REPRODUCTION
REVIEW

The role of syncytins in human reproduction and reproductive organ cancers

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Abstract

Human life begins with sperm and oocyte fusion. After fertilization, various fusion events occur during human embryogenesis and morphogenesis. For example, the fusion of trophoblastic cells constitutes a key process for normal placental development. Fusion in the placenta is facilitated by syncytin 1 and syncytin 2. These syncytins arose from retroviral sequences that entered the primate genome 25 million and more than 40 million years ago respectively. About 8% of the human genome consists of similar human endogenous retroviral (HERVs) sequences. Many are inactive because of mutations or deletions. However, the role of the few that remain transcriptionally active has not been fully elucidated. Syncytin proteins maintain cell–cell fusogenic activity based on *env* gene-mediated viral cell entry. In this review, we summarize how syncytins and their receptors are involved in fusion events during human reproduction. The significance of syncytins in tumorigenesis is also discussed.

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Introduction

Cell fusion is an essential event during mammalian development for fertilization, placentation, formation of an immune defense system, development of skeletal muscle, and differentiation of macrophages into bone-resorbing osteoclasts (Larsson *et al.* 2008). It is also important for tissue repair and cancer development and progression (Vassilopoulos *et al.* 2003, Larsson *et al.* 2007*a*). Cell fusion follows cell adhesion by receptor–ligand interactions, signaling molecules, and alpha helical bundles formed by fusogenic proteins. Among the many proteins involved in fusion events, syncytin proteins form similar alpha helical bundles that bring membranes close together and are encoded by endogenous retroviral genes in humans (Larsson *et al.* 2008, Evans 2012). Syncytin proteins, syncytin 1 and syncytin 2, belong to the human endogenous retrovirus (HERVs) family and were initially identified in human placenta due to their fusogenic activities. Syncytin A and B, homologous to human syncytins, have also been identified in mice (Dupressoir *et al.* 2005) and are essential for normal murine placental development (Dupressoir *et al.* 2009, 2011).

Fertilization requires fusion of sperm and oocyte. Following implantation, the placenta forms and supports development of the embryo/fetus during intrauterine life. Fusion of mononuclear villous cytotrophoblast to form syncytiotrophoblast is essential for normal placental function (Esnault *et al.* 2008). We review, here, the role of the syncytins and their receptors in human reproduction and in reproductive organ tumors.

Human endogenous retroviruses (HERVs)

Infection of a host cell by an exogenous retrovirus causes the integration of retroviral DNA into the host cell's genome. In a case of germ cell infection, the inserted retroviral DNA can be subsequently inherited in a Mendelian fashion, and they are then termed 'endogenous retroviruses' (Prudhomme *et al.* 2005, Denner 2010, Stoye 2012, Mager & Stoye 2015). Endogenous retroviral elements comprise approximately 8% of the human genome (Harris 1998, Griffiths 2001). Full-length HERVs are composed of 5′ and 3′ noncoding regions designated long-terminal repeats (LTRs), group-specific antigen (*gag*), polymerase (*pol*) and envelope (*env*) genes (Fig. 1A). LTRs contain elements related to transcription initiation and termination such as enhancers, promoters and polyadenylation signals (Lower *et al.* 1996, Griffiths 2001, de Parseval & Heidmann 2005, Prudhomme *et al.* 2005). Thus, the effect of HERVs can be associated with the influences on transcriptional activation or repression by LTR enhancers, alterations of tissue specificity, and increased expression of related genes (de Parseval & Heidmann 2005). The *gag* gene encodes core structural proteins such as matrix, capsid and nucleocapsid that are participated in viral RNA encapsulation and particle formation. The *pol* gene encodes viral enzymes such as reverse transcriptase and integrase that are needed

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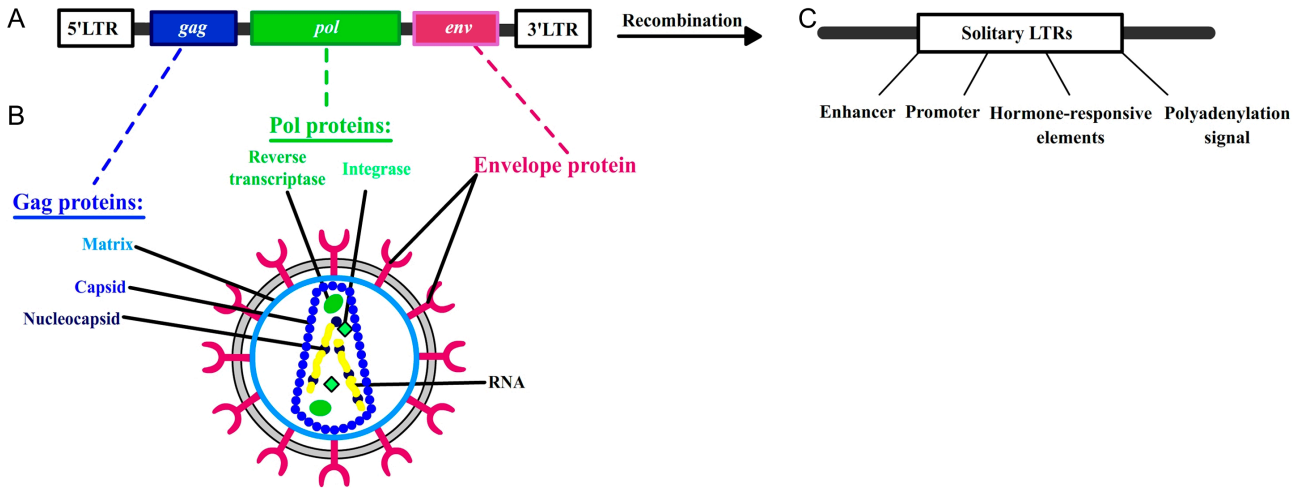

Figure 1 Generic structure of a full-length human endogenous retrovirus (A), its encoded proteins in general retrovirus structure (B), and solitary LTRs (C). 5′ and 3′ noncoding long-terminal repeats (LTRs), group-specific antigen gene (*gag*), polymerase (*pol*), and envelope gene (*env*).

for transcription of the viral RNA into double-stranded DNA and integration of the DNA produced by reverse transcriptase into the host's genome respectively. Finally, the *env* gene encodes viral envelope glycoprotein that is important for receptor recognition and membrane fusion (Lower *et al.* 1996, Prudhomme *et al.* 2005) (Fig. 1B).

env gene-mediated virus–host cell fusion is well studied. However, this fusion machinery can also lead to the fusion of neighboring cells in a receptor-dependent manner (Kjeldbjerg *et al.* 2010), similar to that of the viral entry to host cell (Izumida *et al.* 2015). Briefly, precursor ENV protein is processed by furin, or other proteases, into surface (SU) and transmembrane (TM) subunits. The SU subunit binds to its receptor in a neighboring cell, allowing the TM subunit to mediate membrane fusion between two cells (Kjeldbjerg *et al.* 2010).

In contrast to full-length ERVs, most ERV sequences lose the internal viral genes as a result of recombination between 5′ and 3′ LTRs and form 'solitary LTRs' (Griffiths 2001). Solitary LTRs are composed of enhancers, promoters, hormone-responsive elements and polyadenylation signals similar to all LTRs and can affect the expression patterns of neighboring genes (Vinogradova *et al.* 1997) (Fig. 1C). Most retroviral sequences contain in-frame stop codons or deletions that make them nonfunctional either at the transcriptional or post-transcriptional level. However, some retroviral elements still have large open reading frames (ORF) and retain their potential transcriptional capacity with important functions for the host's physiology (Rote *et al.* 2004, Denner 2010, Stoye 2012, Dewannieux & Heidmann 2013).

Syncytin proteins: two important endogenous retroviral gene products

A systematic analysis of the human genome showed that 18 *ENV* genes encode a full-length ORF (de Parseval *et al.* 2003, Villesen *et al.* 2004). Among them, two retroviral gene products with crucial roles during mammalian development, syncytin 1 (HERV-W) and syncytin 2 (HERV-FRD), are encoded by *ENV* genes. These proteins were initially identified in human placenta (Mi *et al.* 2000, Blaise *et al.* 2003). Syncytin proteins are mainly expressed in the trophoblastic layer, which is an important contributor of normal placental architecture and trophoblast turnover (Mi *et al.* 2000, Malassine *et al.* 2007, Rawn & Cross 2008). Syncytin 1 is a glycoprotein with cell fusogenic activity. It binds to its receptor, SLC1A5/ASCT2/RDR (a neutral amino acid transporter and type D mammalian retrovirus receptor), and promotes the fusion of cytotrophoblast cells to form the multinucleated syncytiotrophoblast layer (Blond *et al.* 2000, Malassine *et al.* 2005). Syncytin 2 entered the primate genome earlier than syncytin 1 (Voisset *et al.* 1999, Blaise *et al.* 2003, de Parseval *et al.* 2005). As this fusogenic retroviral gene was identified after syncytin 1, it was designated syncytin 2 (Blaise *et al.* 2003, de Parseval *et al.* 2005). Its receptor is a member of carbohydrate transporter superfamily MFSD2 (major facilitator superfamily domain containing 2) (Esnault *et al.* 2008). Syncytin 2 also plays roles in immunosuppression (Blaise *et al.* 2003, Mangeney *et al.* 2007, Rawn & Cross 2008). Both syncytin proteins are less polymorphic when compared with other envelope proteins (de Parseval *et al.* 2005).

The role of syncytin proteins in human reproduction

Although many retroviral sequences are defective because of genetic modifications, syncytin sequences have been conserved, possibly due to beneficial reproductive functions (Bjerregaard *et al.* 2006). According to the 'Baton pass' hypothesis proposed by Imakawa *et al.* (2015), multiple successive endogenous retrovirus (ERV) variants incorporate into mammalian genomes in

a locus-specific manner and take over the cell-fusion roles. Thus, some ERV insertions increased cell fusion in trophoblast resulting in enhanced reproductive success in placental mammals (Esnault *et al.* 2013, Lavialle *et al.* 2013, Imakawa *et al.* 2015). To date, many publications show that syncytins are involved in cell fusion (Mi *et al.* 2000, Frendo *et al.* 2003, Vargas *et al.* 2009, Liang *et al.* 2010), cell cycle (Huang *et al.* 2013), apoptosis (Knerr *et al.* 2007, Knerr *et al.* 2008, Huang *et al.* 2014*b*), and immunosuppression events (Mangeney *et al.* 2007) in human placenta. Also, altered expression of syncytin proteins have been reported in placental pathologies (Lee *et al.* 2001, Kudo *et al.* 2003, Chen *et al.* 2006, Ruebner *et al.* 2010, Vargas *et al.* 2011, Holder *et al.* 2012, Soygur *et al.* 2016) and different cancers (Larsson *et al.* 2007*a*, Strick *et al.* 2007, Maliniemi *et al.* 2013, Mo *et al.* 2013). As syncytins are initially defined in human placenta, we summarize their functions there, first.

Syncytin 1 (HERV-W; ERVW-1) and human placentation

The role of retroviral proteins, especially syncytins, in trophoblastic fusion process and placental morphogenesis was hypothesized about 15 years ago (Mi *et al.* 2000, Blaise *et al.* 2003). Mi *et al.* (2000) first identified syncytin 1 in the syncytiotrophoblast layer of human placental villi (Mi *et al.* 2000). They showed that when syncytin 1 is transfected into COS cells (CV-1 in origin and carrying the SV40 genetic material), syncytia formed consisting of many aggregated nuclei surrounded by an extended cytoplasm (Mi *et al.* 2000). When BeWo cells (trophoblast-derived choriocarcinoma cell line) are induced by forskolin to fuse and form syncytiotrophoblast-like cells, a five-fold increase in BeWo cell fusion is correlated with increased syncytin (ERVW-1) transcription (Mi *et al.* 2000). Thus, a role of syncytin 1 in placental cytotrophoblast fusion and its fusogenic properties *in vitro* is demonstrated (Mi *et al.* 2000). Following the discovery of syncytin 1, Blond *et al.* (2000) showed that transfection of different cell lines with syncytin 1 results in syncytia formation via the interaction of syncytin 1 and its receptor, SLC1A5 (Blond *et al.* 2000). SLC1A5 expression is reported in villous (Soygur *et al.* 2016) and extravillous trophoblast (Malassine *et al.* 2005).

After identification of the fundamental fusion role of syncytin 1 and upstream components in this signaling pathway, such as CD9 and cAMP/PKA (Muroi *et al.* 2009), many reports have shown the presence of syncytin 1 in the basal membrane of syncytiotrophoblast (Lee *et al.* 2001), cytotrophoblast (Blond *et al.* 2000, Smallwood *et al.* 2003, Muir *et al.* 2006, Soygur *et al.* 2016), and some stromal cells in the core of placental villi (Holder *et al.* 2012, Soygur *et al.* 2016). It is of interest to note that the presence of syncytin 1 in the stromal core of villi may further indicate some additional non-fusogenic functions of syncytin 1. The presence of syncytin 1 in the apical microvillous membrane of villous trophoblast is also reported by our laboratory (Soygur *et al.* 2016). Malassine *et al.* (2005) showed syncytin 1 expression in all cell types of the extravillous phenotype lineage (Malassine *et al.* 2005). Cytotrophoblast in the tips of villi can differentiate into another type of trophoblast called the extravillous trophoblast. However, extravillous trophoblast cells are anchored and invade into the deeper layers of the decidua and maternal vascular bed (Cartwright *et al.* 2010). As cell–cell fusion of extravillous trophoblast does not occur at the maternal–fetal interface, syncytin 1 expression in extravillous trophoblast arouses great interest. Glial cells missing 1 (GCM1) is an important placental transcription factor as chorionic trophoblast cells in *Gcm1*-deficient placentas do not fuse to form syncytiotrophoblast (Anson-Cartwright *et al.* 2000). Wang *et al.* (2012) identified the GCM1 target gene, HtrA4 (high-temperature requirement protein A4), and reported that HtrA4 protein mediates placental JAR (choriocarcinoma cell line) and BeWo cell invasion by cleaving the extracellular matrix (ECM) protein fibronectin. More importantly, their study also demonstrated that HtrA4 suppresses the cell–cell fusion mediated by syncytin 1 in transfected human embryonic kidney 293T cell line for the first time. Binding of HtrA4 PZD domain to the SU subunit of syncytin results in decreased syncytin 1 expression on the cell surface. While HtrA4 decreases syncytin 1-mediated cell fusion, it also supports the invasion of JAR and BeWo cells *in vitro*. Overall, the results indicated the importance of HtrA4 and syncytin 1 in extravillous trophoblast differentiation by preventing cell fusion and promoting invasion in extravillous trophoblast (Wang *et al.* 2012).

On the other hand, Huang *et al.* (2013) investigated the role of syncytin 1 in trophoblast proliferation (Huang *et al.* 2013). In this study, syncytin 1 knockdown by siRNA significantly inhibited BeWo cell growth and DNA synthesis *in vitro*. Analysis of G1/S cell cycle checkpoint regulators in syncytin 1 knockdown BeWo cells showed that there were decreased CDK4, E2F1, PCNA and c-Myc levels in contrast to increased p15 protein level after siRNA transfection. At 72-h post-transfection in syncytin 1 knockdown BeWo cells compared with control groups, there was an increased percentage of cells in G1 phase and a decreased percentage in S and G2/M phases. These researchers therefore showed that syncytin 1 knockdown causes cell cycle arrest at the G1 phase (Huang *et al.* 2013). Because mononucleated cytotrophoblastic cells leave the cell cycle to differentiate into multinucleated syncytiotrophoblasts, they no longer have the ability to proliferate (Benirschke & Kaufmann 2000). In case of insufficient syncytin 1 protein, cell cycle arrest may occur in cytotrophoblasts. The inadequate proliferation of cytotrophoblast and the absence of continuous fusion

with syncytiotrophoblast may result in impairment of the syncytiotrophoblast layer. Therefore, one can speculate that syncytin 1 is possibly not only involved in the fusion of cytotrophoblast but also the proliferation of the cytotrophoblast via cell cycle. These independent properties of syncytin 1 (fusogenic and non-fusogenic) can maintain a balance between the 'cytotrophoblast pool' and the syncytiotrophoblast layer during human placental development.

Tolosa *et al*. (2012) showed a possible immune regulatory function of syncytin 1 *in vitro* (Tolosa *et al*. 2012). It is known that Th1 cytokines (e.g., TNF-α, IFN-ɣ and IL-2) have harmful effects on the fetus and are downregulated during pregnancy (Raghupathy 1997). Tolosa *et al*. (2012) reported that lipopolysaccharide/phytohemagglutinin (LPS/PHA)-stimulated Th1 cytokine responses (TNF-α and IFN-ɣ) and chemokine CXCL10 are inhibited by a syncytin 1 recombinant ectodomain in a human blood culture system. Moreover, CRH (corticotropin-releasing hormone) treatment of BeWo cells increases secreted exosomal syncytin 1 protein expression but not cellular syncytin 1. These results suggest that the presence of syncytin 1 in placental exosomes might provide a mechanism for syncytin 1 to reach and interact with target cells of the maternal immune system during pregnancy (Tolosa *et al*. 2012).

Further studies are carried out to understand the potential roles of syncytin 1 in placental pathologies such as preeclampsia (PE) (Lee *et al*. 2001, Chen *et al*. 2006, Vargas *et al*. 2011, Holder *et al*. 2012), intrauterine growth restriction (IUGR), and gestational diabetes mellitus (GDM) (Soygur *et al*. 2016). PE is a pregnancy-related disorder that affects approximately 2–7% of all pregnancies (Acien *et al*. 1990). In PE pregnancies, poor replacement of spiral artery wall by endovascular trophoblasts and insufficient placental circulation result in oxidative stress, hypoxia and endothelial dysfunction (Benirschke & Kaufmann 2000). PE is diagnosed based on arterial hypertension and proteinuria in pregnancy (Wilson *et al*. 2003). Many reports have shown decreased expression and aberrant localization of syncytin 1 in PE placentas compared with healthy controls (Lee *et al*. 2001, Chen *et al*. 2006, Langbein *et al*. 2008, Vargas *et al*. 2014, Zhuang *et al*. 2014). Chiang *et al*. (2009) showed decreased levels of GCM1, syncytin 1 and placental growth factor, which are all crucial for syncytiotrophoblast formation and placental vasculogenesis in PE placentas. While hypoxia enables activation of glycogen synthase kinase 3 beta (GSK-3β) in PE placenta, the PI3K–Akt pathway is inactivated under hypoxic condition in PE placental cells. Activated GSK-3β phosphorylates GCM1, promotes its ubiquitination, and is then degraded by the SCF^{FBW2} E3 ligase. As a result of disruption of the GCM1 transcription network, its target genes, syncytin 1 and placental growth factor, are decreased in a parallel manner (Chiang *et al*. 2009). Studies have also indicated a relationship between syncytin 1 and apoptosis in PE placentas (Ishihara *et al*. 2002, Huang *et al*. 2014*b*). Syncytin 1 knockdown in BeWo cells results in increased apoptosis in this carcinoma cell line of trophoblastic origin. Surprisingly, apoptosis in BeWo cells is mediated by apoptosis-inducing factor (AIF), which is independent of caspase. Decreased syncytin 1, increased AIF and increased calpain1 protein levels in apoptotic cells of human PE placentas have also been shown (Huang *et al*. 2014*b*). Thus, changes in cell cycle progression and apoptosis caused by altered syncytin expression may cause abnormalities in PE placentas.

The role of syncytin 1 in intrauterine growth-restricted (IUGR) placentas has also been investigated. The chorionic villi surface areas are reduced compared with age-related controls, and a smaller interface between maternal and fetal tissues is observed in IUGR placentas (Biswas *et al*. 2008). Moreover, an abnormal cellular development of trophoblast and increased trophoblast apoptosis are also seen (Ishihara *et al*. 2002). Ruebner *et al*. (2010) showed decreased syncytin 1 levels in IUGR placentas, which may contribute to placental dysfunction in IUGR (Ruebner *et al*. 2010). Although deregulation of syncytin 1 in PE placental pathology has been comprehensively studied, the role of syncytin 1 in fetal growth retardation is less certain. Thus, further functional studies are needed to highlight the regulatory mechanisms of syncytin 1 in IUGR placentas.

A recent report has identified the first host cell-encoded inhibitor protein, termed suppressyn, for mammalian cell fusion. Like syncytin, suppressyn is HERV-derived, placenta-specific and conserved during evolution. Suppressyn protein inhibits syncytin 1-induced cell fusion by binding with the syncytin 1 receptor, SLC1A5, but does not affect syncytin 2-mediated syncytialization (Sugimoto *et al*. 2013). Unfortunately, the role and regulatory mechanisms of syncytin 1 in different placental pathologies are still not known clearly. Thus, identification and involvement of suppressyn in syncytin 1-mediated cell fusion during placentation might provide a useful approach to better understand the underlying molecular mechanisms in placental pathologies.

Syncytin 2 (HERV-FRD; ERVFRD-1) and human placentation

Syncytin 2 was initially characterized in human placenta by screening human sequence databases for endogenous envelope retroviral elements (Blaise *et al*. 2003). When 16 candidate *ENV* retroviral genes were cloned in a eukaryotic expression vector and fusogenic activity was determined in transfected mammalian cells, syncytin 2 (ERVFRD-1) was discovered. Esnault *et al*. (2008) showed that syncytin 2 interacts with a different receptor (MFSD2) than syncytin 1 (Esnault *et al*. 2008). Further studies analyzed the amino acid sequence of syncytin

2 and demonstrated an immunosuppressive domain (Mangeney *et al.* 2007). This domain may protect the fetus against the maternal immune system (Rawn & Cross 2008, Lavialle *et al.* 2013). Data reported by Mangeney *et al.* (2007), using an *in vivo* tumor rejection assay, also supports the idea that syncytin 2, but not syncytin 1, has immunosuppressive activity. Tumorigenicity potential was assessed after syncytin 1- or syncytin 2-transduced MCA205 cells were engrafted to mice. Even though syncytin 2-transduced MCA205 cells formed large long-lasting tumors, syncytin 1-transduced MCA205 cells formed small tumors that were rapidly eliminated (Mangeney *et al.* 2007).

Many publications have tried to identify how syncytin 2 expression is regulated during healthy placental development. A recent study has demonstrated the regulation of syncytin 2 promoter activity via a CRE/AP-1 motif (Toufaily *et al.* 2015). bZIP (basic leucine zipper) transcription factors such as CREB2 (cAMP-response element binding protein 2) and JunD interact with the CRE/AP-1 motif and induce syncytin 2 expression in BeWo cells and primary villous cytotrophoblast isolated from term placentas (Toufaily *et al.* 2015). Malassine *et al.* (2007) and Esnault *et al.* (2008) showed that syncytin 2 expression is restricted to some cytotrophoblast cells in human first-trimester and term placenta respectively (Malassine *et al.* 2007, Esnault *et al.* 2008). In first-trimester placenta, syncytin 2 protein expression is detected in cuboidal cytotrophoblast cells, whereas it is located in flat cytotrophoblast at term (Malassine *et al.* 2008). Moreover, it has also been shown that its transcript levels decrease *in vitro* when cytotrophoblast cells form syncytiotrophoblast. The presence of the MFSD2 transcripts in the syncytiotrophoblast layer is consistent with the 'in fusion' process of cytotrophoblasts into the syncytiotrophoblast (Esnault *et al.* 2008). We also showed the cellular localization of syncytin 2 and MFSD2 in syncytiotrophoblast, some of the stromal cells, and endothelium (Soygur *et al.* 2016). The different expression pattern from previous studies may suggest additional roles for syncytin 2 other than their fusogenic activity. Altered expression of both syncytin 1 and syncytin 2 envelope proteins are reported in PE (Vargas *et al.* 2011). The data indicated a correlation with disease severity in isolated primary trophoblast cells from control and PE placentas. A more dramatic decrease in syncytin 2 is seen compared with syncytin 1 (Vargas *et al.* 2011). Additionally, altered syncytin 2 localization in trisomy 21-affected vs control placentas has also been reported (Malassine *et al.* 2008).

The presence of syncytin proteins at the surface of placental exosomes has been demonstrated (Tolosa *et al.* 2012). More recently, a possible relationship between placental exosome levels and PE pregnancies was also suggested (Vargas *et al.* 2014). Exosomes are small vesicles (30–100 nm) responsible for intercellular communications and several biological processes (Valadi *et al.* 2007). Both syncytin 1 and syncytin 2 are detected at the surface of exosomes produced by placenta-derived villous trophoblasts and are taken up by other cell types (Tolosa *et al.* 2012, Vargas *et al.* 2014). Decreased syncytin 2 levels in serum-derived exosomes are found in PE versus normal pregnant women. As placental exosomes have been suggested to contribute to fetomaternal immunotolerance during pregnancy, the presence of syncytin 2 in placental exosomes might indicate its immunosuppressive role in exosome-mediated immunotolerance in pregnant women (Vargas *et al.* 2014).

We studied the expression of syncytin proteins in placentas from women with gestational diabetes mellitus (GDM). GDM is characterized by abnormal glucose tolerance with onset, or first recognition, during pregnancy (O'Sullivan *et al.* 1985). GDM is associated with an increased rate of early pregnancy loss, morbidity, mortality, macrosomia and various metabolic abnormalities (Mondestin *et al.* 2002, Ruchat *et al.* 2013, West *et al.* 2013). Aside from its detrimental effects on mother and fetus, most placentas from GDM pregnancies show characteristic histological changes, such as villous immaturity and fibrinoid necrosis (Daskalakis *et al.* 2008). Our study showed reduced syncytin 2 and MFSD2 expressions in diabetic versus control human term placentas (Soygur *et al.* 2016). As previously indicated, the regulation of syncytin 1 in placental pathologies has been elucidated. However, the underlying molecular mechanisms for the deregulation of syncytin 2 in various placental pathologies are not yet clarified.

The role of syncytin proteins in fertilization

Fertilization is one of the most important cell fusion events in mammalian development. It involves multiple steps in which mature gametes meet and fuse at the correct time and place (Bjerregaard *et al.* 2014). Although human spermatozoa undergo many maturation processes during spermatogenesis, they leave the testis as immature cells functionally. Before fertilization, sperm pass through the epididymis where they gain forward motility and undergo further surface modifications, known as capacitation, in the female genital tract (Nixon *et al.* 2007).

Fertilization mainly consists of three steps: (1) acrosome reaction, (2) binding and penetration of zona pellucida (ZP), and finally (3) membrane fusion of sperm and oocyte (Kierszenbaum 2002). Fusogenic molecules play a role in sperm binding and penetration of the zona pellucida (Evans 2012). Although molecules that are involved in sperm:ZP binding are well characterized, the molecules for sperm and oocyte membrane fusion are still unknown. Many studies have tried to explain the role of ADAM proteins (a disintegrin and metalloproteinase), integrins, tetraspanins, and Izumo and Juno proteins in

sperm and oocyte membrane fusion using knockout mice (Cho *et al.* 1998, Nishimura *et al.* 2001, Inoue *et al.* 2005, Nixon *et al.* 2007). As both mouse and human oocytes express ERV proteins on the oolemma and their expression decreases significantly after fertilization, it has been suggested that ERV genomes could play a role in sperm–egg binding and fusion (Nilsson *et al.* 1999).

Syncytin 1 in fertilization

Bjerregaard *et al.* (2014) first demonstrated the presence of syncytin 1 and its receptor, SLC1A5, in human gametes (Bjerregaard *et al.* 2014). They reported that syncyin 1 is expressed at both the mRNA and protein levels in human spermatozoa. It mainly localizes in the acrosomal region of the spermatozoa or at the equatorial segment. A slight staining of the midpiece and tail is also noted. Syncytin 1 receptor is observed in the acrosomal and tail regions. On the contrary, syncytin 1 expression is not detected in human oocytes. However, syncytin 1 receptor is present in oocytes and its mRNA expression increases proportionally with oocyte maturation (Bjerregaard *et al.* 2014). The presence of syncytin 1 and its receptor in human spermatozoa and the presence of the syncytin 1 receptor in human oocytes might reflect a potential fusogenic role in fertilization. A remarkable study conducted by Muroi *et al.* (2009) reinforced the possibility of syncytin 1 involvement in human fertilization. This study showed that the CD9 protein, a member of tetraspanin family, regulates GCM1 and syncytin 1 expressions via cAMP/PKA signaling in BeWo cells. CD9 is involved in membrane fusion of sperm and oocyte (Le Naour *et al.* 2000). Although the regulation of syncytin 1 expression by CD9 has not been proved in the membrane fusion of sperm and oocyte, it seems reasonable to investigate the potential role of syncytin 1 in fertilization.

To determine the role of syncytins in development, Dupressoir *et al.* (2009) knocked out the syncytin A gene (homologous to the human syncytin 1) in mice and showed that heterozygous syncytin $A^{+/-}$ mice are viable, fertile and without phenotypic defects. However, syncytin $A^{-/-}$ knockout mice die by E14.5 (Dupressoir *et al.* 2009). As it is not possible to produce syncytin 1-deficient human spermatozoa and systemic deletion of syncytin A in mice results in lethality, conditional deletion of the syncytin A gene in germ cells will need to be carried out to enlighten the roles of syncytins in fertilization.

Syncytin 2 in fertilization

There is no information regarding the presence of syncytin 2, or its receptor, in human gametes. As indicated previously, syncytin 2 possesses fusogenic activity and the immunosuppressive properties of syncytin 2 could potentially modulate immunological attacks on the oocyte membrane after sperm fusion. The immunosuppressive character of syncytin 2 may also have an effect on blocking further sperm–oocyte fusion (Prudhomme *et al.* 2005). However, further functional studies are needed to clarify the possible role of syncytin 2, and its receptor, during sperm:oocyte membrane fusion.

Syncytins in human reproductive organs and reproductive organ tumors

Mi *et al.* (2000) examined 23 different human tissues for syncytin gene expression by using Northern blots and a weak expression pattern is reported in the testis besides a high expression in the placenta (Mi *et al.* 2000). Placenta- and testis-specific syncytin expression might be explained by DNA methylation. HERVs, as retrotransposons, are epigenetically regulated (Griffiths 2001) and DNA methylation generally suppresses their activity (Kudaka *et al.* 2008). As both human sperm and placenta show lower methylation levels than other tissues (Zhang *et al.* 1987, Nelissen *et al.* 2011), hypomethylation in placenta and testis might explain why these tissues are a rich source of actively transcribed HERVs. de Parseval *et al.* (2003) also analyzed the expression of 16 retroviral envelope genes, including syncytin 1 and syncytin 2, in 19 healthy tissues. Testis is found to be the only organ expressing all retroviral envelope genes at different mRNA levels (de Parseval *et al.* 2003). Additionally, Trejbalova *et al.* (2011) showed epigenetic deregulation of transcription and splicing of syncytins in testicular seminomas. Finally, Gimenez *et al.* (2010) analyzed the HERV transcriptome, including ERVW-1, in different normal and tumorigenic samples by using bisulfite sequencing and reported that six HERV-W loci are overexpressed in testicular cancer. Therefore, additional understanding of syncytins will give us more comprehensive information about their roles in male reproduction.

Studies have suggested that the human ovary exhibits absent/low expression of syncytin 1 (Menendez *et al.* 2004, Huang *et al.* 2014*a*). Menendez *et al.* (2004) reported increased syncytin 1 expression in malignant versus nonmalignant ovarian samples. This report also showed that syncytin 1 was hypomethylated in human ovarian cancers (Menendez *et al.* 2004). In line with the hypomethylated LTR region in the syncytin 1 promoter in endometriotic tissues, altered regulation of two DNA methyltransferase enzymes (DNMT3B and DNMT3B7) is also reported (Zhou *et al.* 2014). When considering that DNMT3B and DNMT3B7 are isoforms with and without methyltransferase activity, respectively, downregulation of DNMT3B and upregulation of DNMT3B7 could be responsible for epigenetic deregulation and syncytin 1 upregulation in endometriotic tissues (Zhou *et al.* 2014). Consequently, alterations in epigenetic regulation of syncytin 1 may

Table 1 A brief summary of the literature with syncytins in the area of placental research.

Reference	Syncytins	Outcome
Mi *et al*. (2000)	Syncytin 1	First identification of the fusogenic role of syncytin 1. Cytoplasmic syncytin 1 expression in syncytiotrophoblast at mRNA level and its fusogenic activity via cell culture experiments *in vitro*.
Blond *et al*. (2000)	Syncytin 1	Syncytin 1 and type D mammalian retrovirus receptor interaction during fusion process. Localization of syncytin 1 mRNA in cytotrophoblast and syncytiotrophoblast in human placenta.
Lee *et al*. (2001)	Syncytin 1	Decreased syncytin 1 mRNA in PE. Determination of different localization patterns of syncytin 1 protein (apical syncytiotrophoblast microvillous membrane) from normal placenta in PE.
Knerr *et al*. (2002)	Syncytin 1	Decreased syncytin 1 mRNA levels in PE and HELPP syndrome compared with normal placenta.
Yu *et al*. (2002)	Syncytin 1	Syncytin 1 gene expression regulation by GCMa (placenta-specific transcription factor) in human choriocarcinoma cell lines (BeWo and JEG3).
Smallwood *et al*. (2003)	Syncytin 1	Decreased syncytin 1 protein expression at term compared with first-trimester human placenta. Immunoreaction in extravillous trophoblasts, syncytiotrophoblast and cytotrophoblasts.
Frendo *et al*. (2003)	Syncytin 1	Increased syncytin 1 mRNA and protein expression in primary cytotrophoblast differentiation into syncytiotrophoblast *in vitro*. Decreased trophoblast fusion and differentiation when syncytin 1 protein inhibition is enforced by using specific antisense oligonucleotides.
Kudo *et al*. (2003)	Syncytin 1	Decreased syncytin 1 expression at low oxygen conditions and hypoxia.
Blaise *et al*. (2003)	Syncytin 2	First identification of syncytin 2.
Malassine *et al*. (2005)	Syncytin 1	Presence of syncytin 1 protein in cytotrophoblastic cell columns, interstitial extravillous trophoblastic cells, multinucleated giant cells and endovascular trophoblast in early pregnancy. SLC1A5 expression in the extravillous phenotypes.
Muir *et al*. (2006)	Syncytin 1	Syncytin 1 protein immunolocalization in syncytiotrophoblast, cytotrophoblast, invading interstitial trophoblast cells, endovascular trophoblast, and placental bed giant cells of first- and second-trimester placenta and choriocarcinoma cell lines.
Chen *et al*. (2006)	Syncytin 1	Determination of syncytin 1 and its receptor mRNA levels in different gestational ages. Reduced syncytin 1 mRNA in the cytotrophoblasts cultured under hypoxia and decreased protein expression in PE compared with normal placenta.
Malassine *et al*. (2007)	Syncytin 2	Syncytin 2 immunolocalization in some cytotrophoblast cells in first-trimester placenta. Decreased syncytin 2 transcripts during *in vitro* differentiation of isolated villous trophoblastic cells.
Mangeney *et al*. (2007)	Syncytin 2	Demonstration of immunosuppressive activity of syncytin 2.
Knerr *et al*. (2007, 2008)	Syncytin 1	Anti-apoptotic function of syncytin 1 by using CHO and HEK293 cells *in vitro*.
Langbein *et al*. (2008)	Syncytin 1	Lower cell–cell fusion index, decreased syncytin 1 gene expression, and increased apoptosis in cultured cytotrophoblasts and primary placental tissues from PE /HELLP-associated IUGR placentas.
Kudaka *et al*. (2008)	Syncytin 1 and syncytin 2	Determination of lower syncytin 1 and syncytin 2 transcription levels in PIH than normotensive pregnant women. Detection of syncytin 1 transcripts in both cytotrophoblasts and syncytiotrophoblast. Syncytin 2 transcripts in only cytotrophoblasts.
Malassine *et al*. (2008)	Syncytin 2	Different syncytin 2 immunolocalization in trisomy-21-affected placentas compared with normal placenta in second trimester of pregnancy. Decreased syncytin 2 transcript levels during fusion of cytotrophoblasts into syncytiotrophoblast.
Chen *et al*. (2008)	Syncytin 2	Determined increased syncytin 2 mRNA and protein levels as gestation progressed in normal placenta. Decreased expression levels in PE compared with normal placentas.
Esnault *et al*. (2008)	Syncytin 2	Discovery of MFSD2 as syncytin 2 receptor.
Chiang *et al*. (2009)	Syncytin 1	Disrupted GCM1 transcription network and reduced syncytin 1 levels in PE.
Muroi *et al*. (2009)	Syncytin 1	Syncytin 1 regulation by CD9 membrane protein in BeWo cells.
Vargas *et al*. (2009)	Syncytin 1 and syncytin 2	Comparison of the expression and functional implication of syncytin 1 and syncytin 2 in various trophoblast and BeWo cells.
Liang *et al*. (2010)	Syncytin 2	Identification of GCM1 as a critical factor for controlling cell fusion via transcriptional regulation of syncytin 2 and *MFSD2A* gene expression in placenta.
Ruebner *et al*. (2010)	Syncytin 1 and syncytin 2	Decreased syncytin 1 and syncytin 2 levels in IUGR placenta compared with normal term placenta. A similar decrease observed in cultured cytotrophoblasts from IUGR placenta compared with normal term placenta.
Vargas *et al*. (2011)	Syncytin 1 and syncytin 2	Reduced syncytin 1 and syncytin 2 levels correlated with severity of PE. A dramatic change in syncytin 2 expression.
Holder *et al*. (2012)	Syncytin 1	Decreased syncytin 1 mRNA levels in first trimester compared with term placenta. Altered syncytin 1 expression in PE, IUGR, and PE with IUGR.
Wang *et al*. (2012)	Syncytin 1	Identification of the GCM1 target gene, HtrA4, and its interaction with syncytin 1 in suppression of cell–cell fusion.
Tolosa *et al*. (2012)	Syncytin 1	The presence of syncytin 1 in human placental exosomes for the first time.
Huang *et al*. (2013)	Syncytin 1	Identification of syncytin 1 role in cell cycle. Syncytin 1 knockdown resulted in blocking the G1/S transition of cell cycle.
Huang *et al*. (2014*b*)	Syncytin 1	Decreased syncytin 1 level and increased apoptosis by activation of AIF in BeWo cells suggesting its role in PE placenta pathology.

(Continued)

Table 1 Continued.

Reference	Syncytins	Outcome
Zhuang *et al.* (2014)	Syncytin 1	Hypermethylation of syncytin 1 promoter in PE compared with normal placentas. Increased DNMT1 and DNMT3B3 mRNA and protein levels in PE placentas.
Vargas *et al.* (2014)	Syncytin 1 and syncytin 2	The presence of both syncytin proteins in placental exosomes and the altered syncytin 2 levels in serum-derived exosomes in women with PE compared with normal pregnant women.
Toufaily *et al.* (2015)	Syncytin 2	The regulation of syncytin 2 promoter activity via a CRE/AP motif and transcription factors.
Soygur *et al.* (2016)	Syncytin 1 and syncytin 2	Reduced syncytin 2 and MFSD2 expression in gestational diabetic placenta compared with normal placenta.

CHO, Chinese hamster ovary cell line; HEK 293, human embryonic kidney cells; HELLP, hemolysis, elevated liver enzymes, low platelets; IUGR, intrauterine growth restriction; PE, preeclampsia; PIH, pregnancy-induced hypertension.

lead to ovarian pathology. In fact, the hypomethylation of CpG dinucleotides located within the promoter region of syncytin 1 might cause abnormal syncytin 1 expression in human ovary. Hypomethylation could result in increased fusogenic syncytin 1 protein expression, or other non-fusogenic consequences leading to ovarian pathology.

Strissel *et al.* (2012) revealed gene expression analysis of 21 envelope genes in human endometrium by qRT-PCR and immunohistochemistry. The data indicated that syncytin 1 immunolocalizes to the cytoplasm of glandular epithelial cells while syncytin 2 is detected in both glandular epithelial cells and ciliated surface tubal-type epithelial cells (Strissel *et al.* 2012). The expression of envelope genes in pathological conditions such as endometrial carcinoma (EnCa), hyperplasia and polyps is also investigated. Interestingly, syncytin 1 and syncytin 2 are found to be overexpressed at the pT2 tumor stage (tumor invasion into the uterine cervix, but no extension beyond the uterus) vs at the pT1b stage (tumor spreads to one-half or more of the myometrium) (Cancer 2010, Strissel *et al.* 2012). Moreover, the ERV-W 5′ LTR promoter region, which regulates syncytin 1 expression, is found to be hypomethylated in ten patients diagnosed with EnCa. On the contrary, Strick *et al.* (2007) identified TGF-β1 (Transforming growth factor beta 1) and TGF-β3 (Transforming growth factor beta 3) as main regulative factors due to the data that steroid hormone-inducible TGF-β1 and TGF-β3 inhibit cell–cell fusion and reported that induced TGF-β can override syncytin 1-mediated cell–cell fusions in EnCa (Strick *et al.* 2007). Thus, these results may suggest that some of the overexpressed envelope genes (including syncytin 1 and syncytin 2) can be used as markers for pathological conditions such as ovarian and EnCa in women.

Bjerregaard *et al.* (2006) showed the presence of syncytin 1 in breast cancer and breast cancer cell lines and also the syncytin 1 receptor in cancer cells and endothelial cells (Bjerregaard *et al.* 2006). Possibly, syncytin 1 and its receptor are involved in normal and cancer cell fusion. The fusion of normal host and cancer cells generates hybrids that contain a mixture of parental genomes (Mortensen *et al.* 2004). It is widely known that tumorigenicity of hybrids is generally suppressed due to activation of tumor suppressor genes transmitted from normal host cell (Harris *et al.* 1969, Wiener *et al.* 1974, Anderson & Stanbridge 1993). However, tumorigenicity of hybrids may not be suppressed in all cases (Kohler & Milstein 1975). A small fraction of hybrids possesses proliferation capacity, which is different from physiologically normal nonproliferating fused cells. It has been shown that proliferating hybrids (approximately 1% of cells) could be more drug resistant and metastatic than parental cancer cells (Duelli & Lazebnik 2003). Thus, the physiopathology and unique features of hybrids may help us to understand tumorigenesis that result from impaired cell fusion. Syncytin 1 antisense treatment decreases syncytin

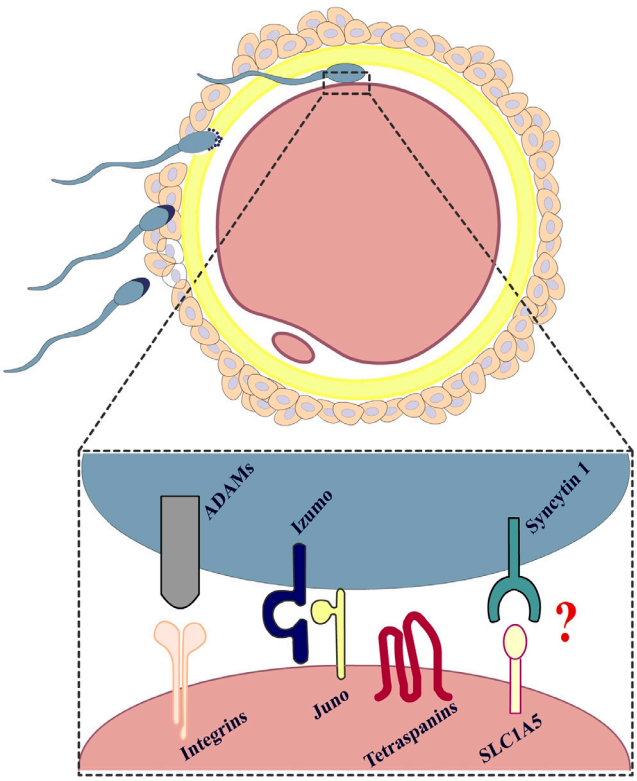

Figure 2 Schematic view of the molecules involving in sperm and oocyte membrane fusion during fertilization (Cho *et al.* 1998, Nishimura *et al.* 2001, Inoue *et al.* 2005, Nixon *et al.* 2007). Please note the presence of syncytin 1 on the sperm plasma membrane and its receptor SLC1A5 on the oolemma of oocyte.

expression and inhibits fusions between MCF-7 (breast cancer cell line) and HUVEC cells (human umbilical vein endothelial cells). In addition, a syncytin 1 inhibitory peptide also inhibits fusions between cancer and endothelial cells *in vitro*. These results are first to show that syncytin is expressed in human cancer cells and are involved in cancer–endothelial cell fusions (Bjerregaard *et al.* 2006). Larsson *et al.* (2007*b*) determined whether syncytin 1 has a prognostic role in breast cancer in 165 premenopausal women with ductal cancers and 54 consecutively operated breast cancer patients (Larsson *et al.* 2007*b*). Syncytin 1 expression is confirmed in 38% of the patients and its expression is demonstrated to be a positive prognostic factor in breast cancer patients (Larsson *et al.* 2007*b*).

Conclusion

In this review, we comprehensively analyzed the role of syncytin proteins in fertilization, placentation, and reproductive organ tumors, all of which are closely linked to human reproduction. Currently, syncytin proteins and their receptors have been studied extensively and found to be required in human placentation (Table 1). The occurrence of embryonic lethality in syncytin $A^{-/-}$ knockout mice proves that syncytin genes are vital for early development. Although human and mouse development, and proteins involved in development may differ, functional experiments via syncytins knockout mouse mutants will definitively determine the function of syncytin genes in mammalian development and physiology (Dupressoir *et al.* 2009, 2011).

Even though the presence of endogenous retrovirus gene products such as ERV-3 (ERV3-1) and MuLV in human and mouse oocytes has been previously shown (Nilsson *et al.* 1999), ERV-3 is polymorphic in human population, thereby disfavoring a possible role during fertilization. However, determination of syncytin 1 in human sperm and its receptor in the human oocyte most likely suggests a role of syncytin 1 in sperm and oocyte fusion during fertilization (Bjerregaard *et al.* 2014). How gamete fusion is carried out by syncytins and their receptors is still unclear (Fig. 2). As syncytin $A^{-/-}$ mice show embryonic lethality, conditional experiments are needed to address this question. On the other hand, identification of possible roles of syncytins in reproductive organ tumors undoubtedly will help overcome the fertility problems related to these tumors.

In conclusion, it is still not known whether HERVs adapt to mammalian physiological needs or the viruses later gain important physiological functions. We believe that clarifying the roles of the fusogenic syncytin proteins in human development will help us to better understand their evolutionary significance and also their important roles in human reproduction and development.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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