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 placentation 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.


Introduction

Cell fusion is an essential event during mammalian development for fertilization, placentaation, 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. 2007a). 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
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 cytrophoblast 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) (Esnauld 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
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), syncytiotrophoblast (CV-1 in origin and carrying the SV40 genetic material), 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 syncytiotrophoblast 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 cytrophoblast but also the proliferation of the cytrophoblast via cell cycle. These independent properties of syncytin 1 (fusogenic and non-fusogenic) can maintain a balance between the ‘cytrophoblast 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-γ 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-γ) 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, Langhein 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 SCFβTRPV2 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. 2014b). 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. 2014b). 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 suppresyn, for mammalian cell fusion. Like syncytin, suppresyn is HERV-derived, placenta-specific and conserved during evolution. Suppresyn 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 suppresyn in syncytin 1-mediated cell fusion during placenta 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 MFS2 transcripts in the syncytiotrophoblast layer is consistent with the ‘fusion’ process of cytotrophoblasts into the syncytiotrophoblast (Esnault et al. 2008). We also showed the cellular localization of syncytin 2 and MFS2 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 syncytin 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 bisulite 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. 2014a). 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.

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(Continued)
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 & Lazebrnik 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

Table 1 Continued.

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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.

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. (2007b) 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. 2007b). 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. 2007b).

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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