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Human endogenous retrovirus K (HERV-K) is expressed in villous and extravillous cytotrophoblast cells of the human placenta

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Abstract

Human endogenous retroviruses (HERVs) have been shown to be important in physiological and pathophysiological processes in humans. Several HERVs have been found to be expressed in the placenta—a tissue with special immunomodulatory functions that is responsible for nutrition of the embryo and the ability of the semiallogenic trophoblast to invade. The envelope proteins of HERV-W (also known as syncytin 1) and HERV-FRD (syncytin 2) were shown to be involved in cell fusion leading to the generation of the syncytiotrophoblast. Syncytin 2 was further shown to have immunosuppressive properties. Herein we analyse the expression of another HERV, HERV-K, which is characterised by open reading frames for all viral genes. Using immunohistochemistry and Western blot analysis, expression of the transmembrane envelope (TM) protein of HERV-K was studied in normal placental and decidual tissues obtained at different gestational ages. The TM protein was expressed exclusively in villous (VT) and extravillous cytotrophoblast (EVT) cells, but not in the syncytiotrophoblast or other cells. The expression of the TM protein of HERV-K in EVT cells was confirmed by Western blot analysis of isolated c-erbB2-expressing cytotrophoblast cells. Thus, this is the first report showing expression of the TM protein of HERV-K in normal human placental tissue with an exclusive expression in cytotrophoblast cells, suggesting a potential involvement of HERV-K in placentogenesis and pregnancy. Since retroviral TM proteins including the TM protein of HERV-K have immunosuppressive properties, expression of the TM protein of HERV-K may contribute to immune protection of the fetus.

1. Introduction

In the last few years our knowledge of the role of endogenous retrovirus-like sequences that account for approximately 8% of the human genome in physiological and pathophysiological processes increased rapidly (for review see [Boeke and Stoye, 1997] and [Denner, 2010]). Many of the human endogenous retroviruses (HERVs) were found to be expressed in different tissues, including the placenta. Most of the HERVs are defective; however, some, such as HERV-K, have retained open reading frames for all viral proteins (Löwer et al., 1996) or at least for the functional envelope protein ([de Parseval et al., 2003] and [Dewannieux et al., 2005]). Different genes of HERV-K such as the *gag* gene, encoding for the core proteins, the *pol* gene encoding for the reverse transcriptase converting the genomic RNA into proviral DNA, and the *env* gene, encoding the envelope proteins involved in receptor recognition and membrane fusion, as well as the accessory proteins Rec and Np9 (Löwer et al., 1993), are expressed in germ cell tumours ([Löwer et al., 1996] and [Götzinger et al., 1996]) and melanomas ([Muster et al., 2003], [Büscher et al., 2005] and [Büscher et al., 2006]). There are several indications that Rec and Np9 may be involved in tumour induction ([Boese et al., 2000], [Armbruester et al., 2002] and [Galli et al., 2005]).

In order to establish a normal pregnancy, proper implantation needs to be tightly regulated by specific cells expressed on the feto-maternal interface. The placenta is made up of chorionic villi covered by a double layer of trophoblast cells. The outer syncytiotrophoblast is formed by fusion of underlying mononuclear cytotrophoblast cells, called villous cytotrophoblast (VT) cells. At the distal end of the chorionic villi, some of the VT cells grow through the syncytial layer to form columns of extravillous

cytotrophoblast (EVT) cells that invade into the maternal decidua (Burrows et al., 1996). These EVT cells are in close contact with different cell types in the maternal decidua, including $CD16^{-}/CD56^{++}$ natural killer and other immune cells that are known to control trophoblast invasion (Bulla et al., 2004). The function of the syncytiotrophoblast on the other hand is to produce hormones and to supply the fetal cells with nutrients (Desforges and Sibley, 2010).

The placenta is a preferential site of expression of different HERV, with HERV-W (syncytin-1) ([Blond et al., 1999], [Blond et al., 2000], [Mi et al., 2000] and [Kim et al., 2008]), and HERV-FRD (syncytin-2) (Malassiné et al., 2005) as first examples of an "enslavement" of endogenous retroviral genes in the reproductive tract of the host. In addition, ERV-3 ([Boyd et al., 1993] and [Venables et al., 1995]) and HERV-E (Yi and Kim, 2007) were also found to be expressed in placental tissue. While syncytin-1 interacts with the D type mammalian retrovirus receptor (ASCT2) ([Blond et al., 2000] and [Lavillette et al., 2002]), which is mainly expressed in VT cells (Hayward et al., 2007), the receptor of syncytin-2 (MFSD2) is specifically expressed in the syncytiotrophoblast (Esnault et al., 2008). Syncytin-1 was shown to mediate the fusion of the villous cytotrophoblast to form the multinucleated syncytiotrophoblast ([Blond et al., 2000] and [Frendo et al., 2003]). Syncytin-1 and syncytin-2 differ in their site of expression within the normal placenta with syncytin-1 localised in both VT and EVT cells (Muir et al., 2006) and syncytin-2 expressed only in VT cells (Malassiné et al., 2007). Syncytin-1 and syncytin-2 are abnormally expressed under pathological conditions, supporting their relevance in reproduction. In preeclampsia, for example, a pathological trophoblast invasion into the maternal decidua, syncytin-1 is localised in the apical rather than the basal aspect of the syncytiotrophoblast ([Lee et al., 2001] and [Knerr et al., 2002]). Nevertheless, it remains unclear whether this observation reflects the cause or the effect of the placental abnormality. Syncytin-2 expression, on the other hand, is altered in placentas of women with trisomy 21, where fusion of VT and maturation of chorionic villi is delayed ([Malassiné et al., 2008] and [Malassiné et al., 2010]).

In addition to the fusogenic property of the retroviral envelope proteins, the retroviral TM proteins are characterised by immunosuppressive properties that may be involved in the pathogenesis of retrovirus-induced immunodeficiency (for review see [Denner, 2000] and [Denner, 2010]). It has been shown that syncytin-2 (the phylogenetically older), but not syncytin-1, is immunosuppressive and that its so-called immunosuppressive (isu) domain is responsible for the immunosuppressive effect (Mangeney et al., 2007). It was also shown that the TM protein and the isu-peptide of HERV-K inhibit proliferation of human peripheral mononuclear cells (PBMCs) and induce an increased release of IL6 and IL10 as well as a decreased IL2 release (J. Denner, unpublished data). The TM protein of HERV-K modulated the expression of more than 300 genes in PBMCs from healthy donors in the same way as the TM protein of the human immunodeficiency virus HIV-1 did (J. Denner, unpublished data).

Herein, we investigate if and where HERV-K is expressed in normal placental tissue where it could be involved in the suppression of the maternal immune system. Therefore, the expression of the TM protein of HERV-K was studied in placentas of different gestational ages and in isolated EVT using immunohistochemistry and Western blot analysis with specific antibodies.

2. Methods

2.1. Tissue specimens and cell lines

The studies were performed with the approval of the Ethics Committee of the Medical Faculty of the University of Würzburg. Placental and decidual tissues were obtained from 10 healthy women undergoing legal therapeutic abortions of an intact pregnancy at 6–12 weeks' gestation, from two women undergoing legal abortion because of chromosomal aberration of the fetus at gestational week 17 and of two cases of pregnancy failure at week 22. Three placentas each of gestational weeks 28, 32 and 36 were collected at the time of a caesarean section carried out because of twin or triplet pregnancies. Three samples from gestational week 39 were obtained through elective caesarean section. Early pregnancy decidual tissue and placenta were obtained by suction curettage; decidual and placental tissue (villous trees) was dissected directly after delivery from placentas of gestational week 17 till term. Subsequently, tissue aliquots were either snap-frozen in liquid nitrogen and stored at −80 °C or fixed for 24 h in 4% PBS-buffered formalin. Two samples of decidua basalis of gestational weeks 8 and 10 were subjected to direct isolation of invasive cytotrophoblasts (EVT). Sections of

frozen placental tissue samples were analysed after haematoxylin-eosin staining to detect samples without signs of necrosis and inflammation. Such samples were then used for protein isolation.

Cell lines PA-1 (human teratocarcinoma), as well as the human choriocarcinoma JAR and JEG cell lines, were obtained from Cell line services (Heidelberg, Germany) and grown in RPMI-1640 medium supplemented with 10% fetal calf serum and gentamycin 25 µg/ml (all: PAA Laboratories GmbH, Cölbe, Germany) at 37 °C in 5% CO_2. Human umbilical vein endothelial cells (HUVEC) were prepared freshly from cords corresponding to the gestational week 39 placentas following standard protocols (Baudin et al., 2007) and expanded in endothelial cell growth medium 2 (Promo Cell, Heidelberg, Germany). Cells were regularly split into new cell culture flasks and harvested for Western blot analysis using trypsin (PAA).

2.2. Single cell isolation

To isolate invasive cytotrophoblast (EVT) cells, fresh decidual tissue was cut into small pieces of approximately 1 mm^3 with a mincing knife and digested for 20 min at 37 °C under slight agitation in PBS with 200 U/ml hyaluronidase (Sigma, Taufkirchen, Germany), 1 mg/ml collagenase type I (Seromed, Berlin, Germany), 0.2 mg/ml DNAse I (2500 U/mg; Sigma) and 1 mg/ml bovine serum albumin/fraction V (Sigma). The cell suspension was filtered through a sterile 50-µm stainless steel wire mesh and washed once in PBS. Mononuclear cells were then separated by a density gradient centrifugation (Leucocyte Separation Medium, PAA) and washed twice in PBS. Invasive EVT cells were characterised by expression of the human epidermal growth factor (EGF) receptor 2 (HER2/neu or ErbB2; Jokhi et al., 1994). Since EVT cells are the only cell type at the feto-maternal interface expressing ErB2, these cells were specifically isolated using anti-ErbB2 antibody coupled with microbeads (mouse IgG1; Miltenyi, Bergisch Gladbach, Germany) and a magnetic assisted cell-sorting (MACS) separator (Miltenyi). Purity of enriched EVT fraction was analysed by flow cytometry and in all cases >90% of the isolated cells were found to be positive for HLA-G (clone MEM-G9, Exbio, Biozol, Eching, Germany). Cells were subsequently used for protein isolation.

2.3. HERV-K specific antibodies

For immunohistochemistry and Western blot analysis, two HERV-K specific antibodies were used: Goat serum 26 was generated immunising a goat with the recombinant ectodomain of the TM protein of HERV-K ([Büscher et al., 2005] and [Büscher et al., 2006]). A TM protein-specific polyclonal antibody preparation was purified by affinity chromatography using a column with recombinant TM protein of HERV-K. In addition, the mouse monoclonal antibody HERM-1811-5 specific for the HERV-K TM protein was purchased from Austral Biologicals, San Ramon, CA, USA. The affinity-purified polyclonal antibody from goat serum 26 was used at 1:50 dilution for immunocytochemistry and at 1:1000 for Western blot analysis. The HERM-1811-5 monoclonal antibody was diluted 1:200–1:500 and 1:5000, respectively (Table 1).

2.4. Epitope mapping

Overlapping peptides corresponding to the ectodomain (aa465 to aa699) of the Env protein of HERV-K (PubMed Accnr. Q69384) synthesised as a cellulose-adsorbed peptide spot library of 15-mer peptides overlapping by 13 amino acids (Jerini Biotools) were used. The affinity purified antibodies from goat serum 26 and the monoclonal HERM-1811-5 were incubated with the membrane for 3 h, washed three times for 15 min with Tris-buffered saline, pH 7.5 containing 0.1% Tween 20 (Sigma) and incubated for 2 h with the appropriate peroxidase-conjugated anti-goat secondary antibody diluted 1:2000 (DAKO, Hamburg, Germany). Binding was detected using a chemiluminescence detection solution (ECL, Amersham Pharmacia Biotech).

2.5. Western blot analysis

For protein extraction, 2×10^6 cells or 25 sections cut from frozen placenta (8 µm thick) were lysed in 100 µl RIPA buffer (25 mM Tris/HCl pH 7.6; 150 mM NaCl; 1% NP-40; 1% Na-deoxycholate; 0.1% SDS), the total protein amount was quantified using the Bradford method and 100 µg of total protein per lane were resolved by 10% SDS-PAGE. After blotting onto nitrocellulose membrane and blocking with 5% non-fat dry milk in PBS, 0.05% (w/v) Tween 20, the membrane was incubated with the corresponding HERV-K-specific primary antibody at the appropriate dilution (Table 1) at 4 °C overnight, followed by incubation with horseradish peroxidase-coupled donkey anti-goat IgG (Santa Cruz, Heidelberg, Germany) diluted 1:2500, or goat anti-mouse IgG (KPL Medac, Wedel, Germany) diluted 1:7500, respectively. Detection was performed by a homemade ECL method. In brief, 50 mg of luminol sodium salt (Sigma) were dissolved in 200 ml 0.1 M Tris/HCl (pH 8.6) (ECL-solution A). For ECL-solution B, 11 mg of p-coumaric acid were dissolved in 10 ml of DMSO (both Sigma). Prior to the work, 3 ml of solution A were mixed with 0.3 ml of solution B and 0.9 µl of hydrogen peroxide, 30% (Merck, Darmstadt, Germany). Protein bands were visualised by autoradiography.

2.6. Immunohistochemistry

For immunohistochemical staining, 2- to 3-µm tissue sections were cut from paraffin-embedded tissue, placed onto APES (3-amino-propyltriethoxy-silane; Roth, Karlsruhe, Germany) coated slides, dewaxed in xylene, rehydrated in graded ethanol and distilled water. For antigen retrieval, sections were subjected to heat pretreatment by boiling in 0.01 M of sodium citrate buffer (pH 6.0) for 10 min in a microwave oven (600 W/s). Endogenous peroxidase was blocked by incubation in 0.1% hydrogen peroxide in PBS for 5 min. The slides were then incubated with the HERV-K specific primary antibodies diluted at optimised concentration (Table 1) in “antibody diluent” (Dako, Hamburg, Germany), washed in Tris-buffered saline (TBS; 25 mM Tris/HCl, pH 7.4, 137 mM NaCl, 2.7 mM KCl) followed by the “labelled streptavidin biotin” (LSAB+) anti-mouse/rabbit/goat detection system (Dako). DAB (Dako) was used as the chromogen and cells were counterstained with haematoxylin (Sigma). To identify invasive extravillous EVT a monoclonal anti-pan-cytokeratin antibody (clone KL1, Immunotech, Heidelberg, Germany) was adjusted at a concentration of 1:100 on a serial section.

3. Results

3.1. Expression of HERV-K protein as detected by immunohistochemistry

To analyse the expression of the TM protein of HERV-K in the placenta, first of all the specificity of the polyclonal antibody was characterised. Goat serum 26 obtained after immunisation with the recombinant TM protein of HERV-K (Büscher et al., 2005), affinity-purified immunoglobulin from this serum, and the monoclonal antibody HERM-1811-5 were found to react with the recombinant TM protein in a Western blot analysis (not shown) and with specific epitopes using overlapping peptides corresponding to the TM protein (Fig. 1). Whereas the affinity purified antibodies from the polyclonal goat serum 26 recognised at least five different epitopes, the monoclonal antibody detected as expected only one epitope, IFEASK (Fig. 1). This epitope was also detected by the crude goat serum 26, but not by the affinity-purified polyclonal antibody used herein. The latter recognised the epitopes ANQIND and NTSDFC.

Using the affinity-purified antibodies, immunohistochemistry on paraffin-embedded placental tissue of different gestational time points was performed. The TM protein of HERV-K was found expressed in the villous (VT) and extravillous cytotrophoblast cells (EVT; Fig. 2). VT cells enveloping the whole villous stroma were HERV-K positive, while the outer syncytiotrophoblast layer, as well as the villous stroma, remained negative in the villi of early gestation placenta. This staining pattern was found in placental tissues throughout the first trimester of gestation (not shown). Both of the antibodies used showed the same staining pattern (Fig. 2A and B), albeit a higher intensity was found when the monoclonal HERM-1811-5 antibody was used (Fig. 2B). Negative controls with pre-immune serum (Fig. 2C) and isotype control antibodies (Fig. 2D) confirmed the specificity of the antibodies used.

The cytotrophoblast cells in the villi of placental tissue were also positive for HERV-K around mid-gestation, but with reduced staining compared with the first trimester (Fig. 2E). The positive staining was found in the remaining few VT cells of the term-placenta (Fig. 2F). The intensity of the staining in the positive cytotrophoblast cells did not change during pregnancy, indicating a strong and continuous expression of the TM protein of HERV-K in VT cells of all gestational ages. Expression was maintained in the columns of EVT cells (Fig. 2B) and in EVT cells invading the decidua (Fig. 2G). To characterise the invasive EVT within decidua, a serial section of the respective tissue sample was stained for cytokeratin (Fig. 2H, arrow).

3.2. Expression of HERV-K protein as measured by Western blot analysis

To confirm the immunohistochemical data and to characterise the expression of the TM protein of HERV-K in more detail, Western blot analyses were performed. Protein isolates were obtained from either frozen tissue of the placenta obtained at different weeks of gestation or from EVT cells by isolation of ErbB2-positive cells from the early pregnancy decidua. ErbB2, the human epidermal growth factor receptor 2, was found expressed on human EVT cells. To investigate the expression of the TM protein, both types of antibodies were used. The monoclonal antibody HERM-1811-5 detected HERV-K-specific proteins with a molecular weight of 26 kDa and 55 kDa (Fig. 3A), the affinity-purified goat antibodies detected mainly the 55 kDa protein (Fig. 3B). The purified recombinant TM protein of HERV-K and the teratocarcinoma cell line PA-1 were used as positive controls, HUVEC were used as negative controls. Whereas HERV-K-specific bands were detected in all placental samples tested by the purified antibodies, only very weak signals were obtained for the first trimester samples when the monoclonal antibody was used. Both antibodies did not detect any proteins in the negative control and the HUVEC cells. Thus, the TM protein seems to be expressed in the human placenta throughout the entire gestation. Pooled isolated EVT cells from two different samples of the decidua (gestational weeks 8 and 10) showed a clear expression of the TM protein of HERV-K. In addition, Western blot analysis of two trophoblast-derived choriocarcinoma cell lines, JEG and JAR, showed in a clear signal at the expected protein size confirming that HERV-K is indeed expressed in cytotrophoblast cells.

4. Discussion

Here we show for the first time expression of the TM protein of HERV-K in villous cytotrophoblast (VT) and extravillous cytotrophoblast (EVT) cells, but not in the syncytiotrophoblast of the human placenta. As previously shown, syncytin-1 (HERV-W) is also localised in VT and EVT cells ([Frendo et al., 2003] and [Muir et al., 2006]), while syncytin-2 (HERV-FRD) is only expressed in VT cells (Malassiné et al., 2007). There is compelling evidence that syncytin-1 and syncytin-2 are involved in the generation of the syncytiotrophoblast and therefore play an important role in human reproduction ([Blond et al., 2000] and [Mi et al., 2000]). Syncytin-like proteins have also been found in mice (syncytin-A, syncytin-B) that are homologous, but not orthologous to the human syncytins (Dupressoir et al., 2005). Knockout mice for the murine syncytin-A died *in utero*, indicating that this gene is essential for mouse embryonic development (Dupressoir et al., 2009). Endogenous retroviral env genes were also “enslaved” for placental functions in sheep (Black et al., 2010) and rabbits (Heidmann et al., 2009). In the sheep, the envelope gene of endogenous retroviruses related to the Jaagsiekte sheep retrovirus was shown to be involved in placenta evolution and inhibition of their expression by RNA interference compromised conceptus elongation. In the rabbit syncytin-Ory 1 is responsible for the formation of the syncytiotrophoblast. These data indicate that “enslavement” of retroviral genes must have occurred independently and on several occasions during mammalian evolution.

4.1. HERV-K expression in placental tissue and in tumours

To date, HERV-K expression has only been found in stem cell tumours (Löwer et al., 1996), melanomas ([Muster et al., 2003], [Büscher et al., 2005] and [Büscher et al., 2006]) and trophoblast tumours (Herbst et al., 1996). The function of HERV-K during tumour development is still unclear. There is evidence that the accessory proteins Rec and Np9, encoded by different open reading frames in the env gene, might be involved in tumour development ([Galli et al., 2005], [Boese et al., 2000] and [Armbruester et al., 2002]). On the other hand, the expression of HERV-K in tumours may

be a secondary event based on activated transcription factors during tumourigenesis. Due to their immunosuppressive properties expression of the retroviral TM protein on the tumour cells may provide protection from the immune system as shown in experimental mouse tumours models expressing different retroviral TM proteins ([Mangeney and Heidmann, 1998], [Mangeney et al., 2001] and [Blaise et al., 2001]).

Since villous cytotrophoblast cells were also expressing the HERV-K TM protein, whereas syncytiotrophoblast cells remained negative, it could be assumed that HERV-K has a fusogenic potential as well. Thus, a redundancy of function may allow compensation if one of the HERV genes is non-functional. This accounts for example for ENV-3, a gene expressed in the placenta, but which is not functional in 1% of fertile women (de Parseval and Heidmann, 1998).

4.2. Potential immunosuppressive function of HERV-K in the placenta

The adequate regulation of placenta development is temporally and spatially restricted. Inhibition of the trophoblast invasion may lead to diseases like preeclampsia; uncontrolled invasion may lead to placenta accreta, increta or even percreta ([Knerr et al., 2002] and [Lavillette et al., 2002]).

The suppression of the maternal immune system during pregnancy is unique, allowing the trophoblast to invade and protecting the embryo from immunological rejection, while inhibiting viral and bacterial infections at the same time. The fact that EVT cells expressing c-erbB2 are regularly found in intimate contact with maternal immune cells, suggests a potential relevance of HERV-K in the induction of the immunosuppression. Our findings that the TM protein of HERV-K has immunosuppressive properties that are very similar to those of the TM protein of HIV-1 and other retroviruses ([Denner, 2000] and [Denner, 2010]) support this suggestion.

5. Conclusion

In addition to the TM protein of the human endogenous retroviruses HERV-W (syncytin 1) and HERV-FRD (syncytin 2), the TM protein of HERV-K is also expressed in the human placenta. It is of great interest for the understanding of the gestational process to learn the specific functions of all endogenous retroviruses expressed in the placenta and to analyse their interaction, if any. The fact that the placentas of different mammals differ significantly and that different species “enslaved” TM proteins from different endogenous viruses during evolution, makes it even more difficult to answer these questions.

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Tables and Figures

Table 1. Primary and secondary antibodies used for immunohistochemistry and Western blot analysis.

Antibody	Specificity	Source	Clone	Company	Concentration	Western blot final dilution	IHC final dilution
Primary							
Serum 26	HERV-K TM	Goat	Polyclonal			1:1000	1:50
Affinity purified antibodies	HERV-K TM	Purified from goat serum 26			8 mg/ml	1:1000	1:50
HERM-1811-5	HERV-K TM	Mouse	HERM-1811-5	Austral Biologicals	1 mg/ml	1:5000	1:500
Actin	Beta-actin	Mouse	M8226	Abcam	0.6 mg/ml	1:10,000	n.a.
Cytokeratin	Pan-cytokeratin	Mouse	KL1	Immunotech	0.15 mg/ml	n.a.	1:100
Isotype control	Control	Mouse	IhG2a	DAKO	0.1 mg/ml	n.a.	1:100
Secondary							
Anti-goat	Goat IgG		Polyclonal	Santa Cruz	0.4 mg/ml	1:2500	n.a.
Anti-mouse	Mouse IgG		Polyclonal	KPL	1 mg/ml	1:7500	n.a.
LSAB+	Mouse, goat, rabbit		Kit-system	DAKO	Ready to use	n.a.	Undiluted

LSAB, labelled streptavidin biotin; n.a., not applied; IHC, immunohistochemistry.

Figure 1. Results of the epitope mapping of antibodies specific to the TM protein of HERV-K. The sequence of the entire TM protein gp36 is shown, the sequence of the recombinant protein used for immunisation is shown in black and the Cys-Cys-loop in bold. The epitopes recognised by the goat serum 26 (dark grey), the affinity-purified antibodies (light grey) and the monoclonal antibody (black) are indicated by boxes.

Figure 2. Immunohistochemical detection of the TM protein of HERV-K in formalin-fixed tissue of the human placenta and the decidua using the affinity purified antibodies from goat serum 26 (A) and the monoclonal HERM-1811-5 (B, E, F and G). In addition, the pre-immune serum from goat 26 (C), the monoclonal IgG isotype control antibody (D) and a pan-cytokeratin antibody (H) were used. The white head arrows mark the syncytiotrophoblast, the black arrows the villous trophoblasts (VT). (A) The affinity purified antibodies from goat serum 26 stained the villous cytotrophoblasts (VT, black-headed arrow) and did not stain the syncytiotrophoblasts (white-headed arrow) in the seventh gestational week placenta. (B) Immunohistochemistry using the monoclonal antibody HERM 1811-5 confirmed the staining of the VT cells and the absence of staining in the syncytiotrophoblast. Whereas syncytia (*) were negative, the VT columns starting to become extravillous trophoblast (EVT) (white-headed arrow) show strong expression of the TM protein of HERV-K. (C) Pre-immune goat serum 26 and (D) isotype control antibody corresponding to the mouse monoclonal antibody did not stain placental cells at the seventh gestational week. (E) In a 22-week placenta the VT still show strong HERV-K expression, while the syncytiotrophoblast cells were negative. (F) Villi of a mature placenta (gestational week 39) show only a few remaining VT cells positive for HERV-K. (G) In the early gestation (eighth week) decidua basalis the invasive EVT cells (arrows) are positive for HERV-K. (H) Serial section stained with cytokeratin to mark the EVT (arrows), * marks the endometrial gland. Magnification ×400 in all cases, the magnification bar represents 50 µm, brown: DAB, blue: counter stain with haematoxylin.

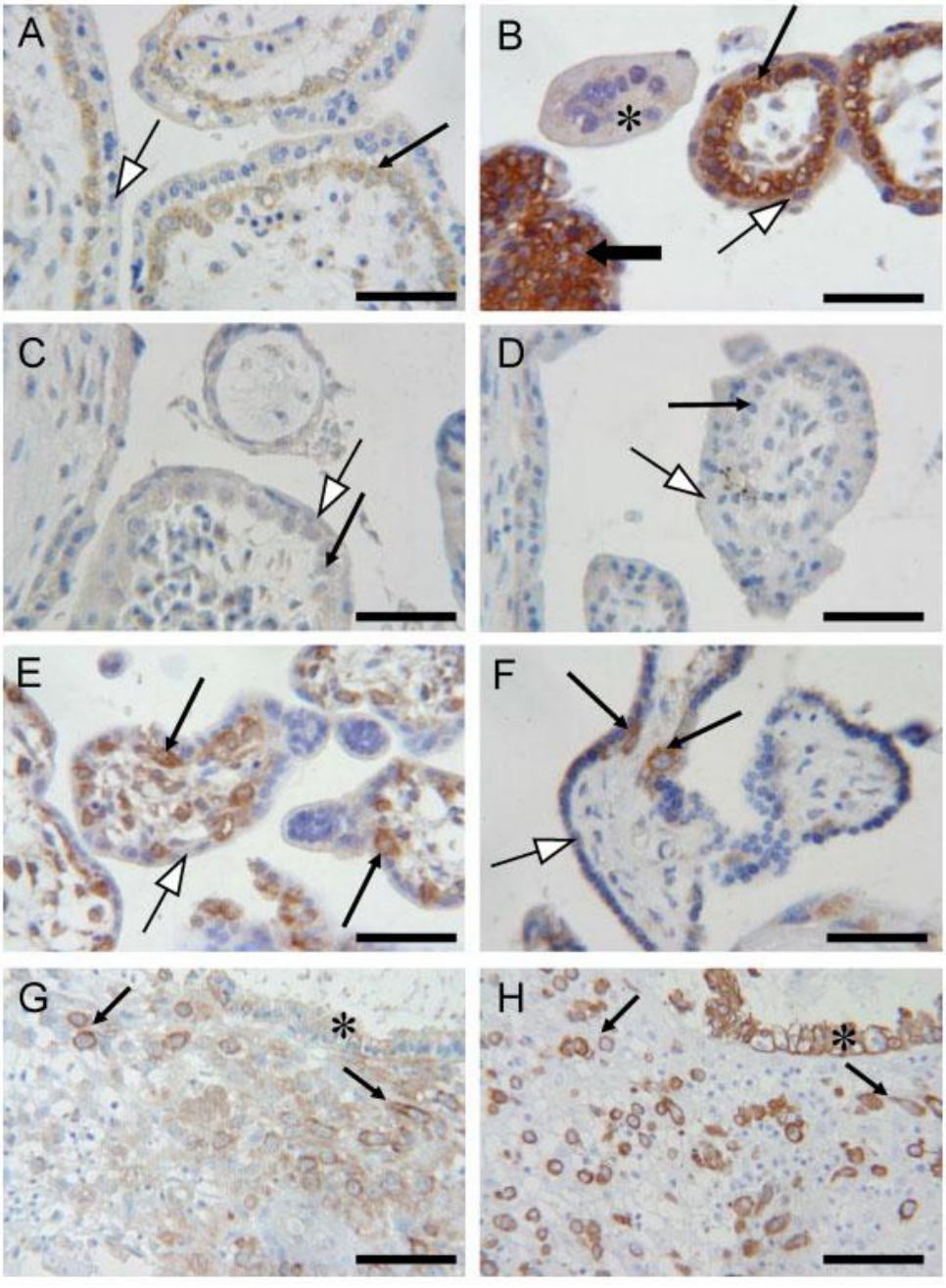

Figure 3. Western blot analysis of placental tissues throughout pregnancy, weeks 6–39, HUVEC cells, isolated pooled extravillous cytotrophoblasts (EVT) from weeks 8 and 10, teratocarcinoma cell line PA-1, the recombinant TM protein (TM), negative control (bovine serum albumin), as well as the choriocarcinoma cell lines JEG and JAR. (A) applying the mouse monoclonal antibody HERM-1811-5, (B) the affinity purified antibodies from goat antiserum 26 and (C) an antibody reactive with beta-actin. The recombinant HERV-K transmembrane envelope protein (TM) served as a positive control.