

Expression of the Thomsen-Friedenreich (TF) Tumor Antigen in Human Abort Placentas

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Abstract. The Thomsen-Friedenreich antigen (TF), or more precisely epitope, has been known as a pancarcinoma antigen. It consists of galactose- β 1-3-N-acetylgalactose. We have already described the expression of TF in the normal placenta. TF is expressed by the syncytium and by extravillous trophoblast cells. In this study, we investigated the expression of TF in the abort placenta. Frozen samples of human abort placentas (12 placentas), obtained from the first and second trimesters of pregnancy and, for comparison, samples of normal placentas (17 placentas) from the first, second and third trimesters of pregnancy, were used. Expression of TF was investigated by immunohistochemical methods. For identification of TF-positive cells in abort placentas, immunofluorescence methods were used. Evaluation of simple and double immunofluorescence was performed on a laser scanning microscope. Furthermore, we isolated trophoblast cells from first and third trimester placentas and evaluated cytokeratin 7 and Muc1 expression by immunofluorescence methods. We observed expression of TF antigen in the syncytiotrophoblasts layer of the placenta in all three trimesters of pregnancy in normal and abort placentas evaluated by immunohistochemical methods. There was no expression of TF antigen in the decidua of abort placentas. Immunofluorescence double staining of TF antigen and cytokeratin 7 showed reduced expression of both antigens in the abort decidua and co-expression of both antigens in the syncytiotrophoblast layer of normal and abort placentas. TF

expression in the syncytiotrophoblast was reduced in abort placentas. In the isolated trophoblast cells, no TF expression was found, however, Muc1 expression was visualized. Expression of TF antigen was reduced in the first and second trimester abort decidua compared to the normal decidua during the same time of pregnancy. TF antigen was restricted to the syncytiotrophoblast and extravillous trophoblast cells in the decidua. Abort placentas expressed TF antigen on the syncytiotrophoblast layer, but with lower intensity compared to normal placentas. We found a significantly reduced co-expression of TF antigen and cytokeratin 7 in the decidua of abort placentas. These data suggested a reduction of extravillous trophoblast cells in the decidua of abort placentas. In addition, we found higher numbers of CD45-positive cells in the abort decidua compared to normal placentas.

The Thomsen-Friedenreich (TF) antigen (or, more precisely, epitope) has been known for a long time as a pancarcinoma antigen (1). The TF antigen is a carbohydrate epitope related to blood group antigens and consists of galactose- β 1-3-N-acetylgalactose (Gal β 1-3GalNAc-). Its expression is restricted to specific carrier proteins in certain cells and organs. In epithelial cells, the TF epitope is carried by mucins, e.g. Mucin1 (Muc1), a family of highly glycosylated proteins present on the surfaces of many glandular epithelial cells. TF carbohydrate epitopes occur in only limited amounts in normal adult human tissues (2). The TF antigen is also expressed by fetal epithelia and mesothelia. The presence of TF during an early fetal phase, its absence in noncarcinomatous postfetal tissues and its association with carcinoma suggest that TF is a stage-specific oncofetal antigen (3). We have already described the expression of TF in the normal placenta (4). Paraffin-embedded placental and decidual tissues of the first, second and third trimesters were incubated with different

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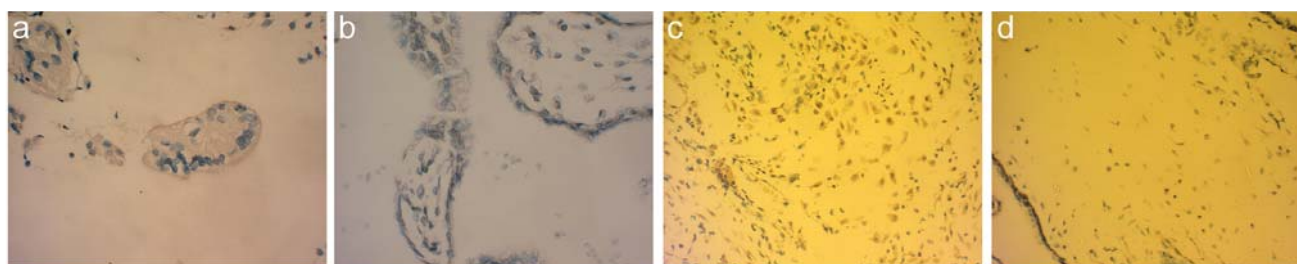


Figure 1. a. TF expression syncytiotrophoblast, 1st trimenon, normal, abruptio. b. TF expression, syncytiotrophoblast, 2nd trimenon, abort. c. TF expression, decidua, 1st trimenon, abruptio. d. lack of TF expression in decidua, 2nd trimenon, abort.

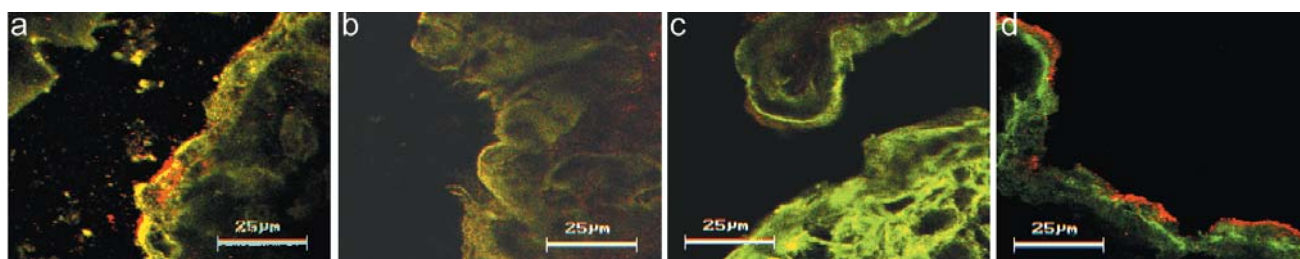


Figure 2. a. Abruptio, 1st trimenon, syncytiotrophoblast, CK 7 pos. (green), TF-positive (red). b. Abort 2nd trimenon, syncytiotrophoblast, CK 7-positive (green), TF-positive (red). c. Term placenta, syncytiotrophoblast, CK 7-positive (green), TF-low-positive (red). d. Deciduitis, abort 1st trimenon, syncytiotrophoblast, CK 7-positive (green), TF-positive (red). Figures 2a-2d double immunofluorescence.

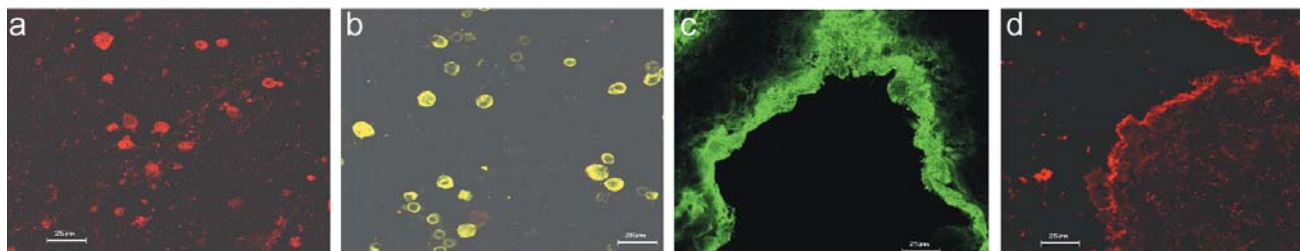


Figure 3. a. isolated trophoblast cells in vitro, abruptio 1st trimenon, CK 7-positive (green), TF-negative. b. Isolated trophoblast cells in vitro, abruptio 1st trimenon, Muc 1-positive (red). c. Immunofluorescence on frozen placental tissue slides, abruptio 1st trimenon, syncytiotrophoblast, CK 7-positive (green). d. Immunofluorescence on frozen placental tissue slides, abruptio 1st trimenon, syncytiotrophoblast, Muc 1-positive (red).

monoclonal antibodies (A78-G/A7 and HH8) directed against the TF epitope and stained with an immunohistochemistry system. We found a strong expression of the TF epitope in the first trimester of pregnancy. In addition, we identified expression of the TF antigen in the second trimester of pregnancy, but only in a few cases was there positive staining in the third trimester of pregnancy. TF is expressed by the syncytium and by extravillous trophoblast cells (5). In the first trimester of pregnancy, we found strong expression of the TF antigen and Muc 1 at the apical side of the syncytiotrophoblast

directed towards the maternal blood. This expression was consistent in the second trimester of pregnancy and, to a lesser degree, in the third trimester. In addition, we found positive staining for the TF antigen and Muc 1 on extravillous trophoblast cells in the decidua during the first and second trimesters of pregnancy. Trophoblast tumour cells of the cell line BeWo, which form a syncytium *in vitro*, were also positive for TF antigen and Muc 1, whereas Jeg3 cells, which are unable to form a syncytium, expressed only Muc 1. In this study, we investigated the expression of TF in the abort placenta.

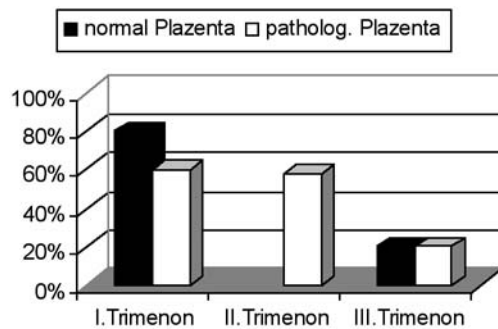


Figure 4. Expression of TF antigen is reduced in first and second trimester abort placentas (red column) compared to the normal placenta (blue column) during the same period of pregnancy. Results were obtained by immunohistochemistry and immunofluorescence methods.

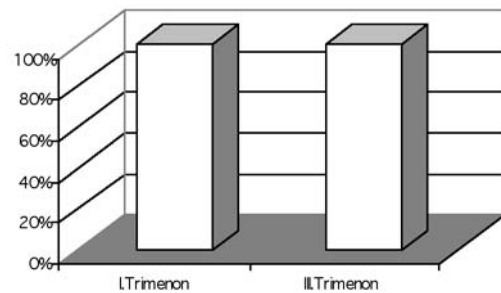


Figure 5. Expression of Muc 1 on freshly isolated trophoblast cells is similar in the first and third trimesters of pregnancy.

Materials and Methods

Immunohistochemistry. Frozen samples of human abort placentas (12), obtained from first and second trimesters of pregnancy and, for comparison, samples of normal placentas (17) from the first, second and third trimesters of pregnancy, were used in the study. Expression of TF was investigated by immunohistochemical methods. For identification of TF-positive cells in abort placentas, immunofluorescence methods were used. Tissue sections were fixed and co-incubated with mouse anti-TF (A78G/A7, IgM) (6) and mouse anti-cytokeratin 7 (IgG). Furthermore, mouse anti-Muc 1 (IgG), mouse anti-CD45 (IgG) and mouse anti-cytokeratin 7 (IgG) also served as primary antibodies. For visualization, samples were incubated with Cy2 anti-mouse IgG and Cy3 conjugated anti-mouse IgM as secondary antibodies. Evaluation of simple and double immunofluorescence was performed on a laser scanning microscope (LSM 410, Zeiss, Germany).

Isolation of trophoblast cells. We isolated trophoblast cells from first (5) and third (8) trimester placentas (abruption- and term-placentas), as described by Jeschke *et al.* (5). Briefly, placental tissue was digested by a standard trypsin-DNase dispersion method of villous tissue, followed by a percoll gradient centrifugation step or by MACS-technique. Trophoblast identity was evaluated by cytokeratin 7 and Muc 1 expression and immunofluorescence methods.

Results

We observed expression of the TF antigen in the syncytiotrophoblasts layer of the placenta in all three trimesters of pregnancy in normal and abort placentas evaluated by immunohistochemical methods (Figure 1a, 1b). There was no expression of the TF antigen in the decidua of abort placentas (Figure 1d). Expression of the TF antigen was observed in normal placentas in the first trimester of pregnancy in the deciduas (Figure 1c). Immunofluorescence double staining of TF antigen and cytokeratin 7 as a marker

of trophoblast identity (7) showed reduced expression of both antigens in the abort decidua and co-expression of both antigens in the syncytiotrophoblast layer of normal and abort placentas (Figure 2a-2d), with reduced TF expression in the abort placenta. Isolated trophoblast cells from the first trimester of pregnancy were characterised by CK 7 immunofluorescence staining (Figure 3a). In the isolated trophoblast cells, we did not find TF expression, however, we visualized Muc 1 expression (Figure 3b). Similar expression pattern could be shown on frozen tissue slides of first trimester placentas. Trophoblast tissue expressed CK 7 (Figure 3c) and Muc 1 (Figure 3d). A summary of the TF staining intensities of normal and abort placentas is given in Figure 4. Expression of the TF antigen was reduced in first and second trimester abort placentas (red column) compared to the normal placenta (blue column) during the same time of pregnancy. The results were obtained by immunohistochemistry and immunofluorescence. Expression of Muc 1 by trophoblast tissue did not change in all three trimesters of pregnancy (Figure 5).

Discussion

The presented data show the expression of the TF epitope and its carrier protein Muc-1 in human normal and abort placentas of the first and second trimesters and in normal placentas in the third trimester of pregnancy. Normally, the expression of the TF antigen, which is considered to be an oncodevelopmental marker, and of epithelial mucins (Muc-1), correlate with phenotypic changes that occur during neoplastic transformation (8, 9). These antigens are involved in the functional changes that take place during transformation of epithelial cells into carcinoma cells (10). During invasion of the trophoblast into the decidua, phenotypic changes of epithelial cells are observed. The

formation of the placenta resembles the invasion of malignant tumours in many respects. At the implantation site, foetal-derived trophoblast cells invade deeply into the maternal decidua. Within the decidua, the contact between foetal and maternal cells is most intimate in the first trimester of pregnancy (11).

Expression of the TF antigen was reduced in the first and second trimester abort decidua compared to the normal decidua during the same time of pregnancy. The TF antigen was restricted to the syncytiotrophoblast and extravillous trophoblast cells in the decidua. Abort placentas expressed TF antigen on the syncytiotrophoblast layer. We found a significantly reduced co-expression of TF antigen and cytokeratin 7 in the decidua of abort placentas. These data suggest a reduction of extravillous trophoblast cells in the decidua of abort placentas. In addition, we found increased CD45-positive cells in the abortion decidua compared to normal placentas. Further experiments are necessary to find out whether abortion is caused by missing invasion of extravillous trophoblasts into the decidua or by maternal immune cells attacking these cells which finally lead to the abort. Pregnancy is often compared with a successful transplant, in that the semiallogenic trophoblast cells escape recognition and destruction by the mother's immune system. Our study shows that the TF epitope is expressed by decidual cells only in that phase of pregnancy where an intimate contact of maternal and foetal cells takes place. The trophoblast and especially the syncytiotrophoblast is surrounded by maternal immune cells in all trimesters of pregnancy. We observed an expression of the TF antigen that declines, but continues throughout pregnancy.

In summary, during human gestation, expression of the pancarcinoma TF antigen in normal and abort placentas is described. The expression of the TF antigen decreases with gestational age, which is in agreement with its nature as an oncodevelopmental marker. In abort placentas, there was a significantly reduced expression of the TF antigen in the decidua, which can be related to a reduced expression of this antigen on extravillous trophoblast cells or low numbers of extravillous trophoblast cells in abort placentas. Further investigations are necessary to explain these findings.

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