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Expression, Regulation and Functional Characterization of Matrix Metalloproteinase-3 of Human Trophoblast

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Abstract

MMP-3 has been detected in human placenta and reduced expression of the enzyme was observed in invasive trophoblasts of patients with severe preeclampsia. However, detailed expression pattern, regulation and biological properties of the placental protease have not been elucidated so far. RT-PCR analyses, Western blotting and enzyme activity assays revealed that pro- and active form of MMP-3 were predominantly expressed in purified first trimester villous trophoblasts, in invasive cytotrophoblasts of differentiating explant cultures and in trophoblastic SGHPL-4 cells. Accordingly, immunofluorescence of first trimester placental tissues detected MMP-3 mainly in villous and extravillous cytotrophoblasts. IL-1 β , an inducer of MMP-3 in decidual cells, increased secretion and activity of the protease in trophoblast supernatants in a dose- and time-dependent manner. IL-1 β -stimulated production of the enzyme was suppressed in the presence of inhibitors of MAPK and AKT signalling. Similar to recombinant MMP-3, MMP-3 in supernatants of IL-1 β -stimulated decidual stromal or SGHPL-4 cells degraded IGFBP-1 in vitro resulting in the appearance of cleavage products at approximately 25, 22, 17, 14 and 11 kD. However, cleavage assays using recombinant MMP-2 suggested that the gelatinase may contribute to IGFBP-1 degradation in trophoblast supernatants. Despite its effects on MMP-3 expression IL-1 β failed to significantly alter invasion of SGHPL-4 cells through Matrigel-coated transwells. In conclusion, the data suggest that invasive trophoblast cell models secrete bioactive MMP-3. Inducible expression of the protease involves MAPK and AKT signalling. In addition to the decidua, MMP-3 of trophoblasts may contribute to the regulation of the IGF system by degrading IGFBP-1.

Keywords

Placenta; Trophoblast; MMP-3; IGFBP-1

1. Introduction

Human trophoblast invasion is an indispensable process of placentation and therefore critical for successful pregnancy outcome. Invasive trophoblasts detaching from cell columns of placental anchoring villi migrate into decidualized stroma and arterial vessel of the endometrium and myometrium. In particular, endovascular invasion involving replacement of maternal endothelial cells and trophoblast-mediated apoptosis of vascular smooth muscle cells is thought to result in transformation of spiral arteries [1,2]. This process may ultimately lead to changes in vessel conductivity thereby increasing blood flow into the

placenta which ensures appropriate supply of gases and nutrients for continuous embryonic development. Failures in invasive trophoblast differentiation are associated with pregnancy diseases such as severe forms of preeclampsia and intrauterine growth restriction [3,4]. Shallow invasion of maternal uterine tissue, reduced transformation of spiral arteries and loss of endovascular trophoblasts are hallmarks of the diseases [5]. Although the pathogenic mechanisms remain largely unknown, investigators believe that these defects may account for the incomplete vascular remodelling [6]. Poor perfusion could then cause placental secretion of products provoking endothelial dysfunction [7,8].

Trophoblast invasion is tightly controlled by numerous growth factors and cytokines expressed at the fetal-maternal interface activating diverse signal transduction pathways [9]. As a final consequence, factors promoting trophoblast invasiveness stimulate the release of proteases necessary for matrix degradation [10,11]. Similar to cancer cells, activation of urokinase plasminogen activator [12] and different matrix metalloproteinases (MMPs) are thought to play a prime role [13]. MMPs comprise a large family of structurally related enzymes cleaving a wide range of substrates including numerous matrix proteins, growth factors and proteases [14]. During trophoblast invasion activity of MMPs is controlled in a time and distance-dependent manner since the maternal decidua may block the proteases by producing tissue inhibitors of metallo proteinases (TIMPs) [15].

Amongst MMPs, most studies in trophoblasts are focussed on the role of the gelatinases, MMP-2 and MMP-9 [16–19], since these proteases cleave major components of basement membranes and therefore play a critical role in tumour cell invasion and metastasis [20]. MMP-9 expression and/or activity of trypsin-isolated cytotrophoblasts were shown to increase in the presence of several growth factors and blockage of the enzyme decreased invasiveness [18,19,21–23].

However, several other MMPs were identified in invasive trophoblasts mainly by using immunohistochemistry, but their specific roles remain largely uncharacterised. As an example, different investigators detected MMP-3, also termed stromelysin-1, in placental tissues [24,25] and trophoblast cultures [26,27]. However, regulation and biological properties of the placental protease were not elucidated so far. In particular, MMP-3 was shown to cleave IGFBP-1 [28] which has been described as a regulator of trophoblast motility [29]. Therefore, we here analysed distribution and activity of MMP-3 in placenta and different trophoblast cell systems. Moreover, its putative role in trophoblast invasion and IGFBP-1 cleavage was investigated using SGHPL-4 cells as a model.

2. Materials and methods

2.1. Tissue collection

Placental tissues of uncomplicated early (between 8th and 12th week of gestation, $n = 15$) and late pregnancies (between 38th and 40th week of gestation, $n = 5$) were obtained by evacuation from legal abortions and caesarean section, respectively, with the permission of the ethical committee of the Medical University of Vienna. Informed consent of patients was obtained. Tissues were fixed with formalin and embedded in paraffin for immunohistochemistry or snap-frozen for RNA preparation. Alternatively, first trimester placental material was used for purification of different primary cells or processed for explant culture.

2.2. Cultivation of cell lines

Trophoblastic HTR-8/SVneo and JEG-3 cells were cultivated in RPMI 1640 (GibcoBRL, Life Technologies, Paisley, UK) supplemented with 5% FCS (Biochrom, Berlin, Germany) as described [30]. JEG-3 choriocarcinoma cells, obtained from ATCC, and trophoblastic

SGHPL-4 were grown in DMEM and in a 1:1 mixture of DMEM and Ham's F-12, respectively, supplemented with 10% FCS as previously mentioned [31–33]. SGHPL-4 cells exhibit features of extravillous trophoblasts and behave similarly as primary trophoblasts with respect to invasion and vascular remodelling [2,34].

2.3. Purification and cultivation of first trimester cytotrophoblasts and fibroblasts

Villous cytotrophoblasts were isolated from early placentae (between 8th and 12th week, $n = 6$) using enzymatic dispersion, Percoll (5–70%) density gradient centrifugation and immunopurification as described [35,36]. Cell preparation was routinely checked by immunocytochemistry using cytokeratin 7 (clone OV-TL 12/30, 8.3 mg/ml DAKO, Glostrup, Denmark) and vimentin antibodies (clone Vim 3B4, 1.2 µg/ml; DAKO) to detect trophoblasts (>99%) and contaminating stromal cells (<1%), respectively. Pure trophoblasts were resuspended in DMEM containing 10% FCS and cultivated on gelatine-coated (1%) 24 well plates. Villous fibroblasts ($n = 5$) of different first trimester placentae were isolated after gradient centrifugation of trypsinised placental material (between 25% and 35% Percoll) and passed two times in DMEM supplemented with 10% FCS. Fibroblasts were characterised by vimentin immunocytochemistry (100% of cells), a contamination with trophoblasts was excluded by cytokeratin 7 staining. Decidual stromal cells were prepared by enzymatic digestion as described [37]. First trimester decidua ($n = 3$) was minced in 3mm³ pieces and digested with 2 mg/ml Collagenase I (484 IU/ml; Gibco BRL, Life Technologies, Paisley, UK) and 0.5 mg/ml DNase I (Sigma). Supernatants were filtered through an 80 µm nylon sieve to remove undigested material. Isolated cells were seeded in DMEM/F-12 supplemented with 10% FCS (Biochrom). Decidual stromal cells were characterized after first passage by immunocytochemistry and were positive for vimentin (100%) and pan-keratin (1%), but negative for CD45 and CD56.

2.4. First trimester villous explant culture

Pieces of villous tissue of different first trimester placentae ($n = 6$) were dissected under the microscope and cultivated on collagen I coated-dishes allowing for trophoblast outgrowth as described elsewhere [38,39]. For RNA analyses pure extravillous trophoblasts which had migrated from anchoring sites were mechanically separated from villous material after 72 h as previously mentioned [31,40]. To determine soluble MMP-3 in supernatants of pure EVT, villi were removed after 48 h and residual cells were incubated with fresh medium for an additional 48 h.

2.5. RNA extraction and semi-quantitative RT-PCR

RNA was extracted from frozen tissue samples or cultures as described [31]. Integrity of RNA was evaluated using the Agilent Bioanalyzer 2100 (Agilent, Palo Alto, CA, USA). Reverse transcription and PCR were done as previously mentioned [41]. Cycle numbers were optimized within the linear range of individual PCR reactions. Sequences of the forward and reverse primers to identify mRNA expression were: MMP-3 (5'-TCTGATAAGGAAAAGAACAA-3', 5'-CATTTCATTTCACAG-3', 338 bp, annealing at 52 °C, 28 cycles); glyceraldehyde-3-phosphate dehydrogenase (GAPDH; 5'-CCATGGAGAAGGCTGGGG-3', 5'-CAAAGTTGTCATGGATGACC-3', 194 bp, annealing at 52 °C, 20 cycles) was used as loading control.

2.6. Western blot analyses

For analyses of MMP-3 distribution all culture systems were grown for 48 h in the absence of serum. Supernatants were concentrated 40 times by using Amicon Ultra Centrifugal Filter Devices (Millipore). For inducible expression serum-starved (2 h) SGHPL-4 cells were incubated with different doses of interleukin-1β (IL-1β; R&D Systems) for 24, 48 and 72 h.

For analyses of signal transduction serum-starved SGHPL-4 cells were stimulated with 1 ng/ml IL-1 β . In blocking studies cells were pre-incubated with UO126 (10 μ M, Cell Signalling) or LY294002 (10 μ M, Cell Signalling) 1 h before stimulation. Isolation of total protein and Western blot analyses were done as described [31]. Equal amounts of protein lysate (10 μ g) or supernatant (500 ng) were separated on 10% or 12.5% (for analyses of IGFBP-1 degradation products) SDS/polyacrylamide (PAA) gels and transferred onto polyvinylidene fluoride (PVDF)-membranes (Amersham, Hybond-P). After blocking filters were incubated overnight (4 °C) with antibodies against MMP-3 (MAB1339, 1 μ g/ml, Chemicon), p44/42 MAPK (1:1000, Cell Signalling), phospho(Thr202/Tyr204)-p44/42 MAPK (1:1000, Cell Signalling), IGFBP-1 (MAB675, 1 μ g/ml, R&D Systems), AKT (1:1000, Cell Signalling), phospho-AKT (Ser473, 1:1000, Cell Signalling) or GAPDH (1 μ g/ml, Ambion). After 1 h of treatment (room temperature) with secondary antibodies (anti-mouse or anti-rabbit Ig horseradish peroxidase linked, Amersham; 1:50.000) signals were developed by using ECL Plus Western Blotting Detection System (Amersham Pharmacia Biotech).

2.7. MMP-3 activity assay

For analyses of active and total MMP-3 protein different trophoblast cultures were grown for 48 h in the absence of serum. Subsequently, the active form of MMP-3 was detected in 40-fold concentrated supernatants using colorimetric MMP-3 Biotrak Activity Assay System as described by the supplier (Amersham Pharmacia Biotech.). Total MMP-3 was quantitated after in vitro conversion of inactive MMP-3 to its active form using 1 mM p-aminophenylmercuric acetate (APMA). APMA is known to initiate an autocatalytic mechanism by disturbing the interaction of zinc with a critical cysteine residue [42].

2.8. Immunofluorescence

Serial sections (4–5 μ m) of paraffin-embedded first trimester placental tissues ($n = 6$) were prepared as previously described [31,40]. Slides were incubated overnight with primary antibodies followed by 1 h treatment with FITC-conjugated anti-mouse or anti-rabbit antibodies (5 μ g/ml, Molecular Probes, Eugene, Oregon). The following primary anti-human antibodies were utilised: Ki67 (clone Ki-S5, 5 μ g/ml, Chemicon, Temecula, CA), p57KIP2, (C-20, rabbit, 2 μ g/ml, Santa Cruz Biotechnology, Santa Cruz, CA), cytokeratin 7/vimentin (as described above), MMP-3 (MAB1339, 10 μ g/ml, Chemicon). As a negative control, the primary antibody was replaced by buffer or isotype IgG. Sections were counterstained with 1 μ g/ml of DAPI (Roche, Mannheim, Germany) and covered with flouromount G (Soubio, Birmingham, AL). Sections were analysed by fluorescence microscopy (Olympus BX50) and digitally photographed.

2.9. Invasion assay

Transwell invasion assays were carried out using BD BioCoat™ growth factor reduced Matrigel™ Invasion Chambers (BD Bioscience) as previously mentioned [31]. Each 50.000 SGHPL-4 cells were seeded and incubated in the absence or presence of 1 ng/ml IL-1 β or 5 ng/ml epidermal growth factor (EGF), as a positive control. After 24 h cells on the upper side of the inserts were removed by a cotton swap. Cells on the lower surface were fixed in ice-cold methanol and stained with haematoxylin solution (Merck). Filters were excised and mounted in Aquatex (Merck). For evaluation, cells were counted using Olympus Cell Imaging Software.

2.10. IGFBP-1 cleavage assay

Proteolysis of IGFBP-1 was done by modifying previously published protocols [28,43]. SGHPL-4 cells and decidual cells were incubated with 10 ng/ml IL-1 β for 72 h in the

absence of serum. Supernatants were concentrated with Amicon Ultra Centrifugal Filter Devices (Millipore) and protein concentrations were adjusted. For IGFBP-1 cleavage assay 0.5 mg recombinant IGFBP-1 (R&D Systems) were incubated with supernatant (35 µg protein) or each 50 ng recombinant MMP-3, MMP-2 or MMP-9 (R&D Systems) for 18 h (37 °C) in a buffer containing 50 mM Tris-HCl 7.6, 1 mM APMA, 1.5 mM NaCl, 0.5 mM CaCl₂, 1 µM ZnCl₂, 0.01% BRIJ™ 35 (Sigma) and 1 U/ml heparin (Sigma) as mentioned [44]. In blockage studies recombinant MMPs were pre-incubated for 1 h with a general MMP inhibitor, GM6001 (2.5 µM, Chemicon). Reactions were terminated by addition of SDS sample buffer containing 2-mercaptoethanol and subjected to IGFBP-1 Western blot analyses.

2.11. Statistical analyses

Statistical analyses were performed by Student's paired *t*-test using SPSS 14 (SPSS Inc., Chicago, IL). A *p* value <0.05 was considered statistically significant.

3. Results

3.1. Active MMP-3 is produced in villous and extravillous trophoblasts

MMP-3 expression was investigated in different trophoblast models systems (Fig.1). Semi-quantitative RT-PCR analyses revealed that MMP-3 mRNA was produced in placental tissues of different gestational ages, in primary cultures of trophoblast and fibroblast as well as in invasive, extravillous trophoblasts purified from first trimester explant cultures (Fig. 1A). Villous trophoblast-specific MMP-3 expression was high during the first trimester of pregnancy but decreased in later gestation. MMP-3 transcripts were also detectable in trophoblastic SGHPL-4 cells but largely absent from HTR-8/SVneo and JEG-3 cells. Similarly, Western blot analyses of cell culture supernatants indicated that the pro-form of MMP-3 was predominantly secreted from villous and extravillous trophoblasts of early pregnancy and from SGHPL-4 cells (Fig. 1B). Accordingly, pro- and active forms of MMP-3 were highest in these cell types, but undetectable in supernatants of HTR-8/SVneo or JEG-3 cells (Fig.1C). Therefore, SGHPL-4 cells were further used as model to study regulation and function of MMP-3. Immunofluorescence of first trimester placental tissues indicated that MMP-3 is produced in Ki67-positive trophoblasts of cell columns as well as in non-proliferating, p57/KIP2-positive extravillous trophoblasts (Fig. 1D). Within the villous MMP-3 protein predominantly localized to the cytoplasm of villous cytotrophoblasts, its expression decreased during syncytium formation.

3.2. Comparison of basal and IL-1β-dependent MMP-3 expression in SGHPL-4 cells and decidual fibroblasts

Since IL-1 was shown to regulate expression and activity of MMP-9 [18,19], the effect of the cytokine on trophoblastic MMP-3 expression was investigated. Recombinant IL-1β increased MMP-3 mRNA expression (Fig. 2A) and secretion of the 57 kD pro-form (Fig. 2B) in a time- and dose-dependent manner. Similarly, active and total MMP-3 of SGHPL-4 cells were elevated in the presence of different IL-1β concentrations (Fig. 2C). In comparison to trophoblast, MMP-3 isolated from supernatants of decidual fibroblasts [45] was similar with respect to molecular weight, IL-1β-dependent expression and APMA activation (Fig. 2D).

3.3. IL-1β increases MMP-3 expression through AKT and MAPK signalling

Growth factors activating mitogen-activated protein kinase (MAPK) and AKT signalling are known to stimulate trophoblast migration and invasion [9]. Therefore, IL-1β-dependent phosphorylation of these kinases was investigated. Addition of 1 ng/ml IL-1β activated both

p42 and p44 MAPK as well as AKT (Fig. 3A). UO126, a dual MAPK kinase (MEK1/MEK2) inhibitor, or LY294002, an inhibitor of phosphoinositide 3-kinase (PI3K)/AKT signalling strongly decreased IL-1 β -induced MMP-3 expression and/or secretion (Fig. 3B).

3.4. Trophoblast-derived MMP-3 cleaves IGFBP-1

Recently, decidual MMP-3 was suggested to cleave IGFBP-1 which may suggest a role of the protease in regulating IGF-II bio-availability [28]. Therefore, decidual and trophoblast-derived MMP-3 were compared with respect to IL-1 β induction and IGFBP-1 degradation (Fig. 4). In vitro cleavage of recombinant IGFBP-1 could be observed upon incubation with supernatants of SGHPL-4 cells resulting in the production of three larger cleavage products (approx. 25 kD, 22 kD, and 17 kD) as well as of smaller fragments between 14 and 11 kD (Fig. 4A). Enhanced degradation was observed when supernatants of IL-1 β -treated cells were utilised. Treatment of IGFBP-1 with 50 ng/ml of recombinant MMP-3 also produced a prominent cleavage product at 25 kD and weaker signals at 22 kD, 17 kD and 14 kD (Fig. 4A and B). In vitro cleavage required APMA-induced conversion of inactive MMP-3 molecules to active proteins, but could also be observed in untreated supernatants at 6–8-fold higher concentrations (not shown). Similar to SGHPL-4 cells and recombinant MMP-3, supernatants of decidual fibroblasts generated IGFBP-1 cleavage products at approx. 25 kD, 22 kD, 17 kD and 14 kD after IL-1 β treatment and APMA activation (Fig. 4B). Besides MMP-3 other MMPs of SGHPL-5 supernatants may contribute to IGFBP-1 degradation. Cleavage assays in the presence of recombinant MMP-2 and MMP-9 suggest that MMP-2 can also degrade IGFBP-1 which was blocked upon addition of a general MMP-inhibitor (Fig. 4C).

3.5. Trophoblast invasion in the presence of IL-1 β

Invasion of SGHPL-4 cells through Matrigel-coated transwells was analysed in presence of different IL-1 β concentrations (Fig. 5). Whereas EGF, used as a positive control, stimulated trophoblast invasion 3.8-fold, IL-1 β did not significantly alter invasiveness at 1 or 10 ng/ml. Similarly, 0.5 or 5 ng/ml of the cytokine failed to provoke changes in SGHPL-4 cell invasion (data not shown).

4. Discussion

MMP-3 has a broad spectrum of ECM substrates including fibronectin, laminin, vitronectin, different collagens, as well as other MMPs such as pro-MMP-2 or -9 which are proteolytically activated by the particular enzyme [13]. Similar to other MMPs, MMP-3 is thought to play a role in physiological tissue remodelling processes; however, abnormal expression of the protease was shown to promote tumour progression [46]. Also, MMP-3 can provoke transition of epithelial to mesenchymal cells thereby facilitating invasion and metastasis [47]. Since invasive differentiation of trophoblasts involves gestation-dependent activation of gelatinases [13] and other protease systems, it may not be surprising that MMP-3 could also be involved. Indeed, MMP-3 protein has been consistently found in EVT throughout pregnancy using immunohistochemistry [24,25] and decreased expression of the enzyme was noticed in EVT around spiral arteries of severe preeclamptic patients [48]. Also, three-dimensional growth of the invasive trophoblast cell line SGHPL-4 was shown to provoke MMP-3 expression [26] and invasion-promoting factors such as 3,5,3'-triiodothyronine increased the particular protease [49].

However, activity of MMP-3 in different placental cultures, regulating signalling pathways and biological properties have not been investigated so far. To gain more insights into trophoblast-derived MMP-3 function descriptive analyses (RT-PCR, Western blotting, immunofluorescence) as well as functional assays (enzyme activity, IL-1 β -dependent

regulation, IGFBP-1 cleavage) were performed. MMP-3 mRNA and protein analyses suggested that the zymogen is expressed and secreted from primary villous and invasive cytotrophoblasts and trophoblastic SGHPL-4 cells as well from decidual and villous fibroblasts. Accordingly, immunofluorescence detected MMP-3 in the diverse placental cell types. Interestingly, stromal expression of MMP-3 within the villous core was hardly detectable on tissue sections whereas considerable amounts of the enzyme were noticed in cultured villous fibroblasts. This may suggest aberrant upregulation of the protease upon in vitro cultivation of these cells. Under non-stimulated conditions MMP-3 protein was largely undetectable in supernatants of confluent primary cultures and cell lines. However, approximately 40-fold concentration of the conditioned medium allowed for detection of pro- and active forms of the protease. Enzyme activity assays of primary trophoblasts, fibroblasts and SGHPL-4 cells revealed that approximately one third of total MMP-3 was secreted in its active form. The ratio between active and inactive MMP-3 considerably increased upon induction with IL-1 β . It is assumed that the cytokine may not only induce expression of the pro-form through transcriptional mechanisms [13] but also activate MMP-3-processing proteases. For example, IL-1 β is known to induce the plasmin/plasminogen activator system which activates diverse MMPs including stromelysins [50]. Indeed, IL-1 β dependent upregulation of uPA has also been noticed in cultured trophoblasts [51].

Signalling pathways involving MAPK or PI3 K/AKT are utilised by a variety of growth factors promoting trophoblast invasion [9]. Inhibition of these kinases was shown to diminish growth factor-dependent expression of MMPs and in vitro invasiveness [52,53]. The current study suggests that MAPK and PI3K/AKT signalling are activated upon IL-1 β treatment in trophoblasts. Similar to previous results on gelatinases, inhibition of the kinases decreased MMP-3 expression suggesting that both pathways are critical for IL-1 β -dependent accumulation in cell culture supernatants. Surprisingly, IL-1 β stimulation failed to significantly alter SGHPL-4 cell invasion in vitro. In contrast, IL-1 β was shown to stimulate invasion of first trimester cytotrophoblasts through Matrigel approximately 1.5-fold [23]. We envision several possibilities for this discrepancy. Due to the fact that trophoblast cell lines were generated by transfection with SV40 large T-antigen, these cultures may only partially mimic the response of primary trophoblasts to cytokines. Alternatively, IL-1 β may not only increase proteases necessary for invasion but also induce protease inhibitors. Indeed, IL-1 β -dependent increase of inhibitors of uPA, PAI-1 and PAI-1, was noticed in SGHPL-4 cells (data not shown). Hence, blockage of another protease system necessary for trophoblast invasion may counteract the invasion-promoting effects of MMPs. A shift in the balance of proteases and their inhibitors could explain the subtle differences between SGHPL-4 cells and first trimester cytotrophoblasts.

On the other hand, IL-1 β -stimulated invasion of primary trophoblasts is modest as compared to other growth factors. Also, Th1 cytokine-dependent expression of MMPs could be regarded in the light of abnormal placentation and pregnancy diseases rather than physiological trophoblast invasion. Except for the presumptive role of IL-1 in implantation, inflammation/IL-1-dependent expression of MMPs has been implicated in preterm rupture of membranes, preterm labour and preeclampsia [54,55]. With respect to that, a putative role of Th1 cytokine-induced MMP-3 expression in normal physiology of chorio-decidua and placenta has to be questioned. For example, IL-1 β -dependent expression of MMP-3 in decidual cells is dampened upon progesterone treatment or co-cultivation with trophoblasts [56,57] suggesting that down-regulation of the endometrial enzyme could be beneficial for the progression of pregnancy. These, facts, however, do not exclude the possibility that balanced levels of active MMP-3 secreted from trophoblast/decidua play a role at certain stages of placental differentiation and invasion. For example, MMP-3 was shown to cleave and thereby activate various other regulatory proteins, such as osteopontin, heparin-binding

EGF or endostatin [58–60]. Active forms of these factors were shown to affect trophoblast motility [61–63].

In comparison to decidual fibroblasts, trophoblast-derived MMP-3 shows similar properties with respect to molecular weight, induction by IL-1 β and IGFBP-1 cleavage. Degradation of IGFBP-1 by trophoblast-conditioned medium, decidual fibroblast supernatants or recombinant MMP-3 produced protein fragments of similar sizes, respectively. However, comparison of cleavage products generated by recombinant MMP-3 or SGHPL-5 cell-conditioned medium (Fig. 4A) suggests that other MMPs may contribute to IGFBP-1 proteolysis in trophoblast supernatants. Of the enzymes tested recombinant MMP-2 also degraded IGFBP-1 in vitro suggesting that some of the proteolytic fragments produced by SGHPL-5 supernatants might be attributed to particular gelatinase. Indeed, MMP-2 was also shown to degrade other IGF-binding proteins such as IGFBP-3 and IGFBP-5 [64,65].

In vitro degradation of IGFBP-1 is probably not very effective, since even concentrated supernatants containing active MMP-3 failed to cleave IGFBP-1 the absence of IL-1 β stimulation and APMA activation. We assume that high concentrations of the enzyme are required for MMP-3 auto-activation and IGFBP-1 in vitro cleavage. Indeed, a previous study showed that 1 mg of protein of non-activated decidual cell supernatants and 1 μ g of recombinant MMP-3 were required for IGFBP-1 degradation [28], whereas in this study 35 μ g protein of supernatant and 50 ng recombinant MMP-3 (both APMA activated) were sufficient.

Degradation products of IGFBP-1 were shown to be unable to bind IGF-I [28] suggesting that MMP-3 could indirectly promote trophoblast invasion by increasing bio-availability of IGFs at the fetal–maternal interface. However, the role of IGFBP-1 in trophoblast is still under discussion [29]. The binding protein contains RGD sequences allowing for interaction with integrin $\alpha 5 \beta 1$ [66]. Depending on the trophoblast in vitro system addition of IGFBP-1 was shown to inhibit or stimulate trophoblast invasion [67,68]. Hence, MMP-3-mediated degradation of IGFBP-1 may also negatively affect trophoblast invasion.

In conclusion, the results suggest that signalling cascades controlling trophoblast motility stimulate production and secretion of active MMP-3 from invasive and non-invasive cytotrophoblasts. Trophoblast-derived MMP-3 may directly or indirectly contribute to trophoblast motility for example through regulation of the IGF/IGFBP system. Alternatively, abnormal activation of MMP-3 in the presence of TH1 cytokines such as IL-1 β could be involved in pregnancy complications. Additional studies are required to more precisely delineate the role of placental MMP-3 under physiological and pathological conditions.

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References

- [1]. Pijnenborg R, Bland JM, Robertson WB, Brosens I. Uteroplacental arterial changes related to interstitial trophoblast migration in early human pregnancy. *Placenta*. 1983; 4:397–413. [PubMed: 6634666]
- [2]. Harris LK, Keogh RJ, Wareing M, Baker PN, Cartwright JE, Aplin JD, et al. Invasive trophoblasts stimulate vascular smooth muscle cell apoptosis by a fas ligand-dependent mechanism. *Am J Pathol*. 2006; 169:1863–74. [PubMed: 17071607]

- [3]. Khong TY, De Wolf F, Robertson WB, Brosens I. Inadequate maternal vascular response to placentation in pregnancies complicated by pre-eclampsia and by small-for-gestational age infants. *Br J Obstet Gynaecol.* 1986; 93:1049–59. [PubMed: 3790464]
- [4]. Pijnenborg R, Anthony J, Davey DA, Rees A, Tiltman A, Vercruysse L, et al. Placental bed spiral arteries in the hypertensive disorders of pregnancy. *Br J Obstet Gynaecol.* 1991; 98:648–55. [PubMed: 1883787]
- [5]. Zhou Y, Damsky CH, Fisher SJ. Preeclampsia is associated with failure of human cytotrophoblasts to mimic a vascular adhesion phenotype. One cause of defective endovascular invasion in this syndrome? *J Clin Invest.* 1997; 99:2152–64. [PubMed: 9151787]
- [6]. Bower SJ, Harrington KF, Schuchter K, McGirr C, Campbell S. Prediction of pre-eclampsia by abnormal uterine doppler ultrasound and modification by aspirin. *Br J Obstet Gynaecol.* 1996; 103:625–9. [PubMed: 8688387]
- [7]. Burton GJ, Jauniaux E. Placental oxidative stress: from miscarriage to preeclampsia. *J Soc Gynecol Investig.* 2004; 11:342–52.
- [8]. Sargent IL, Borzychowski AM, Redman CW. Immunoregulation in normal pregnancy and pre-eclampsia: an overview. *Reprod Biomed Online.* 2006; 13:680–6. [PubMed: 17169180]
- [9]. Pollheimer J, Knofler M. Signalling pathways regulating the invasive differentiation of human trophoblasts: a review. *Placenta.* 2005; 26(Suppl. A):S21–30. [PubMed: 15837062]
- [10]. Bischof P, Meisser A, Campana A. Paracrine and autocrine regulators of trophoblast invasion—a review. *Placenta.* 2000; 21(Suppl. A):S55–60. [PubMed: 10831123]
- [11]. Lala PK, Hamilton GS. Growth factors, proteases and protease inhibitors in the maternal–fetal dialogue. *Placenta.* 1996; 17:545–55. [PubMed: 8916202]
- [12]. Liu J, Chakraborty C, Graham CH, Barbin YP, Dixon SJ, Lala PK. Noncatalytic domain of uPA stimulates human extravillous trophoblast migration by using phospholipase C, phosphatidylinositol 3-kinase and mitogen-activated protein kinase. *Exp Cell Res.* 2003; 286:138–51. [PubMed: 12729802]
- [13]. Cohen M, Meisser A, Bischof P. Metalloproteinases and human placental invasiveness. *Placenta.* 2006; 27:783–93. [PubMed: 16249026]
- [14]. Fingleton B. Matrix metalloproteinases: roles in cancer and metastasis. *Front Biosci.* 2006; 11:479–91. [PubMed: 16146745]
- [15]. Lala PK, Graham CH. Mechanisms of trophoblast invasiveness and their control: the role of proteases and protease inhibitors. *Cancer Metastasis Rev.* 1990; 9:369–79. [PubMed: 2097085]
- [16]. Bischof P, Friedli E, Martelli M, Campana A. Expression of extracellular matrix-degrading metalloproteinases by cultured human cytotrophoblast cells: effects of cell adhesion and immunopurification. *Am J Obstet Gynecol.* 1991; 165:1791–801. [PubMed: 1750477]
- [17]. Fisher SJ, Cui TY, Zhang L, Hartman L, Grahl K, Zhang GY, et al. Adhesive and degradative properties of human placental cytotrophoblast cells in vitro. *J Cell Biol.* 1989; 109:891–902. [PubMed: 2474556]
- [18]. Librach CL, Werb Z, Fitzgerald ML, Chiu K, Corwin NM, Esteves RA, et al. 92-kD Type IV collagenase mediates invasion of human cytotrophoblasts. *J Cell Biol.* 1991; 113:437–49. [PubMed: 1849141]
- [19]. Meisser A, Chardonens D, Campana A, Bischof P. Effects of tumour necrosis factor-alpha, interleukin-1 alpha, macrophage colony stimulating factor and transforming growth factor beta on trophoblastic matrix metalloproteinases. *Mol Hum Reprod.* 1999; 5:252–60. [PubMed: 10333360]
- [20]. Bjorklund M, Koivunen E. Gelatinase-mediated migration and invasion of cancer cells. *Biochim Biophys Acta.* 2005; 1755:37–69. [PubMed: 15907591]
- [21]. Castellucci M, De Matteis R, Meisser A, Cancellato R, Monsurro V, Islami D, et al. Leptin modulates extracellular matrix molecules and metalloproteinases: possible implications for trophoblast invasion. *Mol Hum Reprod.* 2000; 6:951–8. [PubMed: 11006325]
- [22]. Meisser A, Cameo P, Islami D, Campana A, Bischof P. Effects of interleukin-6 (IL-6) on cytotrophoblastic cells. *Mol Hum Reprod.* 1999; 5:1055–8. [PubMed: 10541568]

- [23]. Librach CL, Feigenbaum SL, Bass KE, Cui TY, Verastas N, Sadovsky Y, et al. Interleukin-1 beta regulates human cytotrophoblast metalloproteinase activity and invasion in vitro. *J Biol Chem.* 1994; 269:17125–31. [PubMed: 8006017]
- [24]. Huppertz B, Kertschanska S, Demir AY, Frank HG, Kaufmann P. Immunohistochemistry of matrix metalloproteinases (MMP), their substrates, and their inhibitors (TIMP) during trophoblast invasion in the human placenta. *Cell Tissue Res.* 1998; 291:133–48. [PubMed: 9394051]
- [25]. Maquoi E, Polette M, Nawrocki B, Bischof P, Noel A, Pintiaux A, et al. Expression of stromelysin-3 in the human placenta and placental bed. *Placenta.* 1997; 18:277–85. [PubMed: 9179921]
- [26]. Lamarca HL, Ott CM, Honer Zu, Bentrup K, Leblanc CL, Pierson DL, Nelson AB, et al. Three-dimensional growth of extravillous cytotrophoblasts promotes differentiation and invasion. *Placenta.* 2005; 26:709–20. [PubMed: 16226120]
- [27]. Logan SK, Hansell EJ, Damsky CH, Werb Z. T-antigen inhibits metalloproteinase expression and invasion in human placental cells transformed with temperature-sensitive simian virus 40. *Matrix Biol.* 1996; 15:81–9. [PubMed: 8837009]
- [28]. Coppock HA, White A, Aplin JD, Westwood M. Matrix metalloprotease-3 and -9 proteolyze insulin-like growth factor-binding protein-1. *Biol Reprod.* 2004; 71:438–43. [PubMed: 15070833]
- [29]. Westwood M. Role of insulin-like growth factor binding protein 1 in human pregnancy. *Rev Reprod.* 1999; 4:160–7. [PubMed: 10521153]
- [30]. Graham CH, Hawley TS, Hawley RG, MacDougall JR, Kerbel RS, Khoo N, et al. Establishment and characterization of first trimester human trophoblast cells with extended lifespan. *Exp Cell Res.* 1993; 206:204–11. [PubMed: 7684692]
- [31]. Pollheimer J, Loregger T, Sonderegger S, Saleh L, Bauer S, Bilban M, et al. Activation of the canonical wntless/T-cell factor signaling pathway promotes invasive differentiation of human trophoblast. *Am J Pathol.* 2006; 168:1134–47. [PubMed: 16565489]
- [32]. Choy MY, Manyonda IT. The phagocytic activity of human first trimester extravillous trophoblast. *Hum Reprod.* 1998; 13:2941–9. [PubMed: 9804259]
- [33]. Choy MY, St Whitley G, Manyonda IT. Efficient, rapid and reliable establishment of human trophoblast cell lines using poly-L-ornithine. *Early Pregnancy.* 2000; 4:124–43. [PubMed: 11723542]
- [34]. Cartwright JE, Kenny LC, Dash PR, Crocker IP, Aplin JD, Baker PN, et al. Trophoblast invasion of spiral arteries: a novel in vitro model. *Placenta.* 2002; 23:232–5. [PubMed: 11945091]
- [35]. Kliman HJ, Nestler JE, Sermasi E, Sanger JM, Strauss JF 3rd. Purification, characterization, and in vitro differentiation of cytotrophoblasts from human term placentae. *Endocrinology.* 1986; 118:1567–82. [PubMed: 3512258]
- [36]. Knofler M, Saleh L, Bauer S, Galos B, Rotheneder H, Husslein P, et al. Transcriptional regulation of the human chorionic gonadotropin beta gene during villous trophoblast differentiation. *Endocrinology.* 2004; 145:1685–94. [PubMed: 14715707]
- [37]. Selam B, Kayisli UA, Garcia-Velasco JA, Arici A. Extracellular matrix-dependent regulation of Fas ligand expression in human endometrial stromal cells. *Biol Reprod.* 2002; 66:1–5. [PubMed: 11751256]
- [38]. Genbacev O, Schubach SA, Miller RK. Villous culture of first trimester human placenta—model to study extravillous trophoblast (EVT) differentiation. *Placenta.* 1992; 13:439–61. [PubMed: 1470605]
- [39]. Vicovac L, Jones CJ, Aplin JD. Trophoblast differentiation during formation of anchoring villi in a model of the early human placenta in vitro. *Placenta.* 1995; 16:41–56. [PubMed: 7716127]
- [40]. Bauer S, Pollheimer J, Hartmann J, Husslein P, Aplin JD, Knofler M. Tumor necrosis factor- α inhibits trophoblast migration through elevation of plasminogen activator inhibitor-1 in first-trimester villous explant cultures. *J Clin Endocrinol Metab.* 2004; 89:812–22. [PubMed: 14764800]

- [41]. Leisser C, Saleh L, Haider S, Husslein H, Sonderegger S, Knofler M. Tumour necrosis factor- α impairs chorionic gonadotrophin beta-subunit expression and cell fusion of human villous cytotrophoblast. *Mol Hum Reprod*. 2006; 12:601–9. [PubMed: 16896069]
- [42]. Housley TJ, Baumann AP, Braun ID, Davis G, Seperack PK, Wilhelm SM. Recombinant Chinese hamster ovary cell matrix metalloprotease-3 (MMP-3, stromelysin-1). Role of calcium in promatrix metalloprotease-3 (pro-MMP-3, prostromelysin-1) activation and thermostability of the low mass catalytic domain of MMP-3. *J Biol Chem*. 1993; 268:4481–7. [PubMed: 8440730]
- [43]. Nakamura M, Miyamoto S, Maeda H, Ishii G, Hasebe T, Chiba T, et al. Matrix metalloproteinase-7 degrades all insulin-like growth factor binding proteins and facilitates insulin-like growth factor bioavailability. *Biochem Biophys Res Commun*. 2005; 333:1011–6. [PubMed: 15964556]
- [44]. Galazka G, Windsor LJ, Birkedal-Hansen H, Engler JA. APMA (4-amino-phenylmercuric acetate) activation of stromelysin-1 involves protein interactions in addition to those with cysteine-75 in the propeptide. *Biochemistry*. 1996; 35:11221–7. [PubMed: 8780527]
- [45]. Schatz F, Papp C, Toth-Pal E, Lockwood CJ. Ovarian steroid-modulated stromelysin-1 expression in human endometrial stromal and decidual cells. *J Clin Endocrinol Metab*. 1994; 78:1467–72. [PubMed: 8200951]
- [46]. Sternlicht MD, Bissell MJ, Werb Z. The matrix metalloproteinase stromelysin-1 acts as a natural mammary tumor promoter. *Oncogene*. 2000; 19:1102–13. [PubMed: 10713697]
- [47]. Przybylo JA, Radisky DC. Matrix metalloproteinase-induced epithelial-mesenchymal transition: tumor progression at Snail's pace. *Int J Biochem Cell Biol*. 2007; 39:1082–8. [PubMed: 17416542]
- [48]. Reister F, Kingdom JC, Ruck P, Marzusch K, Heyl W, Pauer U, et al. Altered protease expression by periarterial trophoblast cells in severe early-onset preeclampsia with IUGR. *J Perinat Med*. 2006; 34:272–9. [PubMed: 16856814]
- [49]. Oki N, Matsuo H, Nakago S, Murakoshi H, Laoag-Fernandez JB, Maruo T. Effects of 3,5,3'-triiodothyronine on the invasive potential and the expression of integrins and matrix metalloproteinases in cultured early placental extravillous trophoblasts. *J Clin Endocrinol Metab*. 2004; 89:5213–21. [PubMed: 15472228]
- [50]. Chakraborti S, Mandal M, Das S, Mandal A, Chakraborti T. Regulation of matrix metalloproteinases: an overview. *Mol Cell Biochem*. 2003; 253:269–85. [PubMed: 14619979]
- [51]. Karmakar S, Das C. Regulation of trophoblast invasion by IL-1 β and TGF- β 1. *Am J Reprod Immunol*. 2002; 48:210–9. [PubMed: 12516631]
- [52]. Prast J, Saleh L, Husslein H, Sonderegger S, Helmer H, Knofler M. Human chorionic gonadotropin stimulates trophoblast invasion through extracellularly regulated kinase and AKT signaling. *Endocrinology*. 2008; 149:979–87. [PubMed: 18063683]
- [53]. Qiu Q, Yang M, Tsang BK, Gruslin A. EGF-induced trophoblast secretion of MMP-9 and TIMP-1 involves activation of both PI3K and MAPK signalling pathways. *Reproduction*. 2004; 128:355–63. [PubMed: 15333786]
- [54]. Weiss A, Goldman S, Shalev E. The matrix metalloproteinases (MMPS) in the decidua and fetal membranes. *Front Biosci*. 2007; 12:649–59. [PubMed: 17127325]
- [55]. Lockwood CJ, Oner C, Uz YH, Kayisli UA, Huang SJ, Buchwalder LF, et al. Matrix metalloproteinase 9 (MMP9) expression in preeclamptic decidua and MMP9 induction by tumor necrosis factor α and interleukin 1 β in human first trimester decidual cells. *Biol Reprod*. 2008; 78:1064–72. [PubMed: 18276934]
- [56]. Bellingard V, Hedon B, Capony F, Defrenne A, Rochefort H, Maudelonde T. Identification of prometalloproteinase-3 as a major protein secreted by human endometrial fibroblasts and inhibited by coculture with trophoblast cells. *Biol Reprod*. 1996; 55:604–12. [PubMed: 8862778]
- [57]. Keller NR, Sierra-Rivera E, Eisenberg E, Osteen KG. Progesterone exposure prevents matrix metalloproteinase-3 (MMP-3) stimulation by interleukin-1 α in human endometrial stromal cells. *J Clin Endocrinol Metab*. 2000; 85:1611–9. [PubMed: 10770206]
- [58]. Agnihotri R, Crawford HC, Haro H, Matrisian LM, Havrda MC, Liaw L. Osteopontin, a novel substrate for matrix metalloproteinase-3 (stromelysin-1) and matrix metalloproteinase-7 (matrilysin). *J Biol Chem*. 2001; 276:28261–7. [PubMed: 11375993]

- [59]. Heljasvaara R, Nyberg P, Luostarinen J, Parikka M, Heikkilä P, Rehn M, et al. Generation of biologically active endostatin fragments from human collagen XVIII by distinct matrix metalloproteases. *Exp Cell Res*. 2005; 307:292–304. [PubMed: 15950618]
- [60]. Suzuki M, Raab G, Moses MA, Fernandez CA, Klagsbrun M. Matrix metalloproteinase-3 releases active heparin-binding EGF-like growth factor by cleavage at a specific juxtamembrane site. *J Biol Chem*. 1997; 272:31730–7. [PubMed: 9395517]
- [61]. Briesse J, Oberndorfer M, Patschenik C, Schulte HM, Makrigiannakis A, Loning T, et al. Osteopontin is colocalized with the adhesion molecule CEACAM1 in the extravillous trophoblast of the human placenta and enhances invasion of CEACAM1-expressing placental cells. *J Clin Endocrinol Metab*. 2005; 90:5407–13. [PubMed: 15956076]
- [62]. Leach RE, Kilburn B, Wang J, Liu Z, Romero R, Armant DR. Heparin-binding EGF-like growth factor regulates human extravillous cytotrophoblast development during conversion to the invasive phenotype. *Dev Biol*. 2004; 266:223–37. [PubMed: 14738873]
- [63]. Pollheimer J, Husslein P, Knofler M. Invasive trophoblasts generate regulatory collagen XVIII cleavage products. *Placenta*. 2005; 26(Suppl. A):S42–5. [PubMed: 15837066]
- [64]. Fowlkes JL, Enghild JJ, Suzuki K, Nagase H. Matrix metalloproteinases degrade insulin-like growth factor-binding protein-3 in dermal fibroblast cultures. *J Biol Chem*. 1994; 269:25742–6. [PubMed: 7523391]
- [65]. Thrailkill KM, Quarles LD, Nagase H, Suzuki K, Serra DM, Fowlkes JL. Characterization of insulin-like growth factor-binding protein 5-degrading proteases produced throughout murine osteoblast differentiation. *Endocrinology*. 1995; 136:3527–33. [PubMed: 7543045]
- [66]. Jones JI, Gockerman A, Busby WH Jr, Wright G, Clemmons DR. Insulin-like growth factor binding protein 1 stimulates cell migration and binds to the alpha 5 beta 1 integrin by means of its ArgGly-Asp sequence. *Proc Natl Acad Sci U S A*. 1993; 90:10553–7. [PubMed: 7504269]
- [67]. Hamilton GS, Lysiak JJ, Han VK, Lala PK. Autocrine-paracrine regulation of human trophoblast invasiveness by insulin-like growth factor (IGF)-II and IGF-binding protein (IGFBP)-1. *Exp Cell Res*. 1998; 244:147–56. [PubMed: 9770358]
- [68]. Irwin JC, Giudice LC. Insulin-like growth factor binding protein-1 binds to placental cytotrophoblast alpha5beta1 integrin and inhibits cytotrophoblast invasion into decidualized endometrial stromal cultures. *Growth Horm IGF Res*. 1998; 8:21–31. [PubMed: 10990441]

Fig. 1. Placental expression and cellular distribution of MMP-3 in different trophoblast model systems. Representative data are shown. (A) Semi-quantitative RT-PCR analyses. (B) Western blot analyses detecting the secreted pro-form (57 kD) of MMP-3 in culture supernatants. (C) Activity assay of soluble MMP-3 identifying active and total MMP-3. Mean values of two different experiments performed in duplicates are shown. (D) Immunofluorescence of serial sections of an 8th week placenta using antibodies against cytokeratin 7 (CK7), vimentin (Vim), Ki67, p57/Kip2 and MMP-3. Of the latter, the respective DAPI stainings are shown. Pictures were taken at a 200-fold magnification. 1000-fold magnification (indicated) was used for depiction of villous MMP-3 expression. Areas of villous stroma (VS), cell column (CC), extravillous trophoblast (EVT) and syncytium (S) are indicated.

Fig. 2.

IL-1 β -dependent induction of MMP-3 in SGHPL-4 cells and decidual fibroblasts.

Representative experiments are shown. n.c., negative control. (A) Semiquantitative RT-PCR analyses of MMP-3 mRNA in SGHPL-4 cells after 48 h of stimulation with IL-1 β . (B) Western blot analyses of soluble MMP-3 in SGHPL-4 supernatants. The specific MMP-3 signal is indicated. (C) MMP-3 activity assay showing active and total MMP-3 in SGHPL-4 cell supernatants. Mean values $\pm$ S.E.M. of three independent experiments performed in duplicates are shown. (D) Western blot analyses of soluble MMP-3 secreted from SGHPL-4 cells and decidual fibroblasts. Basal and IL-1 β -dependent secretion as well as generation of active form of MMP-3 in the presence of APMA are shown. Non-activated, recombinant MMP-3 was used as a positive control.

Fig. 3.

Western blot analyses indicating a role of MAPK and AKT in MMP-3 expression. Cellular extracts or supernatants of SGHPL-5 cells were separated on PAA gels, transferred to membranes and incubated with different antibodies as described above. Representative examples are shown. (A) IL-1 β -dependent MAPK and AKT activation. Specific signals of phosphorylated and non-phosphorylated forms of MAPK and AKT are indicated. GAPDH was used as a loading control. (B) Inhibition of MAPK or AKT diminishes MMP-3 secretion. Cells were treated with inhibitors and incubated in the absence or presence of IL-1 β for 48 h. The soluble 57 kD MMP-3 protein is marked.

Fig. 4.

In vitro cleavage of IGFBP-1 by MMP-3 secreted from SGHPL-4 cells or decidual fibroblasts. Supernatants were isolated from untreated or IL-1 β -treated cultures, concentrated and used for IGFBP-1 in vitro cleavage assay and IGFBP-1 Western blot analyses as described above. n.c., negative control (recombinant IGFBP-1 only). Representative examples are shown. (A) Comparison of IGFBP-1 cleavage products generated either by recombinant MMP-3 or by untreated and IL-1 β -stimulated SGHPL-4 cell supernatants in the absence or presence of APMA. (B) Comparison of IGFBP-1 cleavage products generated by recombinant MMP-3 or IL-1 β -stimulated decidual fibroblasts in the absence or presence of APMA. (C) IGFBP-1 cleavage assay using recombinant, APMA-activated MMP-2, MMP-3 or MMP-9 (each 50 ng) in the absence or presence of a general MMP inhibitor.

Fig. 5.

Invasion of trophoblastic SGHPL-4 cells in the absence or presence of IL-1 β . Invasion assays through Matrigel-coated transwells after stimulation with different concentrations of IL-1 β were performed as described in Materials and methods. EGF was used as a positive control. Bars represent mean values of 3 different experiments performed in duplicates, error bars indicate S.D. Values of untreated cultures were arbitrarily set to 100%. * $p < 0.05$; ns, not significant.