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Milk fat globule epidermal growth factor 8 (MFG-E8): A novel protein in the mammalian endometrium with putative roles in implantation and placentation

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ABSTRACT

Objectives: MFG-E8 is a novel endometrial protein with conserved functions in tissue remodeling and angiogenesis in non-uterine tissues. Our aims were: 1. To examine the presence of MFG-E8 protein in the human endometrium during the window of implantation, in human endometrial cell lines, in human placental tissue at different gestational ages, and in murine implantation sites during early gestation; and 2. To study the regulation of MFG-E8 mRNA expression in mice implantation sites.
Study design: MFG-E8 protein and its receptor integrin αvβ3 were detected by immunostaining in human endometrial biopsies obtained from normal volunteers, in human endometrial cell lines (epithelial: Ishikawa and HEC-1A, stromal: HESC, and endothelial: HEEC), in human products of conception from all trimesters of gestation, and in murine implantation and inter-implantation sites dissected on days 5 and 8 post-coitus. MFG-E8 gene expression was assessed by RT-PCR.
Main outcome measures: Immunohistochemical determination of MFG-E8 in endometrium and products of conception as well as relative MFG-E8 mRNA expression in mice implantation sites.
Results: MFG-E8 protein was present almost exclusively in the epithelial compartment of human endometrium. It was also expressed in the cytotrophoblasts and syncytiotrophoblasts outlining chorionic villi of the human placenta at all trimesters of gestation, and in murine implantation sites. MFG-E8 mRNA was significantly up-regulated in murine implantation sites and with increased gestational age.
Conclusions: MFG-E8 expression in the endometrial epithelium as well as in chorionic villi suggests its possible role in endometrial reorganization during the receptive phase and in events related to normal pregnancy in mammals.

1. Introduction

Although embryo implantation is a critical event in the establishment of pregnancy, little is known about the specific molecular and cellular mechanisms involved in the establishment and maintenance of a receptive endometrium. We have identified a novel protein, Milk Fat Globule Epidermal Growth Factor 8 (MFG-E8, NM005928), up-regulated 3-fold in the human endometrium during the window of implantation [1–3]. This protein has never been described before in the mammalian endometrium nor ever been linked to endometrial physiology.

Human milk fat globule antigens were initially characterized as normal mammary gland epithelial cell markers [4,5], and later used to monitor invasiveness of breast cancer [6], cervical intraepithelial neoplasia and carcinoma [7,8] and ovarian clear cell and endometrioid carcinomas [9] by measuring their circulating blood levels.

MFG-E8 has been described as a multifunctional molecule that participates in several cell surface-mediated regulatory events involving different cell types and tissues. It plays an important role in the maintenance of intestinal epithelial homeostasis and the promotion of mucosal healing [10]. It has been identified as the first extracellular ligand essential for diurnal photoreceptor outer segment fragments phagocytosis in the retina [11]. MFG-E8 is a potent proangiogenic molecule involved in vascular branching

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[12,13]; and it may play a role in binding to the egg to facilitate fertilization by being present in the murine sperm surface [14], and in the anterior caput of the human epididymis [15]. In other tissues, MFG-E8 has critical functions in tissue remodeling and angiogenesis, both in normal and abnormal/cancer tissues by binding to its αvβ3 integrin receptor.

In previous human studies, we demonstrated that: 1. MFG-E8 is almost exclusively localized to the human endometrial epithelium [3]; 2. MFG-E8 expression (mRNA and protein) by endometrial epithelial cells increases towards the end of the window of implantation [16]; 3. Its production in endometrial epithelial cells is up-regulated by prolactin, a known product of the decidualized endometrial stroma [16], as well as by human chorionic gonadotropin (hCG), an early embryonic product [17]; 4. MFG-E8 induces endometrial endothelial cell adhesion and proliferation [18], and that 5. It also increases apoptotic activity of stromal cells by activating caspases [17]. Therefore we postulated MFG-E8 as a regulator of endometrial physiology and trophoblast attachment and invasion through regulation of the critical processes of remodeling, apoptosis, and angiogenesis in humans.

The aims of this study were: (i) to evaluate the presence of MFG-E8 protein and its receptor integrin αvβ3: in the human endometrium during the menstrual cycle, in human endometrial cell lines, in human placental tissue at different gestational ages and in mice implantation sites (IS) during early gestation; and (ii) to examine the expression of MFG-E8 mRNA in murine IS.

2. Methods

2.1. Tissue processing and cell cultures

2.1.1. Human endometrial biopsies

This study was approved by the Institutional Review Board. Endometrial biopsies were obtained from healthy ovulatory women (21–30 years of age) with a suction pipelle (Unimar Pipelle, Cooper Surgical, Shelton, CT, USA), formalin fixed, paraffin embedded, and sectioned [16]. Following previous studies [2], the day of the urinary LH surge (Conception Technologies, San Diego, CA, USA) was assigned LH = 0, and LH + 1 was considered the day of ovulation or cycle Day 14. Subjects were randomized using sealed envelopes to schedule a single endometrial biopsy on cycle day 12 (late proliferative), 16 (pre-receptive), 21 (opening of the WOI) or 24 (closure of the WOI) ($n = 3$ subjects per cycle day).

2.1.2. Human endometrial cell lines

Endometrial epithelial cells (Ishikawa cells, donated by Dr Lockwood from Yale University, New Haven CT, USA), and HEC-1A (ATCC, USA), stromal cells (T-HESC-ATCC) and endothelial cells (HEEC-also donated by Dr Lockwood) were cultured according to the manufacturers' instructions and as published previously [18–22] in Falcon culture slides (Invitrogen, Carlsbad, CA, USA). Briefly, Ishikawa cells were grown in DMEM/F12 supplemented with 10% FBS (Gibco, Carlsbad, CA, USA), 200 nM L-glutamine (Gibco), 100 U/ml penicillin and 100 ug/ml of streptomycin (Gibco). HEC-1A cells were cultured in McCoy's 5A medium (Invitrogen). HEEC cells were grown in EMB media with growth factors (Lonza) and supplied 2% FBS. T-HESC cells were cultured in DMEM/F12 (Sigma) supplemented with 10% charcoal stripped FCS (Atlanta Biologicals, Lawrenceville, GA, USA), 1 mM sodium pyruvate (Gibco), 1% ITS + Premix (insulin, transferring and selenium, BD BioScience, Bedford, MA, USA), 1% penicillin/streptomycin (Invitrogen), and 1.25 μg/ml amphotericin B; at 37 °C in a 95% air–5% CO_2 humidified incubator. After washing with PBS (Gibco), they were harvested with 0.05% trypsin-EDTA (Gibco) and fixed with cold methanol.

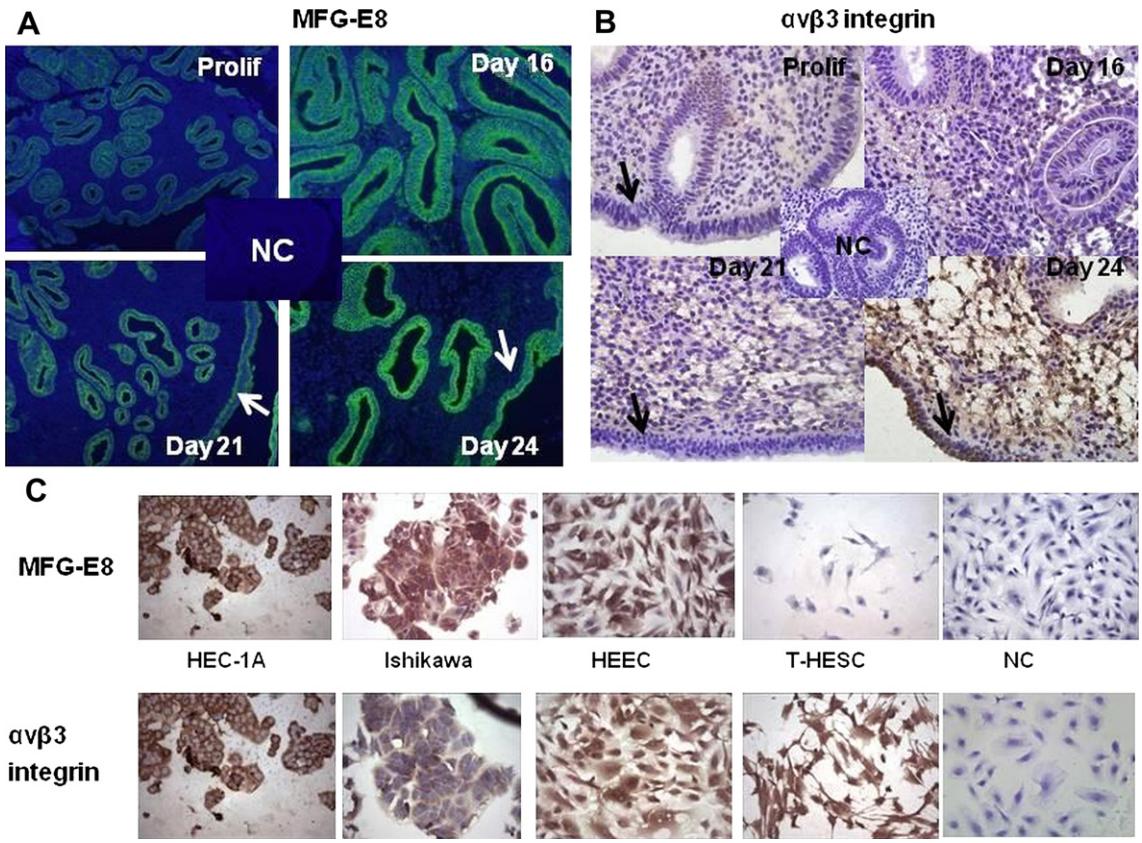

Fig. 1. *Immunolocalization of MFG-E8 and αvβ3 integrin in human endometrial biopsies and in established human endometrial cell lines*. Endometrial biopsies were obtained in the proliferative (day 12) and secretory (days 16, 21 and 24) phases. A. MFG-E8 (1:1000) immunofluorescence localized almost exclusively to the luminal and glandular epithelium, with highest expression on day 24 (200×). NC: negative control (200×). Note robust accumulation of MFG-E8 in apical compartment of epithelial glandular cells. White arrows: luminal epithelium. B. Instead, αvβ3 integrin had a more diffuse distribution in both epithelium and stroma, but also had increased staining on day 24 (1:10, 200×). NC: negative control (200×). Black arrows: luminal epithelium.C. Upper panel: MFG-E8 (1:1000, 200×) had a diffuse, strong pattern of expression in epithelial (Ishikawa and HEC-1A) and endothelial (HEEC) cells studied, and it was absent from the stromal cells (T-HESC). On the other hand, αvβ3 integrin had a diffuse cytoplasmic staining in epithelial (HEC-1A 1:10, Ishikawa 1:50), endothelial (HEEC 1:25) and stromal (T-HESC 1:10) cells (C. Lower panel, 200×). NC: negative control (200×).

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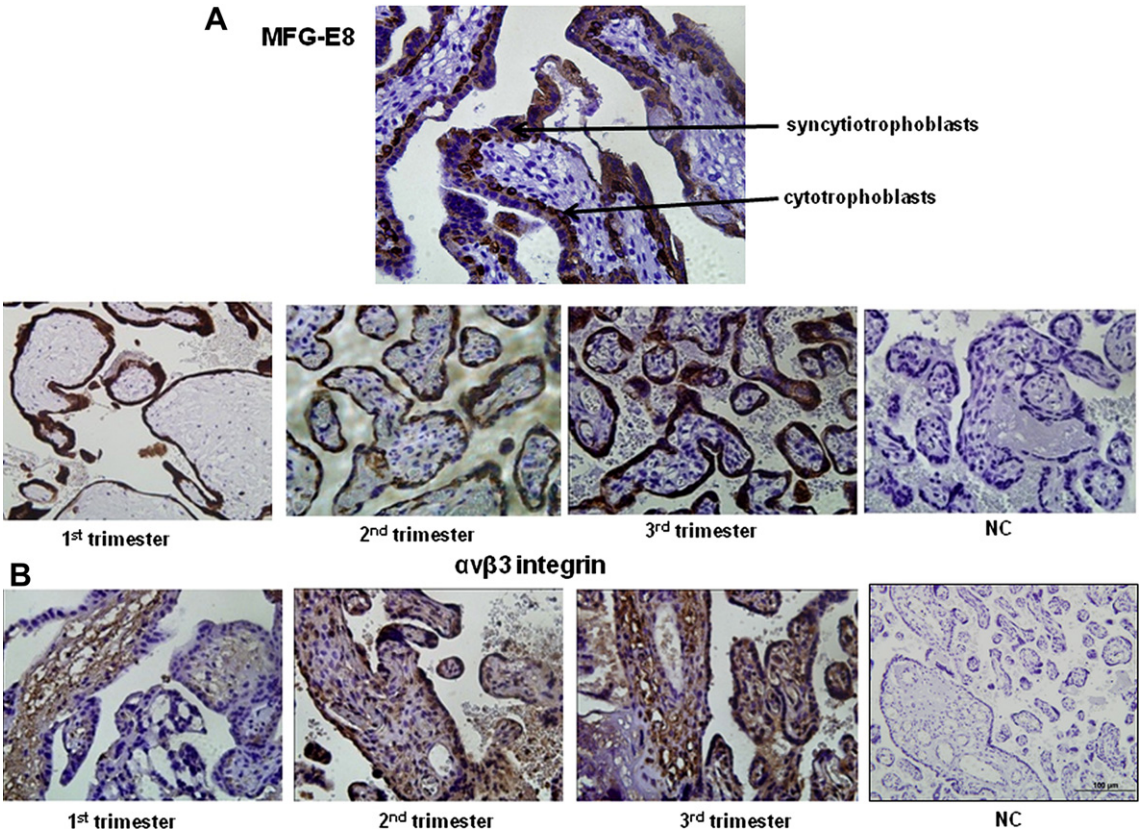

Fig. 2. *Immunolocalization of MFG-E8 and of αvβ3 integrin in human products of conception in all trimesters of gestation.* A. MFG-E8 had a diffuse, strong cytoplasmic pattern in cells outlining the chorionic villi, both in cytotrophoblasts and syncytiotrophoblasts (upper panel, black arrows, 1:1000, 300×). The villi stroma was negative for MFG-E8. Lower panel: MFG-E8 is present in all trimesters of gestation (200×). B. αvβ3 integrin (1:10, 200×) had a diffuse cytoplasmic pattern in cytotrophoblasts, syncytiotrophoblasts and villous stroma, including staining of some blood vessels in all trimesters. NC: negative controls (200×).

2.1.3. Human products of conception

Normal human products of conception ($n = 3$ for 1st and 2nd trimesters, and $n = 10$ for 3rd trimester) were obtained from archived pathology specimens under IRB approval, either after deliveries or by dilatation and curettage. The gestational age of the specimens was kept blinded until after the immunostaining was performed. The formalin fixed/paraffin embedded tissue blocks were sectioned, deparafinized, and stained with hematoxilin-eosin (H&E) as well as with primary antibodies specific to MFG-E8 and integrin αvβ3, and a secondary antibody labeled with a chromogen.

2.1.4. Murine implantation sites

Experiments were conducted in accordance with NIH standards and with approval from the appropriate Animal Care Committees (IACUC #08-010). Adult virgin B6CBA F1/J female mice were mated with fertile males of the same strain to induce pregnancy (day 1 = vaginal plug). Pregnant ($n = 5$ animals per day) and non-pregnant (control, $n = 5$ animals) were sacrificed by cervical dislocation. Implantation sites (IS) and inter-implantation sites (I-IS) were divided by sharp dissection on days 5 and day 8 post-coitus. Half of the tissues were formalin fixed and paraffin embedded for immunostaining and the other half were flash-frozen in liquid nitrogen for RNA extraction.

2.2. Immunohistochemistry

Immunolocalization of MFG-E8 and its receptor αvβ3 integrin in human and mouse cells was performed using the DAKO Envision HRP kit (DAKO, Carpinteria, CA). Endogenous peroxidase activity was quenched with 3% hydrogen peroxide and non-specific binding sites were blocked with 2% normal goat serum (NGS). Fixed cells and tissue sections were incubated with mouse monoclonal primary antibodies (MFG-E8 (ab25251), αvβ3 integrin (ab78289), Abcam, Cambridge, MA, USA) or with rabbit polyclonal anti-MFG-E8 (SC33546, Santa Cruz, CA) or rat monoclonal Ab (ab25251, Abcam), as appropriate, at 4 °C overnight. After washing with PBS, sections were incubated with HRP labeled polymer conjugated with secondary antibody. Non-immune mouse IgG1 (clone NS-1, Sigma, St. Louis, MO) at same dilution was used as negative control. Slides were counterstained with Mayer's hematoxylin (Sigma), dehydrated in a graded series of ethanol, cleared in xylene, and mounted with mounting media. Photographs were taken with an Olympus microscope (Olympus Corp., Tokyo, Japan) and DP-70 camera.

Immunohistochemical intensities were determined by the MetaMorph Image analyzer software (Molecular Devices, Sunnyvale, CA). For analysis, 10 images of 12–22 μm^2 average area taken from each slide of murine IS and I-IS for day 5 and d8 of gestation were quantified, and means compared between groups. Operations performed included separation, binarization, and thresholding of red and blue colors [23].

For human immunofluorescence studies, sections were blocked with 2% normal serum prior to incubation with primary antibody (anti-MFG-E8, anti-αvβ3 integrin, and anti-CD 31 rabbit polyclonal antibody as marker for endothelial cells, ab32457, Abcam) overnight at 4 °C. A goat anti-mouse antibody conjugated with FITC for MFG-E8 or TRITC for CD31 was used as secondary antibody (Santa Cruz Biotechnologies). Nuclei were stained with DAPI (vector Labs, Burlingame, CA). After washing, slides were mounted with anti-fading medium (Vector Laboratories, Burlingame, CA, USA) and analyzed with an SPOT-RT Slider digital camera (Diagnostic Instruments Inc., Sterling, MI, USA).

2.3. RNA isolation and real time RT-PCR

Murine IS and I-IS, and uterine from non-pregnant animals (NPU) were dissected and minced over ice and total RNA was extracted using by Nonidet P-40-guanidine isothiocyanate extraction/silica gel spin column centrifugation (Rneasy^R Mini Kit, Qiagen) and eluted from columns in 30 μl ribonuclease-free water. The quality of total RNA extracted was analyzed on an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and quantification of total RNA was performed on a NanoDrop spectrophotometer (Thermo Scientific, Waltham, MA) [16].

2.3.1. Reverse transcription (RT)

Extracted total RNA was reversed in a thermal iCycler (BioRad, Hercules, CA) as previously described [16]. cDNA solutions were then stored at −20 °C. Preparations without reverse transcriptase were used as negative controls, in which the absence of PCR products indicated a complete lack of contaminating genomic DNA.

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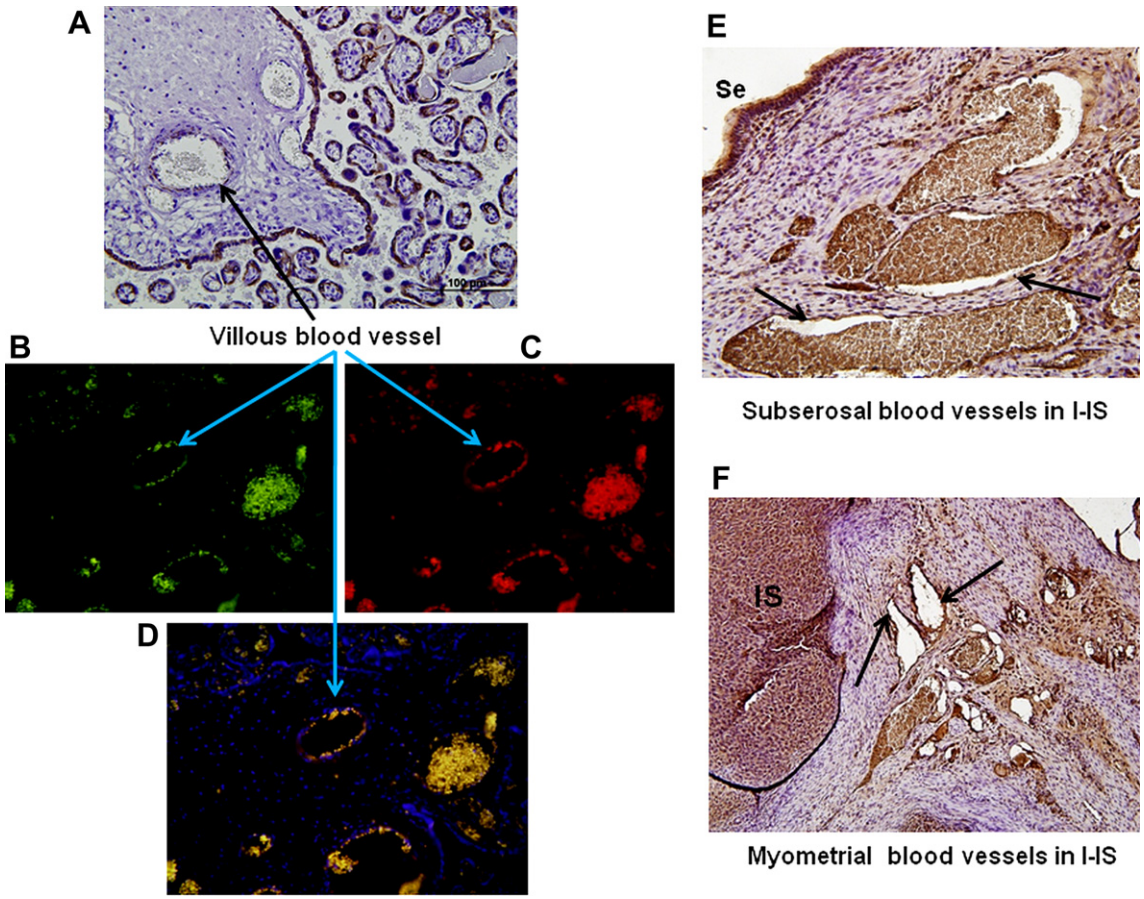

Fig. 3. *MFG-E8 is present in human placental blood vessels and in murine uterine and placental blood vessels.* A. In addition to the intense presence in the epithelium, MFG-E8 was localized by immunostaining to blood vessels present in the core of human chorionic villi (arrow, 100×). B. Blood vessel in villa (arrow) stained for MFG-E8 fluorescence (green fluorescence, 200×). C. Same blood vessel (arrow) stained for CD31 fluorescence (red fluorescence, 200×). D. MFGE-8 colocalized within endothelial cells (arrow) stained for CD31: merger of green (MFG-E8), red (CD 31) and blue nuclei (DAPI) 200×. E and F. MFG-E8 was localized by immunostaining to murine subserosal and myometrial uterine vessels (arrows), respectively (pc d8, I-IS, 200×).

2.3.2. Real time PCR

Quantitative real time RT-PCR was performed using a Lightcycler Fastart DNA Master Plus SYBR green I and a Lightcycler 2.0 instrument (Roche Applied Science, Indianapolis, IN) with 0.5 μM of each sense and antisense primers (except 18 S which was used at 0.3 μM). Amplification parameters were as previously described by us [16]. Negative control consisted of PCR water replacing the cDNA solution (no template control, NTC). Amplification specificity was confirmed by sequencing of all PCR products purified using QIAquick PCR purification kit (Qiagen). RNA levels were determined using a standard curve and the values obtained were normalized against those found for 18 S RNA to account for differing amounts of starting material. The threshold cycle (Ct) was used for determining the relative expression level of MFG-E8 by normalizing to the Ct of 18 S as previously described [24,25]. The method of delta–delta cycle threshold (ddCT) was used to calculate the relative fold-change. The normalized Ct was compared with the Ct in the NPU. Three animals were used at each time point and 6–8 IS and I-IS samples were obtained from each animal.

2.3.3. Data analysis

Statistical analysis was performed by analysis of variance and least significant difference post hoc tests, and by *t*-test for Metamorph imaging and for PCR. $p < 0.05$ values were considered statistically significant.

3. Results

3.1. MFG-E8 and its receptor αvβ3 integrin immunolocation in human endometrial tissue and in human endometrial cell lines

MFG-E8 was present almost exclusively in the epithelium (both luminal and glandular) in human endometrial biopsies and it was absent in stromal cells. The protein showed a strong epithelial distribution with increased staining from the proliferative to the secretory phase, with maximal expression on days 21–24 encompassing the window of implantation (Fig. 1A). Note robust accumulation of MFG-E8 in apical compartment of epithelial glandular cells. As we have previously reported [16], the effect of cycle day on MFG-E8 epithelial expression was statistically significant ($p < 0.00002$), whereas the result of stromal immunostaining was not different. In addition, the epithelial cell expression of MFG-E8 was significantly higher on days 21–24 (receptive phase) compared with the pre-receptive phase (day 12–16, $p < 0.002$). Integrin αvβ3 was present in both epithelial and stromal cells, with increased immunostaining on day 24, in parallel to MFG-E8 (Fig. 1B).

MFG-E8 was also strongly present in the cytoplasm of all human endometrial epithelial (HEC-1A and Ishikawa) and endothelial (HEEC) cells studied (Fig. 1C upper panel), but it was absent from the human endometrial stromal cells (T-HESC). Its receptor αvβ3 integrin had a widespread cytoplasmic distribution in all endometrial cells studied (Fig. 1C, lower panel).

3.2. MFG-E8 and its receptor are present in human placenta at all gestational ages

In the human placenta, MFG-E8 protein was localized outlining the chorionic villi but not in the villous stroma. MFG-E8 stained both syncytio- and cytotrophoblasts with a diffuse and strong

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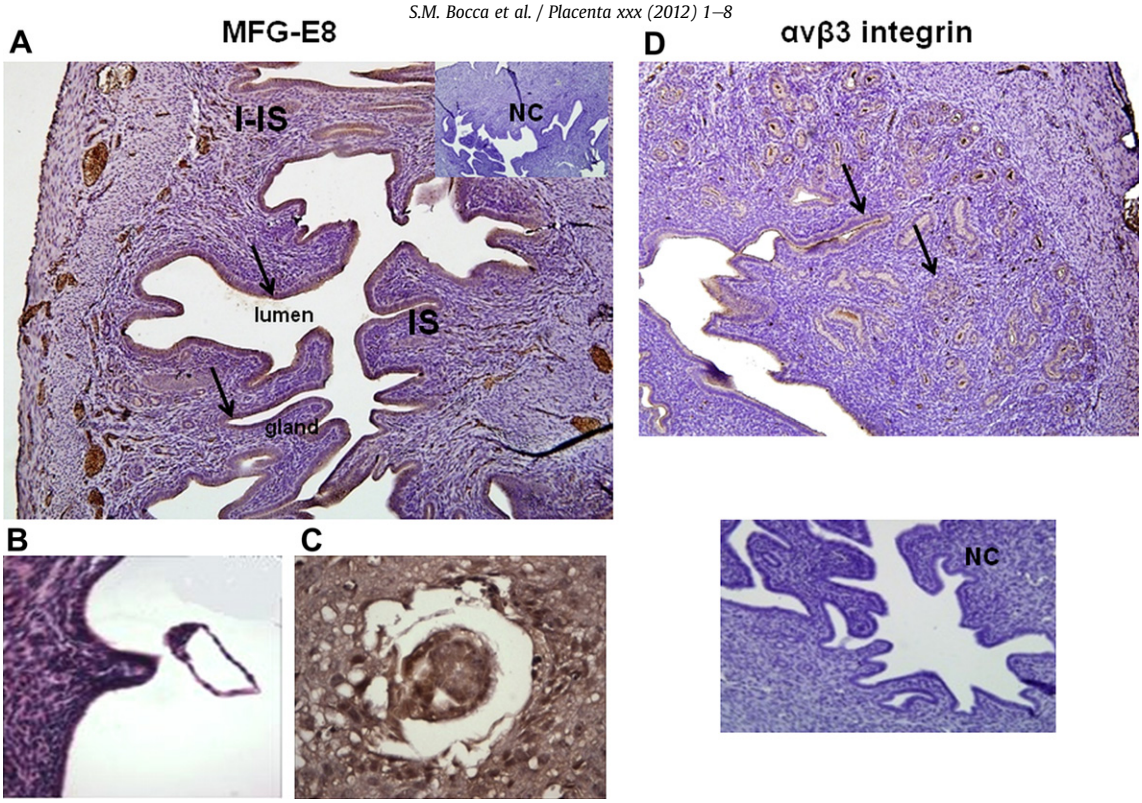

Fig. 4. *Immunolocalization of MFG-E8 and integrin αvβ3 in murine implantation sites 5 days post-coitus*. A. MFG-E8 staining: longitudinal section of a mouse uterine horn on day 5 post-coitus showing implantation (IS), inter-implantation (I-IS) sites and lumen (1:1000, 200×). Note strong MFG-E8 epithelial staining at the surface and glandular epithelium of the IS. B. Uterine horn section showing an implanting blastocyst on d5 (H&E, 100×). C. A d5 implanted blastocyst (400×) stained for MFG-E8. D. Integrin αvβ3 staining: there was a more diffuse staining throughout epithelium and stroma (arrows, 100×). NC: negative controls (100×).

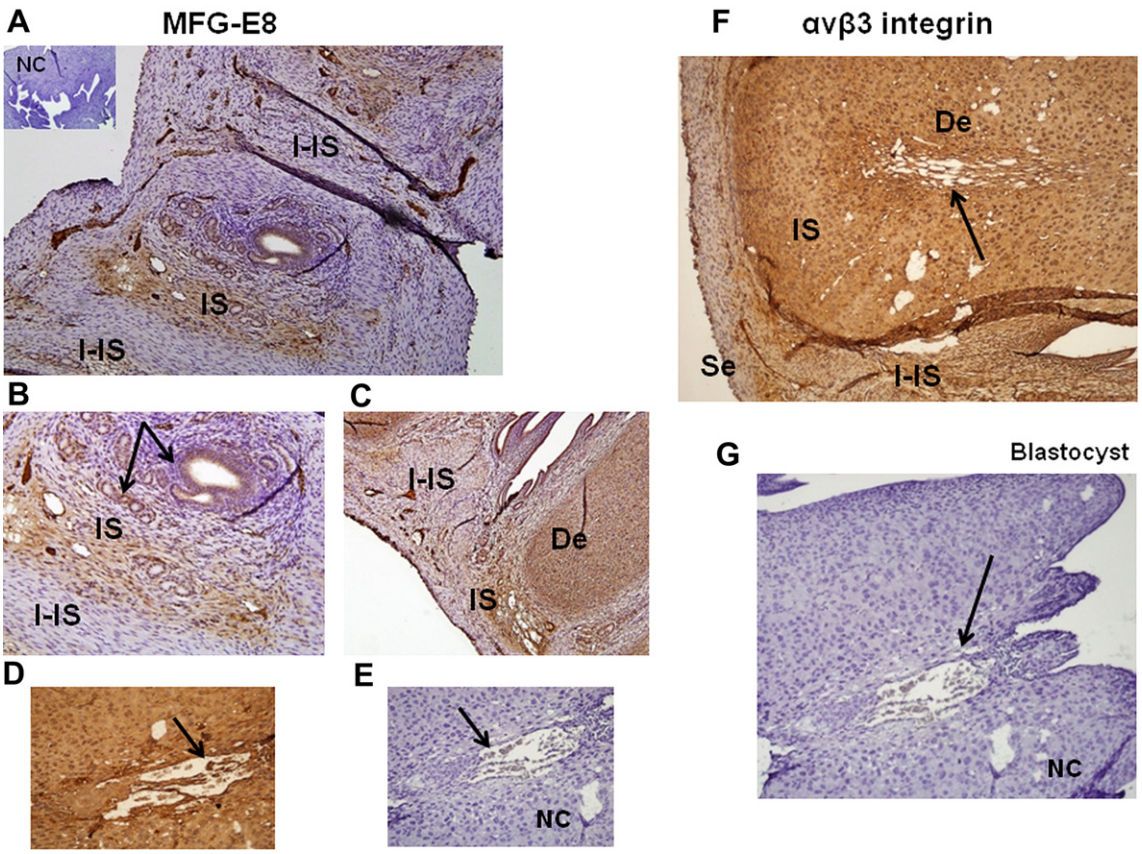

Fig. 5. *Immunolocalization of MFG-E8 and its receptor αvβ3 integrin in murine implantation sites 8 days post-coitus.* A. MFG-E8 (1:1000) staining: longitudinal section of a murine uterine horn on day 8 post-coitus showing implantation (IS) and inter-implantation (I-IS) sites (100×). NC: negative control (100×). B. MFG-E8 (1:1000) was strongly localized to the glandular epithelium (arrows, 200×), to the decidua at the IS (C, 200×) and to the implanting blastocyst (D, arrow, 400×). E. NC: negative control (200×). The I-IS stroma did not stain for MFG-E8 (B, C). F. Integrin αvβ3 (1:25) had a diffuse staining throughout IS, I-IS and blastocyst (arrow) (Se: serosa, De: decidua, 100×). G. NC: negative control (200×). Arrow shows implanted blastocyst.

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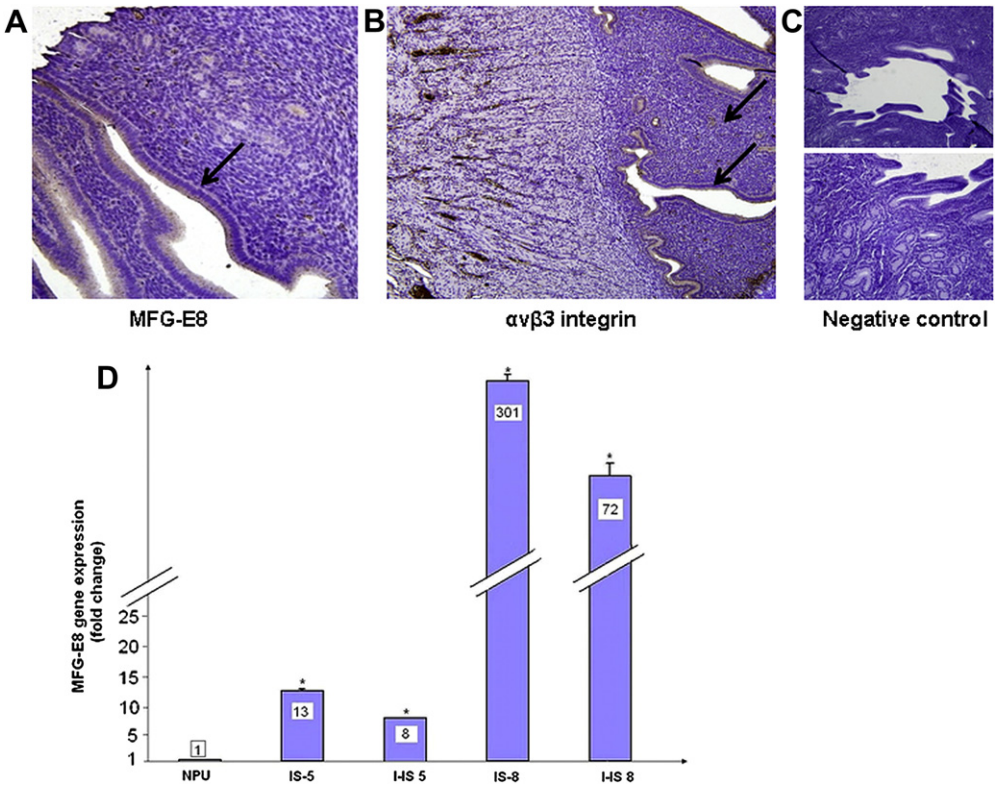

Fig. 6. *Immunolocalization of MFG-E8 and its receptor αvβ3 integrin in the mouse non-pregnant uterus (NPU), and MFG-E8 mRNA expression in murine NPU, implantation (IS) and inter-implantation (I-IS) sites.* In the mouse NPU, MFG-E8 (1:250) had a relatively weak staining almost exclusive to the endometrial epithelium (A, arrow, 200×), whereas αvβ3 integrin (1:50) was present in both epithelium and stroma (B, arrows, 200×). C. NC: respective negative controls (100×). D. MFG-E8 mRNA expression in non-pregnant uterus (NPU), IS and I-IS on post coital days 5 and 8 examined by real time PCR using 18 S rRNA as internal control to normalize cycle threshold (Ct). Values represent mean ± standard deviation of fold-change. *: significant differences versus non-pregnant uterus.

cytoplasmic pattern (Fig. 2A, upper panel). This pattern was similar in all three trimesters of gestation (Fig. 2A, lower panels).

Integrin αvβ3 also localized to the placental tissues throughout gestation with a more variable cytoplasmic staining intensity in all villous components (cytotrophoblasts, syncytiotrophoblasts, and villous core (Fig. 2B)).

3.3. MFG-E8 is present in blood vessels of human placenta and gravid mouse uterus

MFG-E8 was also associated with the blood vessels present in the chorionic villi (Fig. 3A). Double staining of placental sections for MFG-E8 (Fig. 3B green fluorescence) and the endothelial cell marker CD31 (Fig. 3C, red fluorescence) revealed that MFG-E8 staining coincided with CD31 + endothelial cells (Fig. 3D, merger).

MFG-E8 also localized to the myometrial and subserosal vasculature of the gravid mouse uterus, both at the IS and the I-IS at both days studied (Fig. 3E and F). It was apparent that MFG-E8 positive cells were detected lining the blood vessels (endothelial cells, arrows) as well as within the intravascular content.

3.4. MFG-E8 and its receptor αvβ3 integrin are present in early murine implantation sites

We examined the immunolocalization of MFG-E8 and αvβ3 integrin in pregnant murine uterus at 5 and 8 days post-coitus.

At pc d5, MFG-E8 localized to the luminal and glandular epithelium both at the IS and the I-IS (Fig. 4A). An implanting blastocyst is shown on day 5 (Fig. 4B and C) displaying intense and diffuse MFG-E8 staining (Fig. 4C). Conversely, integrin αvβ3 was localized diffusely through all cell types in the epithelium-stroma (Fig. 4D).

By pc d8, with closure of the lumen at the IS (Fig. 5A), MFG-E8 was more prominent in the glandular epithelium (Fig. 5B) and also in the decidua at the IS (Fig. 5C).

The intensity of MFG-E8 staining was significantly higher ($p < 0.0001$) in the IS compared to the I-IS for pregnancy day 8 but not for day 5 ($p = 0.6$). Integrin αvβ3 was localized diffusely through all cell types (Fig. 5F). An implanting blastocyst at pc d8 (Fig. 5D and E), displayed diffuse MFG-E8 (Fig. 5D), as well as αvβ3 (Fig. 5F) staining.

In the non-pregnant uterus integrin αvβ3 was localized diffusely through all cell types (Fig. 6). Similar to the non-gravid human endometrium (Fig. 1), the non-pregnant murine endometrium stained for MFG-E8 almost exclusively in the epithelium (both glandular and luminal), being absent in the stroma of the non-pregnant uterus (Fig. 6A), whereas αvβ3 integrin stained in both epithelium and stroma (Fig. 6B).

3.5. MFG-E8 mRNA is up-regulated in murine implantation sites

We also examined MFG-E8 mRNA expression in the non-pregnant mouse uterus as well as in murine IS and I-IS (Fig. 6D). Although MFG-E8 mRNA expression was relatively low on day 5 both in IS and I-IS, these values were significantly higher than in the non-pregnant uterus (IS: 13-fold increase, $p = 0.04$, and I-IS: 8.3-fold increase, $p = 0.003$ compared to non-pregnant uterus). MFG-E8 expression was significantly up-regulated on day 8, much more so at the IS (301-fold increase, $p = 0.0002$) than at the I-IS (72-fold increase, $p = 0.006$).

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4. Discussion

To the best of our knowledge, this is the first demonstration of the presence of MFG-E8 mRNA and protein in placental tissue obtained from normal human products of conception as well as in normal murine IS and in the mouse embryo. This work extends our previous characterization of MFG-E8 in human endometrial tissue and primary endometrial cells [16].

There are no available data on the presence of MFG-E8 protein or its possible role in the endometrium of any mammals. There is one report of MFG-E8 expression by microarray analysis being 3.9 times higher in the myometrium of pregnant women compared to the non-pregnant state, suggesting MFG-E8 may have a role during pregnancy [26]. As shown by us, in the human endometrium MFG-E8 protein localized almost exclusively to the epithelial cells, both surface and glandular, with minimal immunostaining observed in the stroma [16]. We found changes in the temporal pattern of MFG-E8 protein expression with highest levels in the secretory phase of the menstrual cycle, and peak staining observed during cycle days 21 and 24, encompassing the window of implantation [16]. This was temporarily coincident with the highest glandular expression of one of its specific ligands, αvβ3 integrin [16,27].

MFG-E8 was also strongly present outlining the human chorionic villi and in murine implantation sites. This is in accordance with the literature showing MFG-E8 as an epithelial cell marker and an epithelial secretory product [22]. MFG-E8 is secreted in microvesicles in several body fluids (milk, seminal plasma) as well as in the peripheral circulation (in patients with systemic lupus erythematosus) [28,29].

The robust and conserved expression of MFG-E8 and its receptor integrin αvβ3, in the receptive endometrium, and in the human chorionic villi and murine implantation sites, strongly suggest a role in endometrial reorganization/remodeling and in implantation and placentation at different stages of pregnancy in mammals. MFG-E8 has also been involved in the protection against rotavirus-induced diarrhea by binding specifically to the virus, thereby blocking viral binding to the host-cell receptors and inhibiting viral replication [30]. Therefore, MFG-E8 might also play a key defensive role in the endometrium.

MFG-E8 mRNA expression also increased significantly towards the end of the window of implantation in the human [16]. In mice, its expression increased 13 times (day 5) and 301 times (day 8) in IS compared with the non-pregnant uterus.

We also found MFG-E8 expressed in a human endometrial endothelial cell line (HEEC) as well as in blood vessels of human chorionic villi and in mouse uterus. We are currently evaluating MFG-E8 possible angiogenic properties in these cells [18]. Silvestre et al., 2005 [12] demonstrated MFG-E8 presence in endothelial cells of human aorta and its crucial role as mediator of VEGF proangiogenic effect *in vivo* and *in vitro* studies in mice. In addition, MFG-E8 has been found to be an essential component of the endothelial-derived microparticles that relay biosignals and modulate arterial wall phenotypes [31].

The human endometrium is a tissue with a very high degree of turnover and reorganization both on a monthly basis (menses) as well as during pregnancy. Most of MFG-E8 pleiotroic effects reported in other tissues such as epithelial repair-remodeling and removal of apoptotic cells [32–34], stromal differentiation for glandular development [35], epithelial protection from infections [29], control of angiogenesis [12,13] and smooth muscle differentiation [36], and attenuation of intestinal inflammation such as seen in murine experimental cholitis by modulation of osteopontin-dependent αvβ3 integrin signaling [37], could have homologs in normal endometrial physiology.

In conclusion, the findings of the present study support functional contributions of MFG-E8 in key events related to normal pregnancy in mammals with possible modulatory roles as an epithelial/stromal, as well as an angiogenic factor, in the endometrium and placenta.

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