Short Communication

The Adhesion Molecule CEACAM1 (CD66a, C-CAM, BGP) Is Specifically Expressed by the Extravillous Intermediate Trophoblast

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CEACAM1 (CD66a, C-CAM, BGP) is an adhesion molecule of the carcinoembryonic antigen family which has been shown to be normally expressed at the apical pole of epithelial cells, including the apical pole of endometrial surface and glandular epithelia. The purpose of the present study was to investigate its expression pattern at the maternal-fetal interface, and thus to determine whether CEACAM1 could be implicated in the human implantation process. For this purpose, we performed immunohistochemistry using the 4D1/C2 monoclonal antibody (mAb) as well as flow cytometry and Western blot on isolated trophoblast populations. On the maternal side of the maternal-fetal interface, CEACAM1 was present in epithelial cells of pregnancy endometrium as well as in small endometrial vessels, whereas it was absent from decidual cells. On the fetal side, CEACAM1 was strongly expressed by the extravillous (intermediate) trophoblast at the implantation site, as well as by extravillous trophoblast cells with invasive phenotype in primary culture, as shown by flow cytometry and Western blot. Expression was also observed in placental villous core vessels but was absent from both villous cyto- and syncytiotrophoblasts throughout the pregnancy. We conclude that, given its specific ex-

pression pattern, CEACAM1 can be a useful marker for extravillous intermediate trophoblast and might be functionally implicated in mediating trophoblast/endometrial and/or trophoblast/endothelial interactions during the trophoblastic invasion of the endometrium. (Am J Pathol 2000, 156:1165–1170)

The trophoblast is the first tissue to differentiate in the mammalian conceptus and its normal development and specific properties are crucial for both implantation and further survival of the embryo. Furthermore, the placenta is unique in its ability to proliferate and invade another tissue in a controlled fashion and is thus a very interesting model for the study of molecular mechanisms involved in these processes, and for differentiating them from those implicated in tumor progression.

During development of the human placenta, the stem cell-like cytotrophoblast proliferates and gives rise to the differentiated syncytiotrophoblast on the villous surface and to the invasive intermediate trophoblast, which invades the maternal tissues and provides the anchoring of the placenta and the conceptus at the maternal-fetal interface.¹ The extravillous trophoblast can be further divided into proximal extravillous trophoblast originating from the anchoring villi; deep interstitial extravillous trophoblast invading the decidual stroma and the myometrium; and endovascular trophoblast, which assumes endothelial-like characteristics.^{2–4}

Starting with the initial contact which is made between the trophoblast and the apical plasma membrane of the endometrial surface epithelial cells, through the invasion of the decidua and the invasion of decidual vessels with gradual colonization of the arterial wall of the spiral arteries, cellular contacts mediated by cell adhesion molecules are of essential importance.

Cell adhesion molecules are important mediators of tissue architecture and cellular polarity which also mod-

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ulate proliferation and differentiation processes. CEACAM1 is a member of the carcinoembryonic antigen family and the immunoglobulin superfamily.⁵ Glycoproteins belonging to this family are expressed in epithelial tissues, such as the colonic mucosa, as well as in cells of the myeloid lineage.⁵ CEACAM1⁶ is the human homologue of the adhesion molecule cell-CAM (C-CAM) of the rat^{7,8} and it has been suggested to function as a ligand for E-selectin.⁹ In contrast to most of the genes of the carcinoembryonic antigen family, the CEACAM1 gene predicts a cytoplasmic domain containing sequence motifs involved in signal transduction^{10,11} which has been shown to associate with pp60 c-src.¹²

The present study was designed to investigate the expression pattern of CEACAM1 in the human placental components and thus its potential implication in implantation and placentation. To investigate the expression of CEACAM1, immunohistochemistry on paraffin-embedded specimens and flow cytometry on isolated trophoblast was performed using the mAb 4D1/C2,^{13,14} which had been previously used to study expression in several normal human tissues,¹⁵ as well as expression in normal and malignant mammary gland and in endometrial neoplasia.^{16,17}

Materials and Methods

Tissue Collection

The tissue material was selected after histological review from the files of the Department of Gynecopathology, University Hospital Eppendorf, Hamburg, Germany, as well as from the Department of Obstetrics and Gynecology, University of Pennsylvania Medical Center, Philadelphia, Pennsylvania. Only normal placentas have been included. For immunohistochemistry, the specimens that had been routinely fixed in 4% buffered formalin and embedded in paraffin were used. Fifty-five samples were analyzed, including 33 first trimester, 10 second trimester, and 12 third trimester placentas.

Immunohistochemistry

Serial sections of 4 to 6 μm were cut from the paraffin blocks and mounted on 3-aminopropyl-triethoxysilan (APES)-coated slides, deparaffinized in xylene, and rehydrated in graded alcohol to Tris-buffered saline (TBS; 50 mmol/L Tris, 150 mmol/L NaCl, pH 7.4). The slides were microwaved for 5×2 minutes in 10 mmol/L citrate, pH 6.0. After cooling down for 20 minutes, the slides were washed in TBS, blocked for 30 minutes at room temperature with normal goat serum (DAKO, Glostrup, Denmark), diluted 1:20 in TBS, and incubated overnight at 4°C with mAb 4D1/C2 at 4 $\mu\text{g}/\text{ml}$ diluted 1:100 in TBS for the detection of CEACAM1. For immunohistochemical characterization of intermediate trophoblasts, slides were additionally incubated with anti-cytokeratin mAb (DAKO) diluted 1:50 in TBS and/or anti-human placental lactogen polyclonal antibody (DAKO) diluted 1:300 in TBS. Non-immune murine serum (DAKO) at the same dilution was

used for negative control. Slides were then reacted with biotin-labeled anti-mouse or anti-rabbit immunoglobulin (IgG), incubated with preformed ABC-complex (Vectastain; Vector Laboratories, Burlingame, CA) and detected with the alkaline phosphatase substrate kit (for CEACAM1 and cytokeratin) or the DAB kit (for human placental lactogen) (Vectastain; Vector Laboratories). The slides were counterstained with hemalaun and mounted with glycerine/gelatin.

Additionally, an alternative detection method was used for the detection of CEACAM1. For this, paraffin-embedded human placental tissue section slides were deparaffinized at 70°C in xylene, rehydrated through a graded ethanol series, and rinsed in phosphate-buffered saline (PBS) (pH 7.4). Sections were treated with 0.1% hydrogen peroxide for 30 minutes at 20°C (to inactivate endogenous peroxidase activity), and washed in PBS. Sections were then digested with prewarmed pepsin (0.65 mg/ml in PBS) at 40°C for 5 minutes, preincubated with 5% blocking serum (normal goat serum; Vector Laboratories), and then incubated with the primary antibody for 1 hour at room temperature. Localization of the primary antibody was performed by incubation of the sections with a biotinylated anti-mouse IgG antibody, and then biotin was detected using an avidin-biotin-peroxidase kit (Vector Laboratories) with diaminobenzidine as the chromogenic substrate. Negative control sections were processed in an identical manner by substitution of the primary antibody with a purified mouse IgG fraction.

Isolation of Invasive and Noninvasive Trophoblast Populations

Cultures of first trimester invasive and noninvasive trophoblast populations were established and characterized as reported earlier.¹⁸ Briefly, 8 to 10 placentas (5 to 12 weeks) obtained after legal termination of pregnancy were washed in sterile phosphate-buffered saline (s-PBS) and areas rich in chorionic villi were selected and minced between scalpel blades and were subjected to three sequential 10-minute treatments with 0.125% trypsin and 0.2 mg/ml DNase I (Boehringer Mannheim, Mannheim, Germany) in s-PBS containing 5 mmol/L MgCl_2 . Cells released from each 10-minute step were pooled and filtered through two layers of muslin, resuspended in 70% Percoll (Pharmacia, Uppsala, Sweden) at a density of 2×10^5 cells/ml, and put under 20 ml of 25% Percoll. Ten ml of s-PBS were put on top of the 25% Percoll and a gradient was established by centrifuging for 20 minutes at 2000 revolutions/minute. Cells from the middle band (density, 1.048 to 1.062 g/ml) of the gradient were pooled, washed in s-PBS, and seeded at a density of 1×10^6 cells/ml of keratinocyte growth medium (KGM) supplemented with 10% fetal calf serum.

Characterization of Isolated Trophoblast Populations

Cells were identified as being trophoblast by immunocytochemical staining using monoclonal antibodies to cyto-

keratin (Dako-cytokeratin, MNF 116 and 35BH11, 1:100; DAKO) as well as E-cadherin (HECD-1; Takara Shuzo Co., Shiga, Japan) which stains only trophoblasts in the placenta¹⁹ and by their differential surface expression of antigens, such as major histocompatibility (using anti-MHC, W6/32, 1:50; DAKO) class 1 and hyaluronic acid (NDOG1, diluted 1:10; Serotec, Kidlington, UK), as previously described.¹⁸

In Vitro Invasion Assay

The invasive characteristics of the extravillous trophoblast cells were determined using an *in vitro* Matrigel invasion assay as described^{18,20} using transwells with a polycarbonate filter of 2.5-cm diameter and 8- μ m pore size. The upper surface of the filter was coated with Matrigel (Collaborative Research, Bedford, MA; diluted 1:20 with KGM). The bottom chamber was filled with 3 ml of KGM containing 10% fetal calf serum. Trophoblast cells, labeled for 72 hours with 10 μ Ci/ml ³H-thymidine in KGM, were trypsinized, washed, and resuspended at a density of 1.0×10^5 cells/ml KGM containing 10% fetal calf serum, and 2 ml of the labeled cell suspension was added to the upper well of the Transwell chamber. After 72 hours of incubation, the invasive index was calculated as the amount of radioactivity (disintegrations/minute) from the lower wells divided by the sum of the total radioactivity from both the upper well and lower wells plus the filter.

FACS Analysis: Flow Cytometrical Analysis of Human Extravillous Trophoblast Cells

Flow cytometry proceeded as described.²¹ The isolated human first trimester extravillous trophoblasts were washed in PBS with 0.1% bovine serum albumin (Sigma Chemical Co., St. Louis, MO), sedimented by centrifugation and incubated with the anti-C-CAM antibody, or the purified mouse IgG3 (control, 100 mg/ml) for 1 hour at 4°C. After washing with PBS, the cell pellet was incubated with fluorescein isothiocyanate-conjugated goat-anti-mouse IgG, for 30 minutes at 4°C in the dark. After washing in PBS, the cells were analyzed using an EPICS XL flow cytometer (Coulter Corp., Hialeah, FL). The analysis was performed at least seven times for each analyzed cell type.

Western Blot Analysis

Extraction of proteins from endometrial tissue (used as a positive control) and villous and extravillous trophoblast cells was carried out in PBS in the presence of 1% NP40 and protease inhibitors as previously described.¹⁷ Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed in 7.5% polyacrylamide gel under reducing conditions, applying 50 μ g of each sample of the concentrated protein extract. After electrophoretic transfer to nitrocellulose and blocking in TBS containing 5% bovine serum albumin for 2 hours, CEACAM1-specific

mAb 4D1/C2 was added to a final concentration of 1 μ g/ml and incubated overnight at 4°C. Detection was carried out with a goat-anti-mouse alkaline phosphatase coupled antibody applying the chemiluminescent substrate and enhancer kit (Pierce, Rockford, IL). Films (Hyperfilm; Amersham, Braunschweig, Germany) were exposed for 15 minutes.

Results

Immunohistochemical Localization of CEACAM1 Expression at the Maternal-Fetal Interface

Immunohistochemistry was performed on paraffin-embedded samples with the mAb 4D1/C2, which specifically recognizes only CEACAM1 but not other antigens of the CD66 cluster^{13–15} and detection was performed using two different procedures, which both led to specific and similar results. Results of the immunohistochemical analysis are presented in Figure 1 (A–F) and are summarized in Table 1.

Expression of CEACAM1 in Endometrial Structures

As can be observed in Figure 1A, CEACAM1 is expressed in the endometrium of first trimester pregnancy in epithelial cells of the endometrial glands, with a strong expression level and an apical localization. CEACAM1 was also expressed at the apical poles of surface epithelial cells (not shown here; this aspect was previously described and shown in Ref. 17). Stromal cells are negative for CEACAM1, as shown in Figure 1A. Interestingly, as can be observed in Figure 1A, small vessels within the uterine stroma are CEACAM1-positive.

Expression of CEACAM1 in Placental Structures

Representative results of immunohistochemical detection of CEACAM1 in placental structures are shown in Figure 1, B to F. As can be observed in Figure 1, B to E, CEACAM1 is not expressed in the villous mesenchyme and neither in villous cyto- nor in syncytiotrophoblasts. Strong specific expression is observed in the intermediate (invasive) trophoblast. Note expression in an intermediate trophoblast island (Figure 1B), in invasive trophoblast at the implantation site (Figure 1, C and D) and in a capping mass (Figure 1E). Using an alternative detection method (DAB-kit, see Materials and Methods) resulting in a brown coloring of the expressed protein also showed specific localization in the same structures (data not shown). This expression pattern was maintained throughout pregnancy, but expression in the intermediate trophoblast could best be observed in samples from the first two trimesters for reasons of tissue availability.

Interestingly, as shown in Figure 1F, CEACAM1 expression was also observed in endothelial cells of placental villous core vessels. Expression in placental vessels appeared to be stronger toward the end of

Figure 1. Immunohistochemical localization of CEACAM1 at the maternal-fetal interface. **A:** Expression of CEACAM1 in endometrium of first trimester pregnancy. Note expression of CEACAM1 (red) at the apical poles of glandular endometrial epithelial cells as well as in small endometrial vessels (v) (%250). Expression of CEACAM1 in placental structures. **B, C, D:** Expression in the intermediate trophoblast (it); note absence of expression in villous cyto- (ct) and syncytiotrophoblast (st); (d) = decidua. **B,** first trimester placenta, %100; **C,** second trimester placenta, %250; **D,** second trimester placenta, %250. **E:** Expression of CEACAM1 in a capping mass (cm); no expression in the cyto- (ct) and syncytiotrophoblast (st) (first trimester placenta, $\times 400$). **F:** Expression of CEACAM1 in placental villous core vessels (v) (third trimester placenta, %400)

pregnancy (Figure 1F shows a sample from the third trimester).

FACS Analysis: Flow Cytometrical Analysis of Human Extravillous Trophoblast Cells

Flow cytometry analysis using the mAb 4D17C2 was performed on cultivated villous cyto- and syncytiotrophoblasts and on extravillous trophoblasts which had been characterized with an invasive phenotype. Data from representative experiments are shown in Figure 2. Flow cytometric analysis confirms expression of CEACAM1 in only extravillous invasive trophoblast, whereas villous cyto- and syncytiotrophoblasts are negative (Figure 2).

Western Blot Analysis of CEACAM1 Expression in Human Villous and Extravillous Trophoblast Cells

Western blot analysis using the mAb 4D17C2 was performed on cultivated villous trophoblasts and on extravillous trophoblasts which had been characterized with an invasive phenotype. Endometrium protein extract was used as a positive control. A representative Western blot is shown in Figure 3. Western blot analysis confirms expression of CEACAM1 in only extravillous invasive trophoblasts, whereas villous trophoblasts are negative (Figure 3). The molecular weight of the protein is the same as in the positive control (endometrium).

Table 1. CEACAM1 Localization in Structures at the Maternal-Fetal Interface

Structure	CEACAM1 expression
Endometrium	
Epithelial cells (surface)	+ (apical localization)
Epithelial cells (glands)	+ (apical localization)
Stroma	—
Small vessels	+
Placenta	
Villous cytotrophoblast	—
Villous syncytiotrophoblast	—
Intermediate trophoblast	+
Placental vessels	+

Discussion

In the present study, we investigated the expression pattern of CEACAM1 at the maternal-fetal interface. The mAb 4D1/C2, which does not cross-react with other members of the carcinoembryonic antigen family and the specificity of which has been analyzed in detail,¹³ was used for immunohistochemical localization of CEACAM1 and for flow cytometric analysis of CEACAM1 expression in isolated trophoblast cell populations. Immunohistochemical expression analysis showed the presence of CEACAM1 immunoreactivity at the apical pole of endometrial epithelial cells of first trimester endometrium, as well as in small endometrial vessels, whereas endometrial stromal (decidual) cells were negative. Regarding placental structures, CEACAM1 was found to be specifically expressed by the intermediate trophoblast, both in capping masses and at implantation sites, whereas villous cyto- and syncytiotrophoblasts were negative. Fur-

Figure 3. Western blot analysis of villous and extravillous trophoblast cells with mAb 4D1/C2 for CEACAM1 expression. vil (first lane), villous trophoblast; ext (second and third lanes), extravillous trophoblast (two different preparations); endo (fourth lane), endometrium (positive control).

thermore, endothelial cells of placental vessels expressed CEACAM1. Expression of CEACAM1 by invasive extravillous trophoblasts was confirmed by performing flow cytometry and Western blot analysis for CEACAM1 on isolated trophoblast populations which had been grown in culture and characterized using both immunocytochemical parameters and an *in vitro* invasion assay. Flow cytometry and Western blot also confirmed the absence of CEACAM1 expression in villous trophoblasts. From this expression pattern, implications for the functional role(s) of CEACAM1 at the maternal-fetal interface can be discussed. In transfection studies, CEACAM1 was shown to mediate homo- and heterophilic binding to other members of the CD66 cluster^{22,23} and it could thus play a role in mediating cell adhesion at the maternal-fetal interface. Specifically, CEACAM1 could be implicated in mediating adhesion during the contact between trophoblasts and endometrial epithelial cells, which also express CEACAM1 (see also¹⁷) and could thus permit a homophilic interaction. Also, CEACAM1 could be implicated in heterophilic interactions with other adhesion molecules at the maternal-fetal interface. Previous studies have shown that several adhesion molecules such as cadherins and integrins (especially $\alpha 4 \beta 1$ and $\alpha v \beta 3$) are specifically expressed at the maternal-fetal interface during the implantation window.²⁴ The invasive trophoblast was also shown to specifically express Mel-CAM, an adhesion molecule of the immunoglobulin family, which might be implicated in mediating such interactions as well.²⁵

Another possible role for CEACAM1 could be in mediating the adhesive interaction between the extravillous trophoblast and the endothelial cells of maternal vessels, thus enabling the invasion of these vessels by trophoblasts, which gain endothelial-like qualities.⁴ Such a role could recently be described for the platelet endothelial cell adhesion molecule-1, which was found to be specifically expressed in extravillous interstitial and endovascular trophoblasts, and was absent from villous cyto- and syncytiotrophoblast.²⁶ Furthermore, CEACAM1 could play a role in angiogenesis of both endometrial and/or placental vessels, which have both been shown to express CEACAM1. This assumption is supported by re-

Figure 2. Flow cytometric analysis of villous and extravillous trophoblast cells with mAb 4D1/C2 for CEACAM1 expression. **A:** Negative control (IgG). **B:** Villous cytotrophoblast. **C:** Villous syncytiotrophoblast. **D:** Extravillous invasive (intermediate) cytotrophoblast. Note the marked shift of the curve to the right in extravillous invasive trophoblast, as compared to villous cyto- and syncytiotrophoblast, which are both nonexpressing.

cent evidence which strongly suggests that CEACAM1 is a potent angiogenic factor.²⁷ Expression of the rat homologue of CEACAM1, cell-CAM105, has previously been described at the trophoderm surface of rat blastocysts,²⁸ as well as at the apical surface of rat uterine epithelium²⁹ which implicates that it could also play a role in the implantation process in this species. However, in rat blastocysts, cell-CAM105 seemed to be lost or masked from the surface of the mural trophoblast cells of adhesive-stage blastocysts,²⁸ indicating that its absence or masking, rather than its presence, might be important for this stage of development in rats. Our study was focused on implantation sites from first trimester human placentas, and thus a direct comparison with these data is not possible. However, the presence of CEACAM1 at the implantation sites in first trimester human placentas implicates that, in humans and at this stage, the presence and not the absence of CEACAM1 is probably important for implantation.

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References

- Shih I-M, Kurman RJ: New concepts in trophoblastic growth and differentiation with practical application for the diagnosis of gestational trophoblastic disease. *Verh Dtsch Ges Path* 1997, 81:266–272
- Burrows TD, King A, Loke YW: Trophoblast migration during human placental implantation. *Hum Reprod Update* 1996, 2:307–321
- Damsky CH, Librach C, Lim KH, Fitzgerald ML, McMaster MT, Janatpour M, Zhou Y, Logan SK, Fisher SJ: Integrin switching regulates normal trophoblast invasion. *Development* 1994, 120:3657–3666
- Zhou Y, Fisher SJ, Janatpour M, Genbacev O, Dejana E, Wheelock M, Damsky CH: Human cytotrophoblasts adopt a vascular phenotype as they differentiate. A strategy for successful endovascular invasion? *J Clin Invest* 1997, 99:2139–2151
- Thompson J, Grunert F, Zimmermann W: Carcinoembryonic antigen family: molecular biology and clinical perspectives. *J Clin Lab Anal* 1991, 5:344–366
- Beauchemin N, Draber P, Dveksler G, Gold P, Gray-Owen S, Grunert F, Hammarström S, Holmes KV, Karsson A, Kuroki M, Lin S-H, Lucka L, Najjar SM, Neumaier M, Öbrink B, Shively JE, Skubitz KM, Stanners CP, Thomas P, Thompson JA, Virji M, von Kleist S, Wagener C, Watts S, Zimmermann W: Redefined nomenclature for members of the carcinoembryonic antigen family. *Exp Cell Res* 1999 (in press)
- Lin SH, Giudotti G: Cloning and expression of a cDNA coding for a rat liver plasma membrane ecto-ATPase: the primary structure of the ecto-ATPase is similar to that of the human biliary glycoprotein I. *J Biol Chem* 1989, 264:14408–14414
- Aurivillius M, Hansen OC, Lazrek MB, Bock E, Öbrink B: The cell adhesion molecule Cell-CAM 105 is an ecto-ATPase and a member of the immunoglobulin superfamily. *FEBS Lett* 1990, 264:267–269
- Kuijpers TW, Hoogerwerf M, Van der Laan LJ, Nagel G, Van der Schoot CE, Grunert F, Roos D: CD66 nonspecific cross-reacting antigens are involved in neutrophil adherence to cytokine-activated endothelial cells. *J Cell Biol* 1992, 118:457–466
- Hinoda Y, Neumaier M, Hefta SA, Drzeniek Z, Wagener C, Shively C, Hefta LJ, Shively JE, Paxton RJ: Molecular cloning of a cDNA coding biliary glycoprotein I: Primary structure of a glycoprotein immunologically cross-reactive with carcinoembryonic antigen. *Proc Natl Acad Sci USA* 1988, 85:6959–6963
- Barnett TR, Kretschmer A, Austen DA, Goebel SJ, Hart JT, Elting JJ, Kamarck ME: Carcinoembryonic antigens: alternative splicing accounts for the multiple mRNAs that code for novel members of the carcinoembryonic antigen family. *J Cell Biol* 1989, 108:267–276
- Brümmer J, Neumaier M, Göpfert C, Wagener C: Association of pp60c-src with biliary glycoprotein (CD66a), an adhesion molecule of the carcinoembryonic antigen family downregulated in colonic carcinomas. *Oncogene* 1995, 11:1649–1655
- Drzeniek Z, Lamerz R, Fenger U, Wagener C, Haubeck HD: Identification of membrane antigens in granulocytes and colonic carcinoma cells by a monoclonal antibody specific for biliary glycoprotein, a member of the carcinoembryonic antigen family. *Cancer Lett* 1991, 56:173–179
- Stoffel A, Neumaier M, Gaida F-J, Fenger U, Drzeniek Z, Haubeck H-D, Wagener C: Monoclonal, anti-domain, and anti-peptide antibodies assign the molecular weight 160,000 granulocyte membrane antigen of the CD66 cluster to a mRNA species encoded by the biliary glycoprotein gene, a member of the carcinoembryonic antigen gene family. *J Immunol* 1993, 150:4978–4984
- Prall F, Nollau P, Neumaier M, Haubeck H-D, Drzeniek Z, Helmchen U, Löning T, Wagener C: CD66a (BGP), an adhesion molecule of the carcinoembryonic antigen family, is expressed in epithelium, endothelium, and myeloid cells in a wide range of normal human tissues. *J Histochem Cytochem* 1996, 44:35–41
- Riethdorf L, Lisboa BW, Henkel U, Naumann M, Wagener C, Löning T: Differential expression of CD66a (BGP), a cell adhesion molecule of the carcinoembryonic antigen family, in benign, premalignant, and malignant lesions of the human mammary gland. *J Histochem Cytochem* 1997, 45:957–963
- Bamberger AM, Riethdorf L, Nollau P, Naumann M, Erdmann I, Götze J, Brümmer J, Schulte HM, Wagener C, Löning T: Dysregulated expression of CD66a (BGP, C-CAM), an adhesion molecule of the CEA family, in endometrial cancer. *Am J Pathol* 1998, 152:1401–1406
- Aboagye-Mathiesen G, Zdravkovic M, Toth FD, Ebbesen P: Effects of human trophoblast-induced interferons on the expression of c-fms/CSF-1R, EGF-R and c-erbB2 in invasive and non-invasive trophoblast. *Placenta* 1997, 18:155–161
- Fischer SJ, Cui T-Y, Zhang L, Hartman L, Grahl K, Guo-Yang Z, Tarpey J, Damsky CH: Adhesive and degradative properties of human placental trophoblast cells in vivo. *J Cell Biol* 1989, 109:891–902
- Graham CH, Hawley TS, Hawley RG, MacDougall JR, Kerbel RS, Khoo N, Lala PK: Establishment and characterization of first trimester human trophoblast cells with extended life-span. *Exp Cell Res* 1993, 206:204–211
- Makrigiannakis A, Coukos G, Christofidou-Solomidou M, Gour B, Radice GL, Blaschuk O, Coutifaris C: N-cadherin-mediated human granulosa cell adhesion prevents apoptosis: a role in follicular atresia and luteolysis? *Am J Pathol* 1999, 154:1391–1406
- Rojas M, Fuks A, Stanners CP: Biliary glycoprotein, a member of the immunoglobulin supergene family, functions in vitro as a Ca²⁺-dependent intercellular adhesion molecule. *Cell Growth Differ* 1990, 1:527–533
- Turbide C, Rojas M, Stanners CP, Beauchemin N: A mouse carcinoembryonic antigen gene family member is a calcium dependent adhesion molecule. *J Biol Chem* 1991, 266:309–315
- Tabibzadeh S, Babaknia A: The signals and molecular pathways involved in implantation, a symbiotic interaction between blastocyst and endometrium involving adhesion and tissue invasion. *Mol Hum Reprod* 1995, 10:1579–1602
- Shih IM, Kurman RJ: Expression of melanoma cell adhesion molecule in intermediate trophoblast. *Lab Invest* 1996, 75:377–388
- Coukos G, Makrigiannakis A, Amin K, Albeda SM, Coutifaris C: Platelet-endothelial cell adhesion molecule-1 is expressed by a subpopulation of human trophoblasts: a possible mechanism for trophoblast-endothelial interaction during haemochorial placentation. *Mol Hum Reprod* 1998, 4:357–367
- Ergün S, Kilic N, Ziegeler G, Hansen A, Nollau P, Goetze J, Wurm-bach J-H, Horst A, Weil J, Fernando M, Wagener C: Human cell-cell adhesion molecule (CEACAM-1): an effective angiogenic factor. 10. International CEA-Workshop, Stockholm, 1999
- Svalander PC, Odin P, Nilsson BO, Öbrink B: Trophoblast surface expression of the cell adhesion molecule cell-CAM 105 on rat blastocysts. *Development* 1987, 100:653–660
- Svalander PC, Odin P, Nilsson BO, Öbrink B: Expression of cell CAM-105 in the apical surface of rat uterine epithelium is controlled by ovarian steroid hormones. *J Reprod Fertil* 1990, 88:213–221