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Mammaglobin 1: not only a breast-specific and tumour-specific marker, but also a hormone-responsive endometrial protein

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Aims: The secretoglobin mammaglobin 1 (MGB1) is strongly expressed in breast tumours, and is therefore used to detect breast cancer metastases, although it has also been detected in other tissues. The aim of this study was to examine MGB1 expression and its hormonal regulation in human endometrium to further investigate the use of MGB1 as a marker molecule.
Methods and results: Mammaglobin 1 expression was assessed immunohistochemically in endometrial samples from 60 normal fertile patients throughout the menstrual cycle, in 49 endometriotic tissue samples, in 15 endometrial adenocarcinomas, and in 36 breast carcinomas. In addition, 25 endometrial samples were analysed by western blot and quantitative real-time reverse transcription polymerase chain reaction. To prove hormonal regulation, primary endometrial epithelial cells were cultured with 17β-oestradiol and promegestone. MGB1 was detected in human endometrial tissue, with peak expression during the luteal phase, in 31% of endometriotic samples, in 53% of endometrial adenocarcinomas, and in 64% of breast carcinomas. MGB1 mRNA expression was increased *in vitro* by hormonal treatment.
Conclusions: Our data show that MGB1 expression is not restricted to normal and malignant breast tissue. Besides its documented occurrence in endometriotic and malignant endometrial tissues, MGB1 is also expressed in normal human endometrium, and such expression is controlled by steroid hormones.

Keywords: endometriosis, epithelial cell culture, human endometrium, mammaglobin 1, steroid hormones

Abbreviations: ALAS1, 5-aminolevulinate synthase 1; ER, oestrogen receptor; MGB1, mammaglobin 1; PR, progesterone receptor; RT-PCR, reverse transcription polymerase chain reaction

Introduction

Mammaglobin 1 (MGB1; synonyms SCGB2A2 and mammaglobin A) was first detected by Watson and Fleming [1,2] during their search for proteins specifically expressed in breast cancer, and was shown to be a member of the secretoglobin superfamily.[3] The protein shares 42% sequence identity with the rat prostatein protein and has a high degree of homology with rabbit and human uteroglobin (SCGB1A1).[3] It consists of 93 amino acids, and has a predicted molecular mass of 10.5 kDa.[2]

Mammaglobin 1 forms a covalent complex with lipophilin B in breast tissue.[4] The complex is predicted to be a globular, α-helical molecule with a pocket for a steroid-like ligand.[5] Although two imperfect oestrogen and androgen response elements have been identified in the promoter of the *MGB1* gene, Watson *et al.* [6] did

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not observe any modulation of MGB1 transcription in receptor-positive breast tumour cell lines such as MCF7 and T47D by oestradiol or progesterone. Computer-assisted structural analysis of the MGB1 protein revealed a transmembrane domain at amino acids 9–18,[7] so that association with the cell membrane was proposed. In fact, cytoplasmic staining, membrane staining and staining of the luminal cell surface have all been observed by immunohistochemistry.

The secretory nature of MGB1 has been shown in several studies: it is released into the medium by cultured MGB1-positive breast cancer cells,[8] and is detectable in the serum of breast cancer patients.[9] In 1999, Zach *et al.*[10] detected, for the first time, circulating mammary carcinoma cells in peripheral blood specimens of breast cancer patients, using a nested reverse transcriptase polymerase chain reaction (RT-PCR) assay for *MGB1*. Thus, it was proposed that MGB1 could be used in breast cancer diagnosis or as a target for antibody-based therapy.[11,12]

Up to the present, only a few studies have been carried out to detect MGB1 in malignant or benign human endometrial tissues.[13–16] The aim of our study was to systematically examine MGB1 expression throughout the menstrual cycle in hormonally regulated normal human endometrium, in endometriotic tissue, and in endometrial adenocarcinoma, to further investigate the use of MGB1 as a marker molecule. In addition, by using primary human endometrial epithelial cells, we investigated the control of MGB1 expression by ovarian steroid hormones.

Materials and methods

HUMAN TISSUE SAMPLES

Endometrial tissue samples were obtained throughout the menstrual cycle from normal fertile women undergoing hysterectomy because of benign uterine diseases. Samples were collected in the Departments of Gynaecology and Obstetrics of the Marienhospital and Luisenhospital in Aachen, the St Antonius Hospital in Eschweiler, and the University Hospital of RWTH Aachen University. All patients had a regular menstrual cycle and did not receive hormones for at least 6 months before surgery. The dating of each specimen was performed by menstrual history, histological examination[17] and hormonal assessment for 17β-oestradiol, progesterone and luteinizing hormone on the day of hysterectomy by routine laboratory diagnostic methods. The use of all specimens was approved by the Ethics Committee of the Medical Faculty of the University of Aachen (EK 347).

Endometriotic and adenocarcinoma samples were provided by the Institute of Pathology, Krankenhaus Düren. Samples of breast carcinoma were obtained by the Institute of Pathology, Hannover.

IMMUNOHISTOCHEMISTRY ON PARAFFIN SECTIONS

In total, 60 uterine specimens were investigated immunohistochemically. Specimens were taken throughout the menstrual cycle: menstrual phase (days 1–5), $n = 10$; mid-follicular phase (days 6–10), $n = 10$; late follicular phase (days 11–14), $n = 10$; early luteal phase (days 15–18), $n = 10$; mid-luteal phase (days 19–22), $n = 10$; and late luteal phase (days 23–28), $n = 10$. In addition, 49 samples of endometriotic tissue and 15 endometrial adenocarcinoma samples were studied in cooperation with the Institute of Pathology, Düren.

Four-micrometre-thick paraffin sections were mounted on APES (aminopropyltriethoxysilane)-coated slides or on Super-Frost-Plus slides (R. Langenbrinck, Emmendingen, Germany), deparaffinized with xylene, and rehydrated in phosphate-buffered saline (PBS). After antigen retrieval in a microwave (10 mM citrate buffer, pH 6.0; 4 × 5 min; 600 W) or pressure cooker (10 mM citrate buffer, pH 6.0; 3 min of boiling), endogenous peroxidase was blocked by incubation with 3% H_2O_2/methanol for 30 min. Immunohistochemical staining was performed using a streptavidin–biotin–peroxidase method (Histostain-Plus Kit; Zymed Laboratories, South San Francisco, CA, USA). After blocking of non-specific binding sites, sections were incubated overnight at 4°C with a mouse monoclonal IgG1 antibody against MGB1 (clone 304-1A5; Zeta Corporation, Sierra Madre, CA, USA) at a dilution of 1:50 (protein concentration: 1 μg/ml) in PBS/1.5% bovine serum albumin. Specific binding of the antibody was visualized by incubation of the sections for 15 min in chromogen solution (aminoethylcarbazole). Finally, slides were washed in deionized water and mounted in glycerol gelatine without additional counterstaining.

Two different negative control experiments were performed: (i) the first antibody was omitted; and (ii) the first antibody was replaced with non-immune mouse IgG at the same concentration. None of the negative controls showed positive staining. The reproducibility of the results was confirmed by repeating the staining process at least twice for each specimen.

For immunohistochemical detection of MGB1 in breast carcinoma ($n = 36$), the Zytochem-Plus HRP Polymer-Kit (Zytomed Systems, Berlin, Germany) was

used, and visualization was performed with the chromogen diaminobenzidine.

Immunohistochemical analysis for oestrogen receptor (ER) and progesterone receptor (PR) was carried out by using the SP1 rabbit monoclonal antibody (Medac, Wedel, Germany) and the PGR636 antibody (Dako, Hamburg, Germany), respectively, both diluted 1:100 in antibody diluents (Zytomed Systems).

Mammaglobin 1 staining in breast carcinomas was evaluated by use of the Allred score.[18] The Allred score is a composite of the percentage of stained cells and their staining intensity. The percentage of stained cells is classified from 0 to 5 (0, no stained cells; 1, 1% stained cells; 2, 1–10% stained cells; 3, 11–33% stained cells; 4, 34–66% stained cells; 5, 67–100% stained cells), and the intensity of cell staining is rated as 1, 2, or 3. The proportion and intensity scores are then added to obtain a total score, which ranges from 0 to 8. The immunoreactive scores of ER and PR are given as reduced Allred scores: 0, Allred 0–2; +, Allred 3–4; ++, Allred 5–6; and +++, Allred 7–8.

An immunohistochemical score was also used for semiquantitative evaluation of MGB1 staining in human endometrial epithelial cells and the luminal secretion of the glands. Quantification was performed as follows: 0, no staining of cells and luminal secretion; 1, low intensity of staining and/or <10% of cells or secretion stained; 2, moderate staining and/or 10–50% of cells or secretion stained; 3, strong staining and/or >50% of cells or secretion stained.

PROTEIN ISOLATION

Twenty-five endometrial samples obtained throughout the menstrual cycle were investigated for MGB1 protein expression: six of the follicular phase (days 6–14), three of the early luteal phase (days 15–18), two of the mid-luteal phase (days 19–22), and 14 of the late luteal phase (days 23–28).

After hysterectomy, the endometrial tissues were immediately snap-frozen in isotonic ammonium hydrogen carbonate buffer (0.15 M NH_4HCO_3). For protein isolation, the samples were quickly thawed at 37°C, and homogenized using an Elwein potter by adding 400–800 μl of ammonium hydrogen carbonate buffer containing the protease inhibitor cocktail 'Complete Mini TM' (Roche, Mannheim, Germany). After homogenization, the samples were centrifuged (10 000 ***g***, 10 min, 4°C), and the supernatant was frozen in aliquots for further processing. The total protein concentration of each sample was measured using the Bio-Rad DC protein assay (Bio-Rad Laboratories, München, Germany).

SODIUM DODECYLSULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE) AND WESTERN BLOT

SDS-PAGE was performed under reducing conditions (5% mercaptoethanol).[19] Fifteen per cent gels were used to separate the proteins according to their molecular mass. Each lane was loaded with 70 μg of protein. Rainbow coloured protein molecular mass marker (14.3–220 kDa) was obtained from Amersham Biosciences (Freiburg, Germany).

The separated proteins were transferred to a poly(vinylidene difluoride) membrane (Immobilon P, pore diameter 0.45 μm; Millipore, Hamburg, Germany) with a semidry electroblotting procedure (2 mA/cm^2) performed for 40 min. The membranes were blocked at 4°C overnight in 5 mM Tris-buffered saline, pH 7.6 (TBS), supplemented with 0.1% Tween and 5% goat serum. Detection of the immobilized proteins was performed by incubating the membrane for 1 h at room temperature with a rabbit anti-MGB1 polyclonal antibody (AgriSera, Stockholm, Sweden), 1 μg/ml, diluted in TBS/0.1% Tween, and then, after washing, for 1 h with horseradish peroxidase (HRP)-conjugated secondary antibody (polyclonal goat anti-rabbit IgG/HRP; DakoCytomation, Glostrup, Denmark) diluted 1:2000 in TBS/0.1% Tween. Immunoreactive proteins were detected by chemiluminescence using the enhanced chemiluminescence (ECL+) kit (Amersham Pharmacia Biotech, Freiburg, Germany), following the manufacturer's instructions.

CELL CULTURE

Primary epithelial cell culture was performed as described in detail previously.[20,21] Endometrial tissue of the follicular phase was minced under laminar flow into fragments of ~1 mm^3, and digested by shaking for 1 h in 0.125% type IA collagenase (470 units/mg; Sigma, Deisenhofen, Germany) at 37°C in Dulbecco's modified Eagle's medium/Ham's F12 (ccPro, Neustadt, Germany) without phenol red, and containing 1% streptomycin, penicillin and fungizone, and 10% charcoal-treated steroid hormone-free fetal bovine serum (ccPro).

Stromal and epithelial cells were separated by two filtration steps.[20] The epithelial cells and small glandular fragments were seeded at a density of approximately $2–5 \times 10^5$ cells/cm^2 in an insert with a transparent Biopore membrane of 0.4-μm pore size (Millicell CM-filters, 12 mm; Millipore, Eschborn, Germany). The membrane was coated with Matrigel (BD Biosciences, Heidelberg, Germany) diluted 1:3 in culture medium without any additions. The culture was performed in a dual-chambered Millicell system.

Different inserts were seeded with the same amounts of epithelial cells and glandular fragments, each obtained

from one single endometrial specimen. One group (two inserts) was treated with the progestin agonist promegestone (17,21-dimethyl-19-norpregna-4,9-dien-3, 20-dione; R5020) (10^{-6} M) in combination with 17β-oestradiol (10^{-8} M; Sigma-Aldrich, München, Germany) (both hormones diluted in ethanol <0.1%), and the other group (two inserts) treated only with the vehicle ethanol constituted the control. The culture medium was changed every 2 days. Endometrial epithelial cell cultures originating from follicular-phase endometrium from 13 different donors were performed. After 4 days of hormone treatment the cell culture was terminated, and the cells were processed in lysis buffer for further RNA isolation using the High Pure RNA Tissue Kit (Roche).

REAL-TIME RT-PCR (LIGHT CYCLER)

Total RNA was isolated from endometrial samples by using the High Pure RNA Tissue Kit (Roche). Endometrial tissue (25 mg) was transferred to 900 µl of lysis/binding buffer, and was homogenized for 90 s with a glass–Teflon homogenizer. Then, 700 µl of water-saturated phenol/chloroform/isoamyl alcohol mix (Ambion, Huntingdon, UK) was added, mixed, and subjected to centrifugation (13 000 ***g***, 2 min, room temperature). The supernatant was mixed with 0.5 volumes of ethanol and loaded onto the column provided with the kit. The following RNA isolation steps were performed according to the kit protocol, with minor modifications: (i) DNAse I treatment was prolonged to 30 min and was performed at room temperature; and (ii) the RNA was eluted twice with 50 µl of elution buffer.

Isolation of RNA from cultured epithelial and stromal cells was performed using the High Pure RNA Tissue kit (Roche), according to the manufacturer's recommendations.

Total RNA (1 µg) was reverse transcribed into cDNA using oligo-$(dT)_{15}$ primer and the first-strand cDNA synthesis (AMV) Kit (Roche).

Real-time PCR was performed with the Light Cycler (Roche). The gene encoding 5-aminolevulinate synthase 1 (ALAS1), which is expressed with ~500 mRNA molecules per cell, was used as the reference gene. *ALAS1* mRNA expression was detected using the Light Cycler-h-ALAS1 housekeeping gene set and the Light Cycler Fast Start DNA Master^{Plus} HybProbe Kit (both Roche), as recommended by the kit protocols. For amplification of the *MGB1* cDNA, the Light Cycler Fast Start DNA Master^{Plus} SYBR Green I Kit (Roche) was used. Primers were created with the primer design program PRIMER3 (http://frodo.wi.mit.edu/). The following MGB1 primer pair was used for real time RT-PCR: forward primer, 3′-tgccatagatgaattgaaggaatg-5′ (NM_002411 position 231–254); and reverse primer, 3′-tgtcatatattaattgcataaacacctca-5′ (NM_002411 position 291–319).

The PCR run was programmed with Light Cycler software Version 3.5(Roche). The run included the following four steps: (i) denaturation and activation of the Taq polymerase at 95°C for 10 min; (ii) amplification and quantification: denaturation at 95°C for 5 s, annealing at 57°C for 5 s, and extension at 72°C for 20 s; (iii) melting curve profile: 95°C for 5 s, cooling to 66°C for 30 s, and slow heating to 95°C (0.1°C/s) with continuous measurement; and (iv) cooling to 40°C. The PCR volume was 20 µl, and consisted of 2 µl of cDNA and 18 µl of PCR mix. The $MgCl_2$ concentration was 3 mM. Water and an equivalent of approximately 60 ng of RNA were employed as negative controls in each PCR reaction.

To determine the efficiency of the PCRs and for the quantitative assessment of *MGB1* mRNA expression, a coefficient file for the *MGB1* and the *ALAS1* PCRs was created. The slope of the *MGB1* PCR was −3.602, and the slope of the *ALAS1* PCR was −3.330. The final quantitative analysis of the PCRs was accomplished with Relquant software, Version 1.01 (Roche), with the use of the *MGB1* and *ALAS1* coefficient files. The results are shown as 'relative units'. PCR product identity was studied by melting curve analysis. In addition, products were assessed on ethidium bromide-containing DNA agarose gels, and the product was exemplarily sequenced (Seqlab, Göttingen, Germany).

STATISTICS

Statistical analysis was performed with GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, CA, USA). Differences in MGB1 expression under hormonal regulation were tested with the unpaired Student's two-tailed *t*-test. To test whether the immunoreactive scores of MGB1 correlated with ER and PR expression and tumour grade, Fisher's exact test was applied. *P*-values <0.05 were accepted as statistically significant. Values are given as mean ± standard error of the mean.

Results

ENDOMETRIUM THROUGHOUT THE MENSTRUAL CYCLE

Mammaglobin 1 immunohistochemical staining was seen in the cytoplasm of glandular epithelial cells in a patchy pattern and in the lumen of the glands. The endometrial stroma was completely negative. MGB1 was detected in the early, middle and late luteal phases; in the follicular phase, the staining of the epithelial cells was weak or nearly negative. Fig-

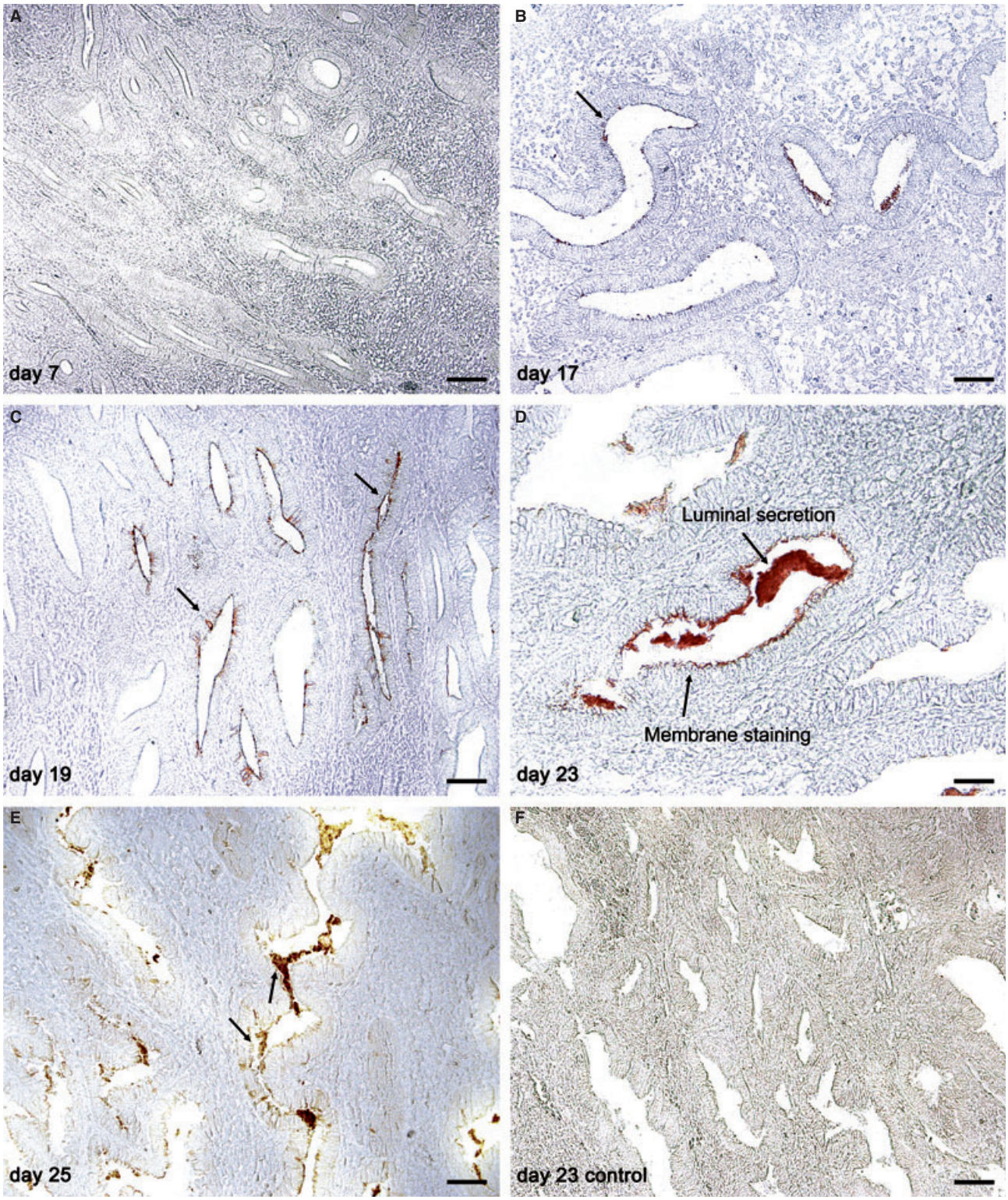

Figure 1. Immunohistochemical detection of mammaglobin 1 in normal human endometrium throughout the menstrual cycle: **A**, Cycle day 7 (follicular phase). **B**, Cycle day 17 (early luteal phase). **C**, Cycle day 19 (mid-luteal phase). **D**, Cycle day 23 (late luteal phase). **E**, Cycle day 25 (late luteal phase). **F**, Representative negative control, cycle day 23. Distinct staining can be observed in the early luteal phase, strong staining in the mid-luteal to late luteal phase, and no or very weak staining in the follicular phase. Scale bars: 100 μm (**A**); 50 μm (**B**,**C**,**E**,**F**); and 25 μm (**D**).

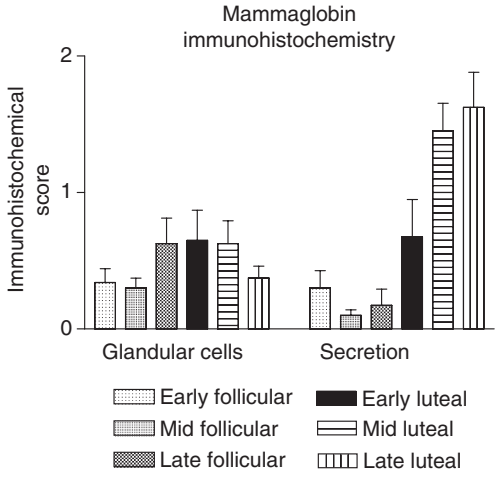

Figure 2. Immunohistochemical score of mammaglobin 1 expression throughout the menstrual cycle in glandular cells and in the secretion in the luminal compartment.

ure 1A–F shows representative sections with MGB1 staining (with a control) for the different phases of the menstrual cycle. Figure 2 shows the semiquantitative immunohistochemical score of the glandular cells and the secretion.

Western blot analysis of endometrial tissue homogenates with the polyclonal rabbit antibody against MGB1 detected a specific band of approximately 10 kDa in the middle and late secretory phases (Figure 3A), confirming the results of immunohistochemistry.

Real-time RT-PCR was performed with endometrial RNA obtained from tissue samples derived from all phases of the menstrual cycle. *MGB1* mRNA expression was absent or weak during the follicular phase (0.15 ± 0.042), whereas expression was significantly higher (0.46 ± 0.096; $P = 0.0069$) in RNA samples derived from secretory-phase endometrium (Figure 3B,C).

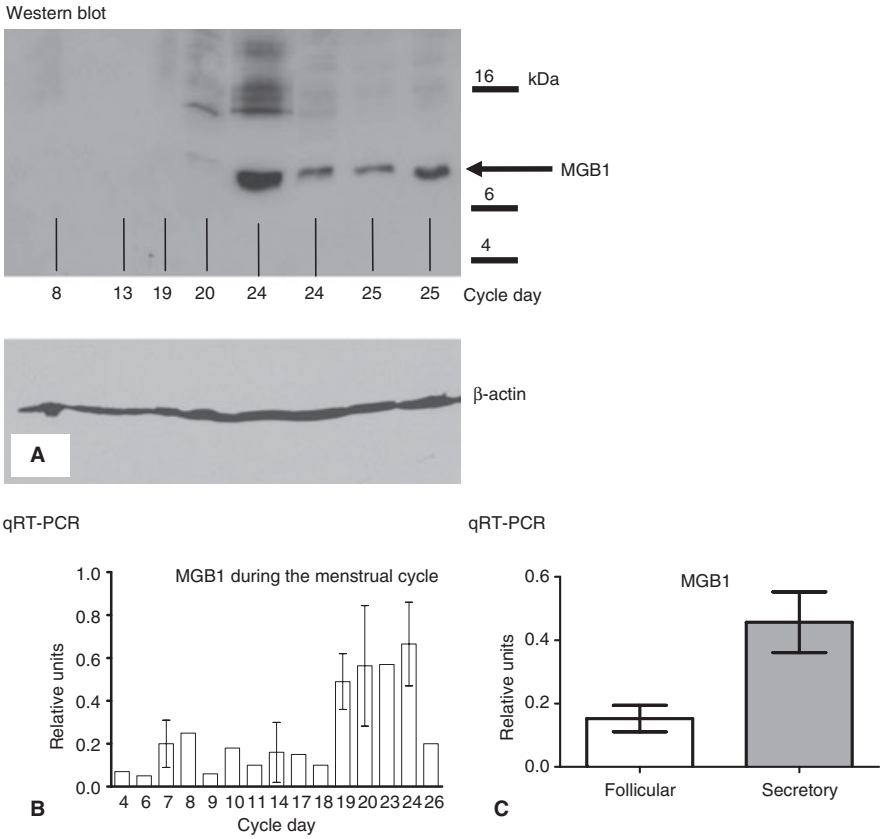

Figure 3. **A**, Western blot analysis. Total proteins (70 μg) of endometrial samples were analysed by sodium dodecylsulphate polyacrylamide gel electrophoresis (15%) at different cycle days. Strong immunodetection for mammaglobin 1 (MGB1) was obtained at ~10 kDa in the mid to late luteal phase (days 20–25) with the rabbit anti-MGB1 polyclonal antibody (AgriSera) at a dilution of 1 μg/ml. There was no staining at cycle days 8, 13, or 19. Loading control line: β-actin. **B**, Quantitative real-time reverse transcription polymerase chain reaction across the menstrual cycle. Strong expression of *MGB1* mRNA in the mid-secretory to late secretory phase was obtained (cycle days 19–24). **C**, Comparison of MGB1 expression in the secretory and follicular phases. MGB1 expression was significantly higher (0.46 ± 0.096 versus 0.15 ± 0.042; $P = 0.0069$) in RNA samples derived from secretory phase endometrium.

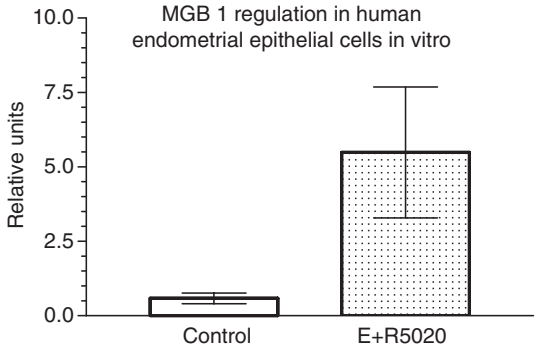

Figure 4. Quantitative real-time reverse transcription polymerase chain reaction in cell culture. Comparison of mammaglobin 1 (MGB1) expression in human endometrial epithelial cells *in vitro* under hormone treatment with 17β-oestradiol and promegestone (R5020) versus control (vehicle: ethanol <0.1%). *MGB1* mRNA expression was found to be significantly up-regulated (5.49 ± 2.19 versus 0.59 ± 0.18; $P = 0.0358$) by the hormonal treatment.

CELL CULTURE

To prove the hormone dependency of MGB1 expression, human primary endometrial epithelial cells were cultured with 17β-oestradiol + promegestone (R5020) to mimic the hormonal situation in the secretory phase of the menstrual cycle. Control cells received only the vehicle (ethanol). Thirteen different cell cultures were performed. *MGB1* mRNA expression was analysed by real-time RT-PCR, and was found to be significantly up-regulated (5.49 ± 2.19 versus 0.59 ± 0.18; $P = 0.0358$) by the hormonal treatment (Figure 4).

ENDOMETRIOTIC TISSUE

Seventy-five endometriotic tissue samples were obtained from archival pathological specimens. After microscopic analysis, 49 samples exhibiting endometrial glandular structures were selected for immunohistochemical evaluation. Fifteen (31%) endometriotic tissue samples showed MGB1 staining in glandular epithelial cells or in the luminal secretion. Stromal cells were negative. Figure 5A,B shows representative staining of two positively stained samples.

ENDOMETRIAL ADENOCARCINOMA

Fifteen endometrioid endometrial adenocarcinoma samples were studied in comparison with the endometriotic tissue samples. Tumours were graded (FIGO) as G1–G3, and of the 15 adenocarcinomas two were well-differentiated (G1), eight were moderately differentiated (G2), and five were undifferentiated (G3). Eight (53%) samples were MGB1-positive; of these, one was G1, four were G2, and three were G3. Some samples, as shown in Figure 5, displayed very strong cytoplasmic and membrane staining (Figure 5C,E) or luminal staining (Figure 5D). Figure 5F represents a negative endometrial adenocarcinoma sample.

BREAST CARCINOMA

Thirty-six ductal invasive breast carcinomas were evaluated for immunohistochemical MGB1 staining. Positive staining was observed in 23 (64%) carcinoma samples. Strong cytoplasmic staining can be seen in Figure 6A,B, but MGB1-negative carcinoma samples were also found (Figure 6C). All breast carcinomas were additionally evaluated for ER expression and 31 for PR expression (Figure 6D,E). There was no significant correlation between steroid hormone receptor status and MGB1 staining.

Tumour grading was obtained for 24 samples (G1–G3). No significant difference was observed between MGB1 expression in G1/2 tumours and that in G3 tumours (Figure 6F).

Discussion

In this study, MGB1 was detected in normal human endometrial tissue throughout the menstrual cycle, in endometriotic tissue, and in endometrial adenocarcinoma, besides the well-described expression in breast carcinoma. Localization was performed by immunohistochemistry of tissue sections, and quantification of MGB1 protein and mRNA expression carried out by western blotting and quantitative RT-PCR. In addition, to prove hormone dependency, primary endometrial epithelial cells were cultured under hormonal stimulation: MGB1 expression was significantly increased after 2–4 days treatment with the progestin R5020 and 17β-oestradiol, a hormonal regime that mimics the hormonal situation in the luteal phase.

Progesterone dependency of MGB1 had already been presumed. In a microarray study, up-regulation of MGB1 was detected in the so-called 'window of implantation' (middle of the luteal phase, dominated by progesterone) compared with the follicular phase, which is dominated by 17β-oestradiol.[22] Other members of the secretoglobin superfamily, such as uteroglobin (SCGB1A1), which show a high level of homology with MGB1, are known to be progesterone-regulated.[23] Uteroglobin, which was first identified in rabbit uteri,[24] was intensively studied as a progesterone-dependent protein in rabbits and in human endometrium,[25–29] and is a very well-characterized protein with regard to its crystal structure and molecular biology.[30,31]

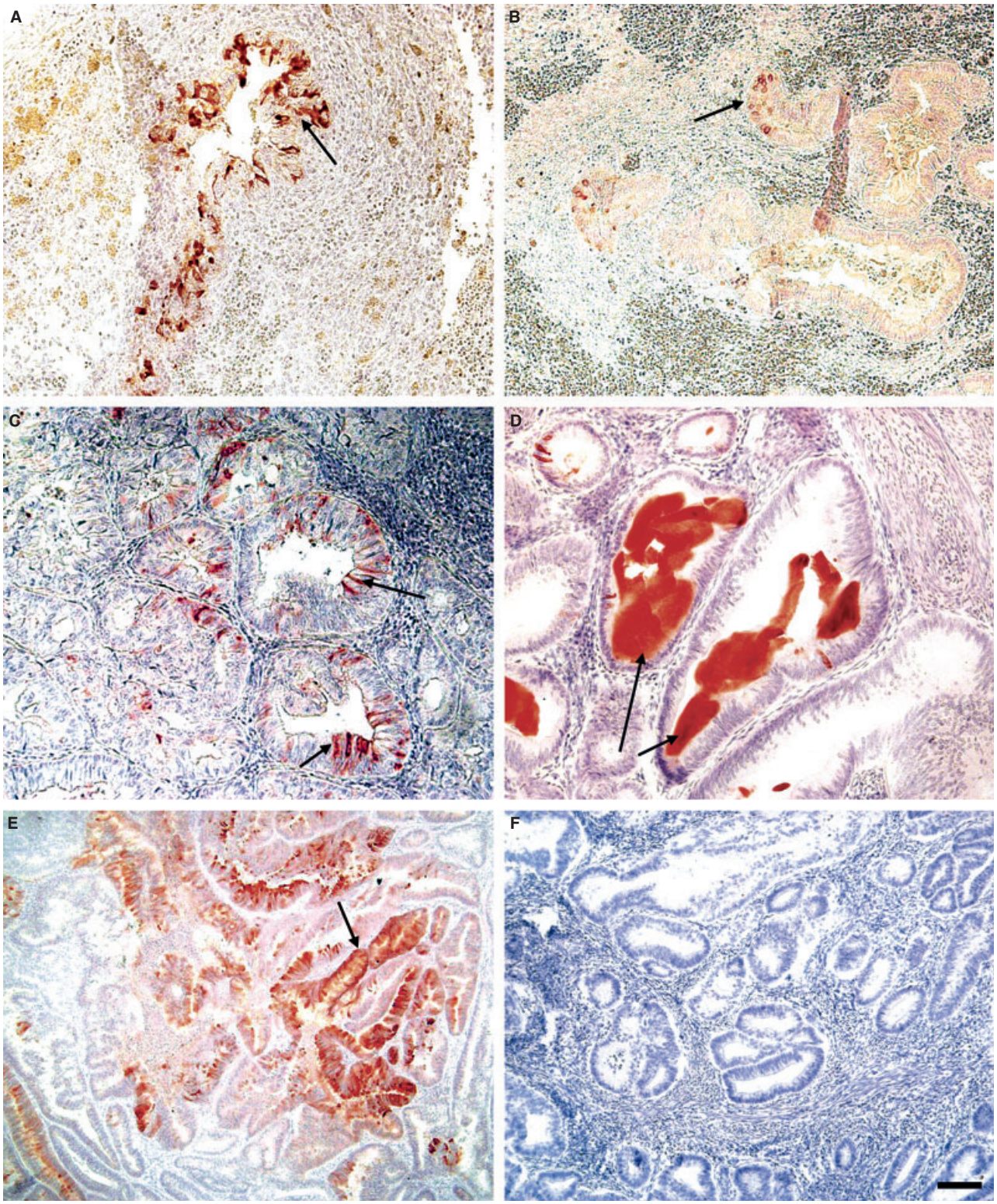

Figure 5. Immunohistochemical detection of mammaglobin 1 (MGB1) in endometriotic tissue (**A**, **B**). Positive staining can be observed in some glands (arrows). $n = 49$; 31% of samples were positive. Immunohistochemical detection of MGB1 in adenocarcinomas (**C**–**F**). Cytoplasmic (**C**,**E**), luminal (**D**) and membrane (**E**) staining can be observed. The section of adenocarcinoma in (**F**) was negative. $n = 15$; 53% of samples were positive. Scale bar: 50 μm.

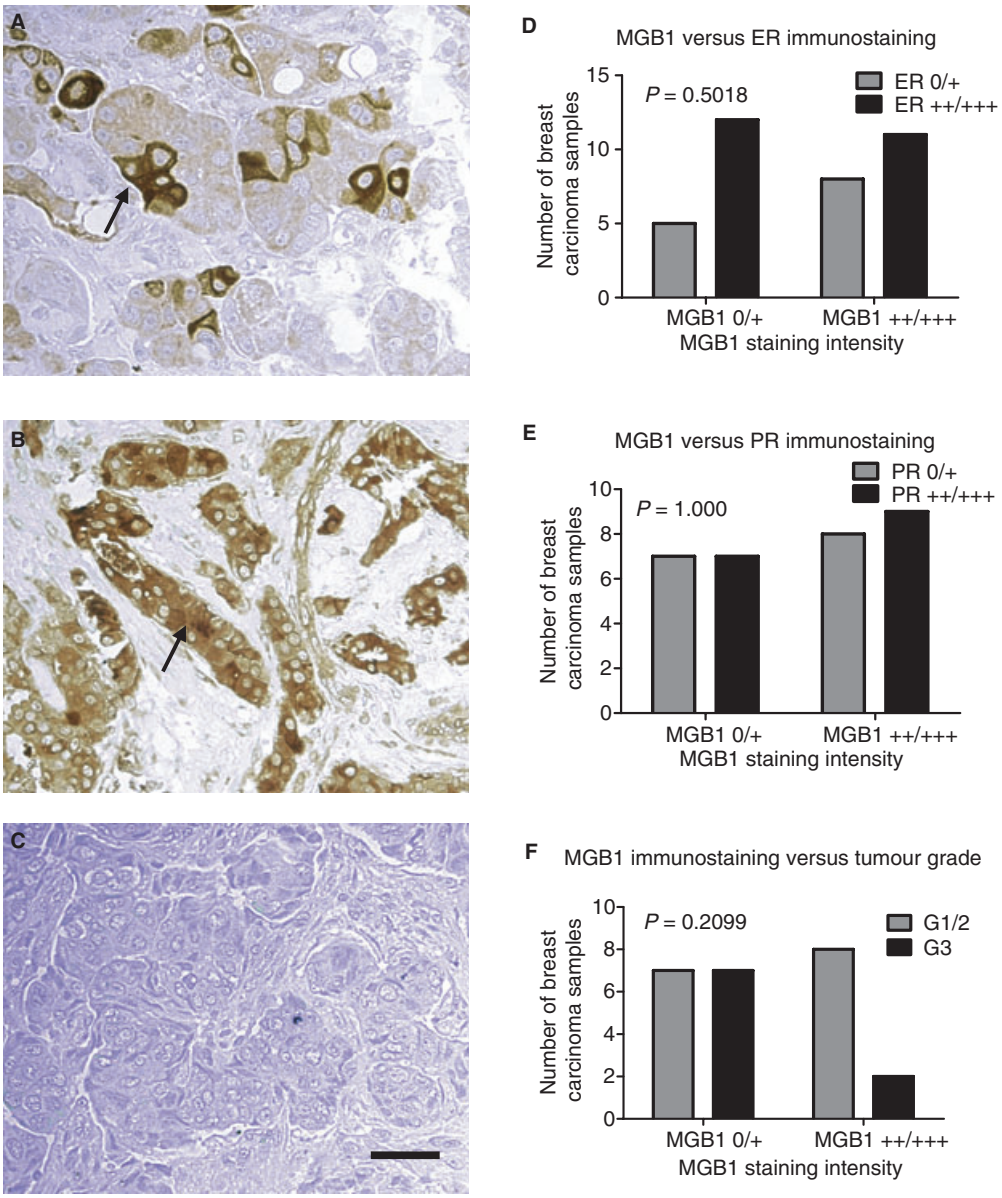

Figure 6. Immunohistochemical detection of mammaglobin 1 (MGB1) in breast cancer (**A–C**). Sixty-four per cent of the studied samples ($n = 36$) were positive. (**D–F**) MGB1 staining intensity in relation to (**D**) oestrogen receptor expression ($n = 36$), (**E**) progesterone receptor expression ($n = 31$), and (**F**) tumour grade ($n = 24$). MGB1 staining intensity did not correlate with steroid hormone receptor expression or tumour grade. Scale bar: 50 μm.

Nevertheless, its physiological function is not known, although it has been proposed to have immunomodulatory and anti-inflammatory functions besides its steroid- and lipophilic-binding capacities. The function of MGB1 is also not precisely known, but it might similarly be involved in steroid and biphenyl binding and in regulating the immune system.[5]

MGB1 has an important role as a specific diagnostic marker for breast cancer,[32] and is used for the identification of metastases originating from these tumours. Han *et al.*[33] investigated MGB1 expression in metastatic carcinoma of lymph nodes from the breast and various other organs; they found MGB1 in 84% of metastatic carcinoma tissue samples originating from breast tumours and 15% of those from non-breast tumours. In addition, MGB1 was detected in the serum of 72% of breast cancer patients by enzyme-linked immunosorbent assay,[8] but only rarely in the

serum of patients with other tumour types. As reviewed by Lacroix,[34] MGB1 is now designated as a standard marker for the detection of disseminated cancer cells by RT-PCR-based methods to clarify the identity of unknown tumour entities. It is also used in the molecular assessment of sentinel lymph nodes.[35–37]

Although MGB1 was initially detected only in the mammary gland,[32] several other tissues have been shown to express MGB1, mainly tissues of the reproductive tract. Such expression was first detected in 2002 in benign and malignant epithelial tissue from the ovary, uterus, cervix and some prostate samples, by the use of nested RT-PCR.[13] In addition, MGB1 has been detected in normal human sweat glands[38] using both a highly specific and quantitative real-time RT-PCR, and immunohistochemistry.

The number of reports confirming MGB1 expression in normal and malignant tissue other than the mammary gland is increasing. Zafrakas *et al.*[15] investigated MGB1 in more than 300 human tumours and corresponding normal tissues, and found it to be abundantly expressed in tumours of the female genital tract, such as endometrial, ovarian and cervical cancer. In a comprehensive immunohistochemical study by Sasaki *et al.*,[39] including 480 tumours from various organs, 55% of salivary gland tumours and 13% of endometrial cancers were positive, besides 48% of breast cancers.

The potential use of MGB1 in gynaecological pathology practice was tested by Onuma *et al.*[14] On the basis of their results, which are in accordance with our present data, they confirmed that MGB1 is not a breast-specific marker but is also variably expressed in benign and malignant endometrial tissues, and therefore concluded that MGB1 can be used as an adjunct marker in gynaecological pathology. In agreement with Wang *et al.*,[40] our data support the conclusion that endometrial origin should be considered as a major differential diagnosis when tumour cells are positive for MGB1.

In summary, we have shown, for the first time, the steroid hormone-induced expression of MGB1 in cell culture experiments with primary endometrial epithelial cells, and obvious hormone-dependent expression of MGB1 during the regular menstrual cycle. One-third of endometriotic tissue samples and 53% of endometrial adenocarcinomas were MGB1-positive. Although the highest expression was observed in breast carcinoma, our study demonstrated that normal endometrial tissue also expresses MGB1, and that its expression is enhanced under progesterone dominance during the secretory phase of the menstrual cycle. The hormonal regulation of MGB1 that we detected might also have some importance for cancer diagnosis. Depending on the cycle date in premenopausal women or on hormone replacement therapy in postmenopausal women, higher or lower expression of MGB1 might also occur in cancer cells and this should be taken into account. In conclusion, the obvious increase in MGB1 expression induced by steroid hormones and its expression in normal endometrial tissue should be considered in future gynaecological diagnostic and therapeutic assessments.

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References

1. Watson MA, Fleming TP. Isolation of differentially expressed sequence tags from human breast cancer. *Cancer Res.* 1994; **54;** 4598–4602.
2. Watson MA, Fleming TP. Mammaglobin, a mammary-specific member of the uteroglobin gene family, is overexpressed in human breast cancer. *Cancer Res.* 1996; **56;** 860–865.
3. Ni J, Kalff-Suske M, Gentz R, Schageman J, Beato M, Klug J. All human genes of the uteroglobin family are localized on chromosome 11q12.2 and form a dense cluster. *Ann. N. Y. Acad. Sci.* 2000; **923;** 25–42.
4. Colpitts TL, Billing-Medel P, Friedman P *et al.* Mammaglobin is found in breast tissue as a complex with BU101. *Biochemistry* 2001; **40;** 11048–11059.
5. Carter D, Douglass JF, Cornellison CD *et al.* Purification and characterization of the mammaglobin/lipophilin B complex, a promising diagnostic marker for breast cancer. *Biochemistry* 2002; **41;** 6714–6722.
6. Watson MA, Darrow C, Zimonjic DB, Popescu NC, Fleming TP. Structure and transcriptional regulation of the human mammaglobin gene, a breast cancer associated member of the uteroglobin gene family localized to chromosome 11q13. *Oncogene* 1998; **16;** 817–824.
7. Zuo L, Li L, Wang Q, Fleming TP, You S. Mammaglobin as a potential molecular target for breast cancer drug delivery. *Cancer Cell Int.* 2009; **9;** 8.
8. Bernstein JL, Godbold JH, Raptis G *et al.* Identification of mammaglobin as a novel serum marker for breast cancer. *Clin. Cancer Res.* 2005; **11;** 6528–6535.
9. Fanger GR, Houghton RL, Retter MW *et al.* Detection of mammaglobin in the sera of patients with breast cancer. *Tumour Biol.* 2002; **23;** 212–221.
10. Zach O, Kasparu H, Krieger O, Hehenwarter W, Girschikofsky M, Lutz D. Detection of circulating mammary carcinoma cells in the peripheral blood of breast cancer patients via a nested reverse transcriptase polymerase chain reaction assay for mammaglobin mRNA. *J. Clin. Oncol.* 1999; **17;** 2015–2019.
11. Zehentner BK, Carter D. Mammaglobin: a candidate diagnostic marker for breast cancer. *Clin. Biochem.* 2004; **37;** 249–257.
12. Zehentner BK, Persing DH, Deme A *et al.* Mammaglobin as a novel breast cancer biomarker: multigene reverse transcription-PCR assay and sandwich ELISA. *Clin. Chem.* 2004; **50;** 2069–2076.
13. Grunewald K, Haun M, Fiegl M *et al.* Mammaglobin expression in gynecologic malignancies and malignant effusions detected by

nested reverse transcriptase-polymerase chain reaction. *Lab. Invest.* 2002; **82;** 1147–1153.
14. Onuma K, Dabbs DJ, Bhargava R. Mammaglobin expression in the female genital tract: immunohistochemical analysis in benign and neoplastic endocervix and endometrium. *Int. J. Gynecol. Pathol.* 2008; **27;** 418–425.
15. Zafrakas M, Petschke B, Donner A *et al.* Expression analysis of mammaglobin A (SCGB2A2) and lipophilin B (SCGB1D2) in more than 300 human tumors and matching normal tissues reveals their co-expression in gynecologic malignancies. *BMC Cancer* 2006; **6;** 88.
16. Kajiwara H, Kumaki N, Hirabayashi K *et al.* A case of oncocytic carcinoma of the endometrium. *Arch. Gynecol. Obstet.* 2009; **279;** 733–738.
17. Noyes RW, Hertig AT, Rock J. Dating the endometrial biopsy. *Am. J. Obstet. Gynecol.* 1975; **122;** 262–263.
18. Harvey JM, Clark GM, Osborne CK, Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J. Clin. Oncol.* 1999; **17;** 1474–1481.
19. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; **227;** 680–685.
20. Classen-Linke I, Kusche M, Knauthe R, Beier HM. Establishment of a human endometrial cell culture system and characterization of its polarized hormone responsive epithelial cells. *Cell Tissue Res.* 1997; **287;** 171–185.
21. Classen-Linke I, Alfer J, Hey S, Krusche CA, Kusche M, Beier HM. Marker molecules of human endometrial differentiation can be hormonally regulated under in-vitro conditions as in-vivo. *Hum. Reprod. Update* 1998; **4;** 539–549.
22. Kao LC, Tulac S, Lobo S *et al.* Global gene profiling in human endometrium during the window of implantation. *Endocrinology* 2002; **143;** 2119–2138.
23. Mukherjee AB, Zhang Z, Chilton BS. Uteroglobin: a steroid-inducible immunomodulatory protein that founded the Secretoglobin superfamily. *Endocr. Rev.* 2007; **28;** 707–725.
24. Beier HM. Uteroglobin: a hormone-sensitive endometrial protein involved in blastocyst development. *Biochim. Biophys. Acta* 1968; **160;** 289–291.
25. Beato M. Proceedings: binding of progesterone to uteroglobin. *Hoppe Seylers Z. Physiol. Chem.* 1974; **355;** 1173–1174.
26. Miele L, Cordella-Miele E, Mukherjee AB. Uteroglobin: structure, molecular biology, and new perspectives on its function as a phospholipase A2 inhibitor. *Endocr. Rev.* 1987; **8;** 474–490.
27. Muller-Schottle F, Classen-Linke I, Alfer J *et al.* Expression of uteroglobin in the human endometrium. *Mol. Hum. Reprod.* 1999; **5;** 1155–1161.
28. Krusche C, Beier HM. Localization of uteroglobin mRNA by nonradioactive *in-situ* hybridization in the pregnant rabbit endometrium. *Ann. Anat.* 1994; **176;** 23–31.
29. Krusche CA, Herrler A, Beier HM. Uteroglobin *in-situ* hybridization: novel monitoring of epithelial differentiation in the rabbit endometrium. *Ann. N. Y. Acad. Sci.* 2000; **923;** 316–320.
30. Miele L, Cordella-Miele E, Mantile G, Peri A, Mukherjee AB. Uteroglobin and uteroglobin-like proteins: the uteroglobin family of proteins. *J. Endocrinol. Invest.* 1994; **17;** 679–692.
31. Pattabiraman N, Matthews JH, Ward KB, Mantile-Selvaggi G, Miele L, Mukherjee AB. Crystal structure analysis of recombinant human uteroglobin and molecular modeling of ligand binding. *Ann. N. Y. Acad. Sci.* 2000; **923;** 113–127.
32. Fleming TP, Watson MA. Mammaglobin, a breast-specific gene, and its utility as a marker for breast cancer. *Ann. N. Y. Acad. Sci.* 2000; **923;** 78–89.
33. Han JH, Kang Y, Shin HC *et al.* Mammaglobin expression in lymph nodes is an important marker of metastatic breast carcinoma. *Arch. Pathol. Lab. Med.* 2003; **127;** 1330–1334.
34. Lacroix M. Significance, detection and markers of disseminated breast cancer cells. *Endocr. Relat. Cancer* 2006; **13;** 1033–1067.
35. Douglas-Jones AG, Woods V. Molecular assessment of sentinel lymph node in breast cancer management. *Histopathology* 2009; **55;** 107–113.
36. Abdul-Rasool S, Kidson SH, Panieri E, Dent D, Pillay K, Hanekom GS. An evaluation of molecular markers for improved detection of breast cancer metastases in sentinel nodes. *J. Clin. Pathol.* 2006; **59;** 289–297.
37. Backus J, Laughlin T, Wang Y *et al.* Identification and characterization of optimal gene expression markers for detection of breast cancer metastasis. *J. Mol. Diagn.* 2005; **7;** 327–336.
38. Sjodin A, Guo D, Hofer PA, Henriksson R, Hedman H. Mammaglobin in normal human sweat glands and human sweat gland tumors. *J. Invest. Dermatol.* 2003; **121;** 428–429.
39. Sasaki E, Tsunoda N, Hatanaka Y, Mori N, Iwata H, Yatabe Y. Breast-specific expression of MGB1/mammaglobin: an examination of 480 tumors from various organs and clinicopathological analysis of MGB1-positive breast cancers. *Mod. Pathol.* 2007; **20;** 208–214.
40. Wang Z, Spaulding B, Sienko A *et al.* Mammaglobin, a valuable diagnostic marker for metastatic breast carcinoma. *Int. J. Clin. Exp. Pathol.* 2009; **2;** 384–389.