethical standards.

Tissue sections were analyzed using monoclonal antibodies to CA 15-3 (clone M411149) and CA 19-9 (clone M602207) (Fitzgerald Industrial International - MA, USA). Trophoblast and decidual cells were identified by immunostaining using monoclonal antibody to cytokeratin-18 (CK-18, Dako Cytomation, Denmark) and to vimentin (clone V6, Sigma, MO, USA), respectively. Endothelial cells were identified by monoclonal antibody to CD34 (Serotec, Oxford, UK; not shown). Primary antibodies were used in the respective dilutions: 1:100 for anti-CA 15-3, 1:40 for anti-CA 19-9, 1:6000 for anti-CK-18, 1:1000 for vimentin and 1:25 for anti-CD34. Antigen unmasking (for CK-18 staining) was performed by boiling the slides in 10 mM citrate buffer, pH 6.0 for 1 min. Immunohistochemistry for all used antibodies was performed as previously described [15], using DAB or Nova Red as chromogen (Vector Laboratories, Burlingame, CA, USA). Omission of the primary antibody resulted in complete absence of staining. Slides were counterstained with hematoxylin, examined and photographed using a Carl Zeiss Axio Imager 1.0 microscope (Jena, Germany), with a Canon A640 Digital Camera System (Tokyo, Japan).

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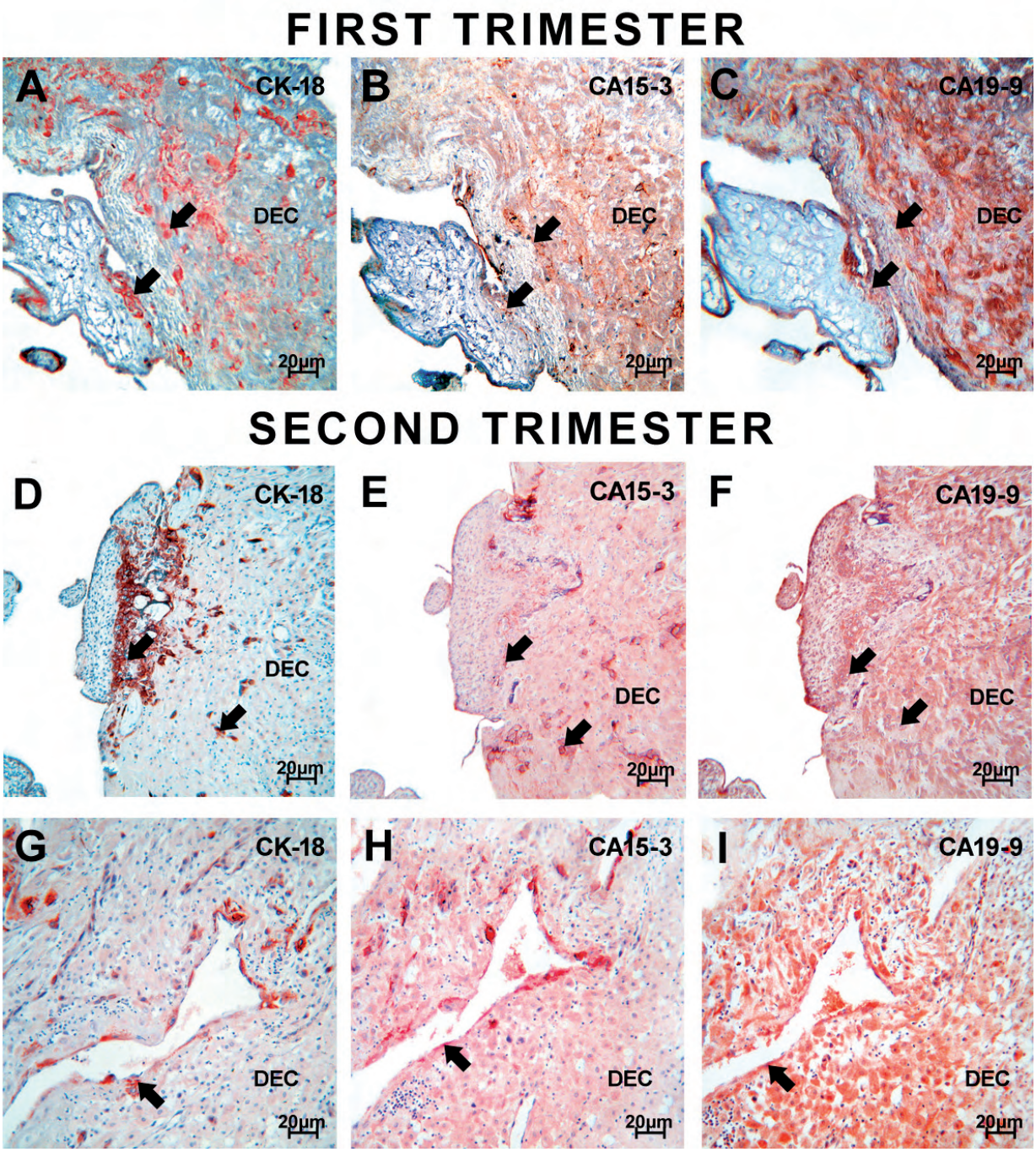

Figure 1. — Immunohistochemical localization of mucin-like epitopes in the first (B, C) and second trimester (E, F, H, I) trophoblast and the decidua. Arrows point to cytokeratin-positive trophoblast cells identified in first (A) and second trimester placentas (D, G), also stained with CA 15-3 in the first (B) and second trimester (E, H), and with CA 19-9 in the first (C) and second trimester (F, I). Decidual cells (DEC) also stained for CA 15-3 (B, E, H) and CA 19-9 (C, F, I). Scale bar represents 20 µm.

Results

Expression of mucin-related glycoepitopes was studied on sections from the first and second trimesters of pregnancy, and at term pregnancy using monoclonal anti-CA 15-3 and anti-CA 19-9 antibodies. In parallel sections, cytokeratin staining was used to identify trophoblast (arrows in Figure 1A, D, G) and vimentin staining to identify decidual cells (Figure 2E). Immunohistochemical analysis of normal placenta demonstrated that trophoblast cells express both mucin/tumor antigens throughout gestation with a distinct staining pattern. In

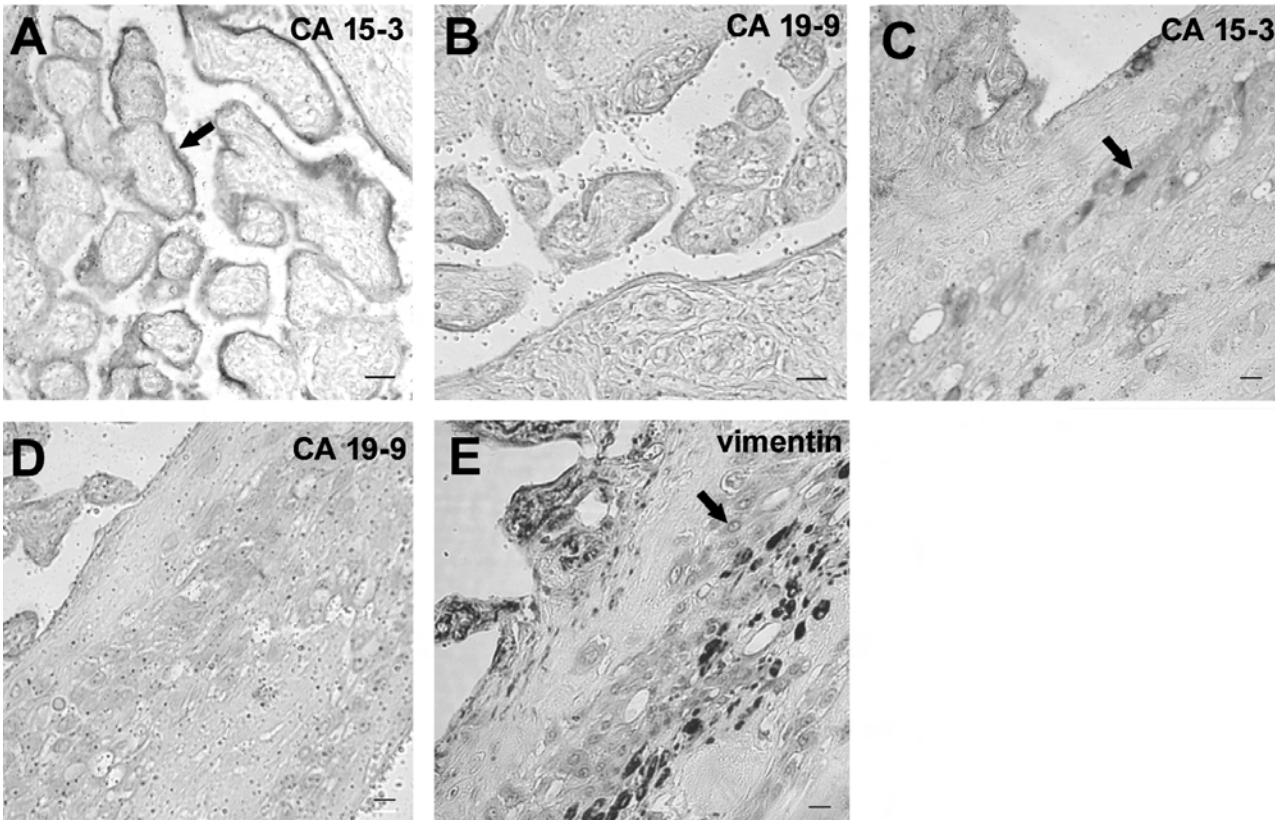

Figure 2. — Immunolocalization of tumor related antigens CA 15-3 (A, C), and CA 19-9 (B, D) at term. Arrows point to syncytiotrophoblast stained with CA 15-3 (A), and trophoblast of placental septae negative to vimentin and positive to CA 15-3 (C). Note absent or weak staining for CA 19-9 (B, D). Decidual cells in placental septae were identified by staining for vimentin (E). Scale bar represents 20 μm.

the first trimester of pregnancy, in all analyzed samples, non-uniform staining with anti-CA 15-3 was detected in trophoblast villous (Figure 1B), ranging from negative to moderate positive staining. Strong staining with CA 15-3 was observed in some, but not all, cytokeratin-positive cells of the cell column, and invasive trophoblast (Figure 1B), while surrounding decidual stromal staining was relatively weak. Regarding CA 19-9 in the first trimester of pregnancy, moderate and non-uniform staining was present in trophoblast villous and in the cell column (Figure 1C). In second trimester placentas staining with CA 15-3 and CA19-9 was detected in the same trophoblast cell types as in the first trimester, as can be observed in panels E and H for CA 15-3, and in F and I for CA 19-9. In addition, trophoblast lining blood vessels were also found to express CA 15-3 (Figure 1H) and CA 19-9 (Figure 1I). At term, there was strong CA 15-3 staining of syncytiotrophoblast (Figure 2A), chorion, and trophoblast of placental septae (Figure 2D). Immunohistochemical analysis demonstrated weak or absent staining for CA19-9 in syncytiotrophoblast (Figure 2B), and weak to moderate staining of chorion and placental septae (placental septae, Figure 2D). Our results also showed that decidual stromal cells express both mucin antigens throughout pregnancy (Figure 1 B, C, E, F, H, I).

Discussion

Mucin MUC1 expressed by human uterine epithelium has been proposed to act as one of the glycoproteins involved in blastocyst attachment [16]. In recent years, studies detected MUC1 in human villous and extravillous trophoblast [6, 7]. Jeschke *et al*. [6] reported using immunohistochemistry strong expression of MUC1 in both first and second trimester placentas, and to a lesser degree in the third trimester, while Shuy *et al*. [7] found weak expression in the first trimester, which increased with gestational age, at mRNA and protein levels. Reported findings of MUC1 expression are somewhat controversial, which might result from the techniques or antibodies used. It is interesting to note that the same finding was obtained for both peptide core and glycoepitope specific antibody [7]. In the present study, localization of two differently glycosylated human MUC1 forms, CA 15-3 and CA 19-9 tumor antigens, in normal placentas during gestation was investigated. We observed that both antigens were present in human trophoblast, but the staining for CA 15-3 was more pronounced than for CA 19-9. On the other hand, decidual stroma was considerably more stained for CA 19-9, and negative to moderately stained for CA 15-3. Invasive trophoblast in the first

and the second trimester of pregnancy were found to consistently express CA 15-3, which is in keeping with the report of Shuy *et al*. [7] with respect to percent of trophoblast stained and relatively weak staining. On the other hand, in our study endovascular trophoblast were also found to express this MUC1 glycoform (CA 15-3), which differs from a previous report by Jeschke *et al*. [6]. Our finding regarding CA 19-9 expression by trophoblast cells is novel, and differs from the previous reports that did not find CA 19-9 antigen in trophoblast cells [13, 14]. There are several glycotopes comprised of short sugar chains which can be found on MUC1, and they include Thomsen-Friedenreich antigen (TF or T antigen), Tn (N-acetylgalactosamine) and sialyl-Tn antigen [2]. The results of immunohistochemical studies in general critically depend on the reactivity of antibodies used. The specific anti-CA 15-3 and CA 19-9 antibodies used here have not been previously used. Data presented in the literature do not relate to the same antibodies, and there is a possibility that the other reported antibodies detected different stages in MUC1 processing.

Overexpression of MUC1 contributes to the malignant phenotype [1]. However, in contrast to the breast and intestinal mucins [17], glycans of placental MUC1 are incompletely known [18]. It has been shown that MUC1 extracted from term placental tissue contains a short glycan structure, T and Tn antigens, similar to tumor carbohydrate antigens [18]. Furthermore, the T antigen associated with MUC1 may play a critical role in cancer cell adhesion to endothelium, through interaction with galectin-3 [19]. Our previous study has shown that gestational trophoblast diseases are associated with increase in galectin-1 and galectin-3 expression compared to the normal trophoblast [20]. There is a possibility that oligosaccharides linked to MUC1 are potential ligands for trophoblast galectins and could thus mediate cell interactions of trophoblast. At present, there is no evidence to show that altered expressions of CA 15-3 or CA 19-9 antigens characteristic for neoplastic tissue are in any way responsible for the development of transformed trophoblast phenotype. However, the presence of MUC1 [7] and CA 15-3 antigen shown here in some, but not all cytokeratin-positive cells of the cell column, invasive and endovascular trophoblast, raises a possibility that this mucin could have an active role in trophoblast cell invasion. It is interesting to note that overexpression of MUC1 was found to decrease invasion of choriocarcinoma cell line JAr [7]. Whether the studied MUC1 glycoforms can be linked to trophoblast cells invasion or not remains to be established.

References

[1] Singh P.K., Hollingsworth M.A.: "Cell-surface associated mucins in signal transduction". *TRENDS in Cell Biol*., 2006, *16*, 467.
[2] Hanisch F.-G., Müller S.: "MUC1: the polymorphic appearance of a human mucin". *Glycobiology,* 2000, *10,* 439.
[3] Aplin J.D.: "MUC-1 glycosylation in endometrium: possible roles of the apical glycocalyx at implantation". *Hum. Reprod.,* 1999 (suppl. 2), 17.
[4] Meseguer M., Aplin J.D., Caballero-Campo P., O'Connor J.E.O., Martin J.C., Remohi J. *et al*.: "Human endometrial MUC1 is up-regulated by progesterone and down-regulated in vitro by the human blastocyst". *Biol. Reprod.,* 2001, *64*, 590.
[5] Carson D.D., Julian J., Lessey B.A., Prakobphol A., Fisher S.J.: "MUC1 is a scaffold for selectin ligands in the human uterus". *Front. Biosci.,* 2006, *11,* 2903.
[6] Jeschke U., Richter D.U., Hammer A., Briese V., Friese K., Karsten U.: "Expression of the Thomsen-Friedenreich antigen and its putative carrier protein mucin1 in the human placenta and in trophoblast cells in vitro". *Histochem. Cell. Biol.,* 2002, *117*, 219.
[7] Shyu M.K., Lin M.C., Liu C.H., Shih J.C., Lee C.N., Chen H.Y. *et al*.: "MUC1 expression is increased during human placental development and suppresses trophoblast-like cell invasion in vitro". *Biol. Reprod.,* 2008, *79*, 233.
[8] Spiro R.: "Protein glycosylation: nature, distribution, enzymatic formation, and disease implication of glycopeptide bonds". *Glycobiology,* 2008, *12*, 43.
[9] Brockhausen I., Kuhns W.: "Glycosylation in cancer and oncogenic transformation". In: Glycoprotein and human disease, Brockhausen I., Kuhns W. (eds.), Heidelberg: Medical Intelligence Unit, Springer, 1997, 157.
[10] Julian J., Carson D.D.: "Formation of MUC1 metabolic complex is conserved in tumor-derived and normal epithelial cells". *Biochem. Biophys. Res. Commun.,* 2002, *293*, 1183.
[11] Duffy M.J.: "CA 15-3 and related mucins as circulating markers in breast cancer". *Ann. Clin. Biochem.,* 1999, *36,* 579.
[12] Steinberg W.: "The clinical utility of the CA 19-9 tumor-associated antigen". *Am. J. Gastroenterol.,* 1990, *85,* 350.
[13] Kobayashi F., Sagawa N., Nanbu Y., Nakamura K., Nonogaki M., Ban C. *et al*.: "Imunohistochemical localization and tissue levels of tumor-associated glycoproteins CA 125 and CA19-9 in the deciduas and fetal membranes at various gestational age". *Am. J. Obstet Gynecol*., 1989, *160,* 1232.
[14] Minas V., Mylonas I., Schiessl B., Mayr D., Schulze S., Friese K. *et al*.: "Expression of the blood-group-related antigens Sialyl Lewis a, Sialyl Lewis x and Sialyl Lewis y in term placentas of normal, preeclampsia, IUGR- and HELLP-comlicated pregnancies". *Histochem. Cell. Biol*., 2007, *128,* 55.
[15] Vićovac Lj, Božić M., Bojić-Trbojević Ž., Golubović S.: "Carcinoembryonic antigen and related molecules in normal and transformed trophoblast". *Placenta*, 2007, *28*, 85.
[16] De Souza M.M., Surveyor G.A., Price R.E., Julian R., Kardon R., Zhou S. *et al*.: "MUC1/episialin: a critical barrier in the female reproductive tract". *J. Reprod. Immunol.,* 1999, *45,* 127.
[17] Cao Y., Blohm D., Ghadimi M., Stosiek P., Xing P.-X., Karsten U.: "Mucins (MUC1 and MUC3) of gastrointestinal and breast epithelia reveal different and heterogenous tumor-associated aberrations in glycosylation". *J. Histochem. Cytochem*., 1997, *45*, 1547.
[18] Paszkiewicz-Gadek A., Porowska H., Sredzinska K.: "Expression of MUC1 mucin in full-term pregnancy human placenta". *Adv. Med. Sci.,* 2008, *53*, 54.
[19] Yu L.-G., Andrews N., Zhao Q., McKean D., Williams J.F., Connor L.J. *et al*.: "Galectin-3 interaction with Thomsen-Friedenreich disaccharide on cancer-associated MUC1 causes increased cancer cell endothelial adhesion". *J. Biol. Chem*., 2006, *282,* 773.
[20] Božić M., Petronijević M., Milenković S., Atanacković J., Lazić J., Vićovac L.J.: "Galectin-1 and galectin-3 in the trophoblast of the gestational trophoblast disease". *Placenta,* 2004, *25,* 797.

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