

Supplementary methods

Primary decidual endothelial cell isolation

Primary human first-trimester decidual endothelial cells were isolated using a modification of the method described by Grimwood *et al* 1995.¹ In brief, maternal decidual tissue from samples obtained from first trimester therapeutic abortions of apparently healthy pregnancies was washed with HBSS, cut into 3mm³ pieces and incubated with 2mg/mL collagenase type 1A (Sigma) in McCoy's 5A medium with 10% (v/v) FCS, 100U/mL penicillin and 0.1mg/mL streptomycin, for 2h at 37°C. The cell suspension was filtered through a 100µm mesh, washed repeatedly with PBS/0.1% (w/v) BSA and further digested in 0.1% (w/v) trypsin (Sigma 1:250) with 0.05% (w/v) DNase I (Roche) for 5 minutes then washed with medium containing 10% (v/v) serum. Ulex europaeus 1 (UEA1, Sigma) was covalently bound to tosylactivated M-450 Dynabeads according to manufacturer's instructions (Dyna, UK). Positive selection of endothelial cells using UEA1-coated dynabeads was performed as described.¹ The selected cells were washed, resuspended in McCoy's medium with 50% (v/v) human serum (pooled male AB), 5ng/mL vascular endothelial growth factor, 16IU/mL heparin, 2mg/mL magnesium sulphate, 100U/mL penicillin and 0.1mg/mL streptomycin and plated in collagen-coated wells.

Immunoprecipitation and western blot analysis of endothelial Fas expression

SGHEC-7 cells (approximately 5×10⁶ cells) or primary decidual endothelial cells (approximately 10⁶ cells) were lysed in 1mL of RIPA buffer (1×PBS, 1% (v/v) NP40, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) SDS) with 0.1mg/mL PMSF, 30µL/mL aprotinin, and 1mmol/L sodium orthovanadate at 4°C for 30 minutes. Lysate was

incubated with 2 μ g of monoclonal anti-Fas (B-10, Santa Cruz) for 1h at 4°C. Protein-G PLUS agarose was added (20 μ L, Santa Cruz) for 16h at 4°C with rotation. The pellet was collected by centrifugation at 1000g for 5 minutes at 4°C, washed three times with RIPA buffer then resuspended in sample buffer. The samples were separated by SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. Following incubation in blocking buffer (10mmol/L Tris, pH 8, 150mmol/L NaCl, 0.05% Tween 20, 10% (w/v) milk powder) for 1h at room temperature, the membrane was incubated with monoclonal anti-Fas (MAB142, R&D) at 1 μ g/mL in blocking buffer for 1h. After washing, the membrane was incubated with anti-mouse IgG peroxidase (A4416, Sigma) at a 1:1000 dilution in blocking buffer for 1h. Detection of membrane bound antibodies was carried out by chemiluminescence (ECLPlus, Amersham).

Western blot analysis of endothelial c-FLIP expression

For analysis of c-FLIP expression, SGHEC-7 endothelial cells were seeded in culture plates and SGHPL-4 extravillous trophoblast cells or SGHEC-7 (to control for cell number) were seeded at the same concentration as the SGHEC-7 cells in tissue culture inserts (Nalge Nunc, 0.4 μ m membrane). After 16h the medium was changed to phenol-red free RPMI/M199 containing with 0.5% (w/v) gelding serum and the inserts were added to the plates (three 25mm inserts/ 9cm plate). After the stated time the endothelial cells on the plate were lysed in RIPA buffer and western blot analysis was carried out. The samples were separated by SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. Following incubation in blocking buffer (10mmol/L Tris, pH 8, 150mmol/L NaCl, 0.05% Tween 20, 10% (w/v) milk powder) for 1h at room temperature, the membrane was incubated with rabbit polyclonal anti-human FLIP (FLICE/caspase 8-inhibitory protein, 1:500, BD PharMingen) which detects the long form of FLIP or monoclonal anti-human FLIP

(1:200, Santa Cruz Biotechnology) which also detects the shorter forms of FLIP for 1h. Anti-rabbit IgG peroxidase (1:6000, A6154, Sigma) or anti-mouse IgG peroxidase (1:5000, A4416, Sigma) were added for 1h. Detection of membrane bound antibodies was carried out by chemiluminescence (ECLPlus, Amersham).

References

1. Grimwood J, Bicknell R, Rees MC. The isolation, characterization and culture of human decidual endothelium. *Hum Reprod.* 1995;10:2142-2148.