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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; 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; 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; Götzinger et al., 1996) and melanomas (Muster et al., 2003; Büscher et al., 2005, 2006). There are several

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indications that Rec and Np9 may be involved in tumour induction (Boese et al., 2000; Armbruester et al., 2002; 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, 2000; Mi et al., 2000; 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; 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; 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; 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; 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, 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, 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).