Abstract. Background and Aim: Altered mucin 1 (MUC1) secretion patterns have been implicated in several cancerous conditions including gastric, colorectal and breast carcinomas. Additionally, an association between the expression of MUC1, Thomsen-Friedenreich (TF) antigen, and binding of gal-1 (gal-1) has been proposed. Therefore, the aims of this study were to determine the frequency and tissue distribution of MUC1, TF and gal-1 binding in endometrioid adenocarcinomas. Materials and Methods: Endometrial carcinomas diagnosed with only one histological tumor form (endometrioid adenocarcinomas) were obtained from 70 patients and classified according to the WHO grading system (G1=50; G2=12; G3=8). An immunohistochemical analysis was performed with specific antibodies against MUC1 and TF and in addition with biotinylated gal-1, followed by a semiquantitative evaluation and statistical analysis ($\chi^2$ test and Spearman’s correlation coefficient). Results: MUC1, TF and gal-1 were observed in human endometrioid adenocarcinomas. The MUC1 and gal-1 immunoreaction increased from G1 to G3, while TF demonstrated a lower intensity in G3 compared to G1, although with no statistical significance. However TF showed a significant correlation with MUC1 ($p=0.019$) in G1 and G2 endometrioid adenocarcinomas, with no observed correlation in G3 tumors. MUC1 and TF demonstrated a significant ($p=0.006$ and $p=0.046$, respectively) down-regulation in surgically staged FIGO III/IV compared to FIGO I/II. Gal-1 binding was up-regulated in FIGO III/IV although with no statistical significance. Interestingly, there was an association between gal-1 binding and lymphangiosis ($p=0.008$). Conclusion: An immuno-histochemical expression of MUC1 and TF and gal-1 binding was demonstrated in human endometrioid adenocarcinomas. Although no significant expression patterns could be demonstrated within different nuclear grading, TF and MUC1 showed a significant correlation in G1/G2 tumors. Therefore, MUC1 and TF might be associated with endometrial malignant transformation. Additionally, MUC1 and TF were down-regulated in stage III/IV tumors, while a higher binding of gal-1 was observed in stage III/IV tumors, suggesting a substantial role of this antigen in endometrial carcinogenesis. Gal-1 binding was associated with lymphangiosis, which is thought to be a poor prognostic marker in endometrial adenocarcinomas. Therefore, MUC1, TF and galectin might have important roles in endometrial pathogenesis and malignant transformation. However, their utilization as specific tumor markers remains unclear and further studies are warranted.

Epithelial mucin 1 (MUC1) is a high molecular weight transmembrane glycoprotein expressed on the apical side of normal epithelial cells. In malignant cells, an increased and often depolarized expression throughout the entire cell cytoplasm has been recorded (1). It is generally believed that MUC1 overexpression by tumor cells facilitates invasive growth and metastasis (2, 3). It was also thought that overexpression of MUC1 disrupts cell-cell and cell-extracellular matrix adhesions (4-6). In addition, MUC1 was shown to contribute to cancer cell escape from immune surveillance as MUC1-expressing cells are less susceptible to T-cell- and NK-cell-mediated lysis (7).

The Thomsen-Friedenreich (TF) epitope has been known for a long time as a tumor-associated epitope (8). The presence of the TF epitope during the early fetal phase, its absence in non-carcinomatous fetal tissues and its association with carcinomas suggest that TF epitope is a stage-specific oncofetal carbohydrate epitope. The TF
epitope is a carbohydrate moiety related to blood group epitopes and consists of galactose-β1-3N-acetyl-\(\text{GalNAc}\). In epithelial cells, the TF epitope is carried by MUC1 on the apical surface of these cells. On tumor cells, MUC1 is post-translationally modified resulting in incomplete O-glycosylation and exposure of the TF epitope (9). In addition, in the first trimester of pregnancy Jeschke et al. found strong expression of the TF epitope and MUC1 on 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 (10). Positive staining for the TF epitope and MUC1 was also found on extravillous trophoblast cells in the decidua during the first and second trimester of pregnancy. Trophoblast tumor cells of the cell line BeWo, which form a syncytium in vitro, were also positive for the TF epitope and MUC1, whereas Jeg3 cells, which are unable to form a syncytium, expressed only MUC1 (11).

Gal-1 (gal-1), a prototype galectin, forms non-covalently associated homodimers under physiological conditions with two carbohydrate recognition domains (CRDs) which preferentially recognize Type I and Type II N-acetyllactosamine residues present on all complex N-linked and many O-linked glycoproteins (12, 13). Through the recognition of cell surface β-galactosidic residues, the lectin displays a wide range of biological activities involving cell adhesion to the endothelium (14, 15). A recent study showed that gal-1 expression increases in the late secretory-phase of the endometrium and in decidua (16). In a former study it was shown that gal-1 recognizes appropriate glycoepitopes on the syncytiotrophoblast and on chorionic carcinoma cells (BeWo) (17). Results demonstrated further that ligation of gal-1 to Galβ1-4GlcNAc and Galβ1-3GalNAc (TF) epitopes on BeWo cells have regulatory effects on hCG and progesterone production.

Because an association between the expression of MUC1, TF and the binding of gal-1 has been proposed, the aims of this study were to determine their frequency and tissue distribution in endometrioid adenocarcinomas.

**Materials and Methods**

**Tissue samples.** Endometrial carcinomas diagnosed with only one histological tumor form (endometrioid adenocarcinomas) were obtained from 70 patients and classified according to the WHO grading system (G1 = 50; G2 = 12; G3 = 8).

**Purification and biotinylation of gal-1 from human placenta.** Gal-1 was prepared from full-term placental tissue by lactose extraction with EDTA-McPBS (20 mM sodium phosphate, pH 7.2, 150 mM NaCl, 4 mM 2-mercaptoethanol, 2 mM EDTA; all BioRad, Munich, Germany) and purified by sequential affinity chromatography on asialofetuin Sepharose 4B (18) followed by affinity chromatography on lactosyl agarose (GE Healthcare, Freiburg, Germany). The protein was then purified to homogeneity by anion exchange chromatography on a Resource Q column (GE Healthcare) (19).

The lectin (1 mg/ml) was biotinylated in phosphate-buffered saline (PBS, pH 8.0) by the addition of 40 µl 10 mM biotinyl-N-hydroxysuccinimide in dimethyl sulphoxide (20). After incubation at room temperature for 1 h, biotinylated gal-1 was affinity-purified on lactosyl agarose (GE Healthcare). The bound fraction was eluted with 50 mM lactose in EDTA-McPBS. Buffer exchange was performed on a Bio-Gel P6 column (BioRad) equilibrated with PBS, pH 7.4 (17).

**Immunohistochemistry.** Immunohistochemistry was performed using a combination of pressure cooker heating and the standard streptavidin-biotin-peroxidase complex with the use of the mouse-IgG-Vectorstain Elite ABC kit (Vector Laboratories, Burlingame, CA, USA). Mouse monoclonal antibodies used for these experiments are listed in Table I. In addition biotinylated gal-1 was used for immunohistochemical detection of gal-1 binding.

Briefly, paraffin-fixed tissue sections were dewaxed using xylol for 15 min, rehydrated in an ascending series of alcohol (70%, 96% and 100%), and subjected to epitope retrieval for 10 min in a pressure cooker using sodium citrate buffer (pH 6.0) containing 0.1 M citric acid and 0.1 M sodium citrate in distilled water. After cooling, sections were washed twice in PBS. Endogenous peroxidase activity was quenched by immersion in 3% hydrogen peroxide (Merck, Darmstadt, Germany) in methanol for 20 min. Non-specific binding of the primary antibodies was blocked by incubating the sections with diluted normal serum (10 ml PBS containing 150 µl horse serum; Vector Laboratories) for 20 min at room temperature. Sections were then incubated at room temperature for 60 min with the primary antibodies. After washing with PBS, sections were incubated in diluted biotinylated serum (10 ml PBS containing 50 µl horse serum; Vector Laboratories) for 30 min at room temperature. After incubation with the avidin-biotin peroxidase complex (diluted in 10 ml PBS; Vector Laboratories) for 30 min and repeated washing steps with PBS, visualisation was performed with substrate and the chromagen 3,3'-diaminobenzidine (DAB; Dako, Glostrup, Denmark) for 8-10 min. Sections were counterstained with Mayer’s acidic haematoxylin and dehydrated in an ascending series of alcohol (50-98%). After xylol treatment, sections were covered. Negative controls were performed by replacing the primary antibody with normal horse serum. Immunohistochemical staining was performed using an appropriate positive control. Positive cells showed a brownish colour and negative controls, as well as unstained cells, were blue.
Evaluation and statistical analysis. The intensity and distribution patterns of specific immunohistochemical staining were evaluated using the semi-quantitative assay as described elsewhere (21) and used to assess the expression pattern of other molecules, like CA-125, steroid receptors, inhibin/activin subunits and cathepsin D (22-26). The IRS score was calculated by multiplication of the optical staining intensity (graded as 0=no, 1=weak, 2=moderate and 3=strong staining) and the percentage of positively stained cells (0=no staining, 1=<10% of cells, 2=11-50% of cells, 3=51-80% of cells and 4=>81% of cells stained). The slides were examined by two independent observers (gynecological pathologist D. M. & N. S.). Sections were examined using a 3CCD color camera (JVC, Victor Company of Japan, Japan) and a Leitz (Wetzlar, Germany) microscope. The results were evaluated using the $\chi^2$ test and the non-parametric Spearman’s correlation coefficient was used for estimating correlations between MUC1, TF and gal-1 (SPSS, Chigaco, IL, USA). Significance was assumed at $p<0.05$.

Results

Immunohistochemical expression of Mucin 1 (MUC1), TF and gal-1 binding in endometrial cancer tissue. MUC1, TF and gal-1 were observed in human endometrioid adenocarcinomas. The MUC1 and gal-1 immunoreaction increased from G1 to G3, while TF demonstrated a lower intensity in G3 compared to G1, although with no statistical significance (Figure 1).

However, TF showed a significant correlation with MUC1 ($p=0.019$) within endometrioid adenocarcinomas G1 and G2, with no observed correlation in G3 tumors (Figure 2a). MUC1 and TF demonstrated a significant ($p=0.006$ and $p=0.046$, respectively) down-regulation in surgically staged FIGO III/IV compared to FIGO I/II (Figure 2b). Gal-1 binding was up regulated in FIGO III/IV although...
Figure 2. Mucin1, TF and galectin expression in endometrioid adenocarcinomas with different grading (a) and FIGO staging (b). Mean±SEM (*p<0.05, χ² test).
with no statistical significance. Interestingly, there was an association between gal-1 binding and lymphangiosis \((p=0.008)\).

**Discussion**

We demonstrated the immunohistochemical expression of MUC1, TF and gal-1 binding in human endometrioid adenocarcinomas. Human endometrial epithelium undergoes progesterone-modulated differentiation during the menstrual cycle (27, 28). MUC1 expression of the female reproductive tissues are dependent on the stage of the menstrual cycle (29-31). Studies have shown that MUC1 expression in endometrial tissues is at its highest in the secretory phases (32), when embryo implantation occurs. MUC1 is associated with the apical surface of epithelial cells and is also secreted, being detectable in uterine fluid, at elevated levels in the implantation phase (33). However, its physiological role is uncertain; it may either inhibit intercellular adhesion by steric hindrance or carry carbohydrate recognition structures capable of mediating cell cell interaction (34). Hey and Aplin showed that endometrial epithelium expresses both Sialyl-Lewis x (SLex) and Sialyl-Lewis a (SLea), with a distribution and pattern of menstrual cycle regulation similar to that of MUC1 (33).

The TF glycopeptide \((\text{Gal\&beta}1\text{-3GalNAc})\) is expressed in more than 85% of human carcinomas (8, 35). In normal adult human tissues, TF epitopes are only expressed in limited amounts and are restricted to a few immunologically privileged sites (36). The TF epitope is, however, expressed on fetal epithelia and mesothelia (37), on transferrin from human amniotic fluid (38) and on trophoblast cells (10). The TF epitope and its carrier protein MUC1 are expressed by syncytiotrophoblasts at the materno-fetal interphase and by extravillous trophoblast cells invading the decidua. It is also found on BeWo trophoblastic tumor cells forming a syncytiotrophoblast \((\text{gal\&beta}1\text{-3GalNAc})\). In a former study we showed that gal-1 recognizes appropriate glycopolitopes on the syncytiotrophoblast and on chorionic carcinoma cells (BeWo) (17). Results demonstrated further that binding of gal-1 to Gal\&beta|1\text{-4GlcNAc} and Gal\&beta|1\text{-3GalNAc} (TF) epitopes on BeWo cells have regulatory effects on hCG and progesterone production and on apoptosis of these cells (39). Gal-1 expression by the bovine blastocyst was described by Mohan et al. (40). Because gal-1 has two carbohydrate recognition domains (CRDs), it is able to mediate both intramolecular and intermolecular cross-linking by binding to more than one sugar residue (41) and supports cell adhesion (42).

Although no significant expression patterns could be demonstrated within different nuclear grading, TF and MUC1 showed a significant correlation in G1/G2 tumors. Therefore, MUC1 and TF might be associated in endometrial malignant transformation. Additionally, MUC1 and TF were down-regulated in stage III/IV tumors, while a higher binding of gal-1 was observed in stage III/IV tumors, suggesting a substantial role of this antigen in endometrial carcinogenesis. Interestingly, gal-1 binding has been associated with lymphangiosis, which is thought to be a poor prognostic marker in endometrial adenocarcinomas. Therefore, MUC1, TF and gal-1 might have important roles in endometrial pathogenesis and malignant transformation. However, their utilization as specific tumor markers remains unclear and further studies are warranted.

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