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REVIEW ARTICLE

Critical role of exosomes in sperm–egg fusion and virus-induced cell–cell fusion

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Abstract In mammals, two integral membrane proteins, sperm IZUMO1 and egg CD9, regulate sperm–egg fusion, and their roles are critical, but yet unclear. Recent studies, however, indicate interesting connections between the sperm–egg fusion and virus-induced cell–cell fusion. First, CD9-containing exosome-like vesicles, which are released from wild-type eggs, can induce the fusion between sperm and CD9-deficient egg, even though CD9-deficient eggs are highly refractory to the fusion with sperm. This finding provides strong evidence for the involvement of CD9-containing, fusion-facilitating vesicles in the sperm–egg fusion. Secondly, there are similarities between the generation of retroviruses in the host cells and the formation of small cellular vesicles, termed exosomes, in mammalian cells. The exosomes are involved in intercellular communication through transfer of proteins and ribonucleic acids (RNAs) including mRNAs and microRNAs. These collective studies provide an insight into the molecular mechanism of membrane fusion events.

Keywords CD9 · Exosome · Fertilization · Membrane fusion · Tetraspanin

Introduction

Fertilization is a sequential event that includes cell–cell adhesion, cell–cell fusion, and activation of cellular signaling, which allows the resumption of the egg cell cycle arrested at the stage of meiotic metaphase II (Fig. 1). In mammals, two kinds of membrane protein families, the cell adhesion molecule "integrin" [1, 2] and the membrane-anchored protease "a disintegrin and metalloprotease (ADAM)" [3–9], were biochemically identified in mammalian eggs and sperm, respectively, and immunocytochemically confirmed to localize on their outer cell membranes. The integrin family, which is expressed in many types of cells in animals, mediates cell–cell and cell–matrix interaction and intercellular communication, including cell adhesion and cell–cell fusion [10–14]. On the other hand, the ADAM family has a characteristic domain that is homologous to an extracellular region of the integrin family [4, 15]. The presence of the domain conserved between the integrin and ADAM families indicated that these two protein families play a role in sperm–egg adhesion and/or fusion [2, 15–18]. As expected, antibodies against the extracellular regions of these protein families were shown to significantly reduce the rate of sperm–egg binding and fusion in mice [1]. However, when genetically manipulated mice were produced, both male and female mice displayed no overt anomalies in both sperm–egg membrane adhesion and fusion [18–22] (Table 1), suggesting that the integrin and ADAM families are not essential for these events.

In the past, many genes predicted to participate in the sperm–egg fusion have emerged in mammals, but contrary to the expectations, most were found to be dispensable [23, 24] (Table 1). The sperm are required to migrate to the oviduct, where the ovulated eggs and sperm meet. Gene disruption experiments have been used to produce at least eight mouse lines with gene disruption for two testis-specific chaperones expressed in the endoplasmic reticulum (ER) (Calmegin and Calreticulin 3) [25–27], a protein disulfide isomerase (PDILT) [28], a tyrosylprotein

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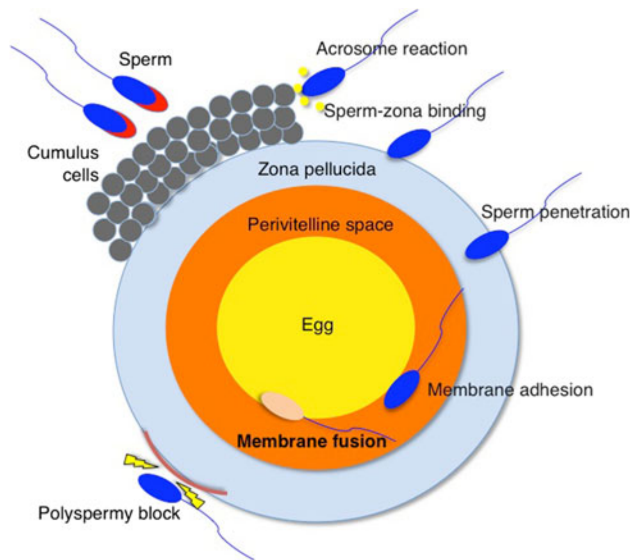

Fig. 1 Series of steps from sperm–egg interaction to fusion during mammalian fertilization. This is an overview of mammalian fertilization. Fertilization is divided into multiple steps: interaction of sperm–somatic cells (termed cumulus cells), binding of sperm to the extracellular matrix (termed zona pellucida), and penetration of the egg. After the sperm penetrates the zona pellucida, it can bind and fuse to the egg cell membrane. Successful fertilization requires not only that a sperm and egg fuse, but also that polyspermy block occurs

sulfotransferase (TPST2) [29], a testis-specific angiotensin-converting enzyme (ACE) [30], and three members of the ADAM family (ADAM1A, ADAM2, and ADAM3) [31–33]. In these mice, the sperm showed impaired migration into the oviduct and lost their zona pellucida-binding (zona-binding) ability when mixed with cumulus-free eggs in vitro. As reported for each of the *ADAM1a*-deficient, *ADAM3*-deficient, and PDILT-deficient lines, these mutant sperm could fertilize cumulus-surrounded eggs. These results indicate that the primary cause for infertility in these mouse strains is not a defect in sperm–zona binding, but the inability of sperm to migrate from the uterus into the oviduct.

ACE3, a testis-specific ACE homologue, is identified as an IZUMO1-interacting protein in sperm [34]. *Ace3*-deficient mice showed that the localization of IZUMO1 spread in a little wider area on sperm, but the elimination of ACE3 did not result in a loss of sperm fertilizing ability [34].

Two monoclonal antibodies (mAb), mMN7 and mMC41, against intra-acrosomal proteins, ACRIN1/MN7 and ACRIN2/MC41, respectively, significantly inhibited fertilization of zona-intact eggs in a dose-dependent manner, but did not influence the fertilization of zona-free eggs [35]. This result suggests that ACRIN1 and ACRIN2 are involved in the sperm–zona pellucida interaction before or during penetration of the zona pellucida.

BASIGIN/MC31/CE9/CD147 is a transmembrane glycoprotein and also acts as a receptor essential for erythrocyte invasion by *Plasmodium falciparum* [36]. A mAb against this protein significantly inhibited fertilization of cumulus-intact, zona-intact, and zona-free rat eggs in a dose-dependent manner. By contrast, sperm–egg membrane binding was not affected [37]. These findings suggest that this protein facilitates sperm–egg fusion, but the gene-disrupted sperm have been not analyzed.

Before membrane adhesion, both sperm and egg retain the cell–cell adhesion complex comprising β-catenin and E-cadherin [38–43]. Once membrane adhesion occurs, β-catenin is immediately ubiquitinated and probably degraded in both the sperm and egg, thereby initiating membrane fusion between these two cells. The absence of β-catenin results in a reduction in the ability of sperm to adhere to an egg, but sperm–egg fusion occurs normally [42]. This result indicates that β-catenin contributes partly to sperm–egg membrane adhesion, but does not play an essential role in this event. N-cadherin is also expressed in the human gonads and gametes [44], but the gene-disrupted sperm have not been investigated.

CD98 is a glycoprotein composed of SLC3A2 and SLC7A5 that forms the large neutral amino acid transporter [45], and is expressed on mouse eggs [46]. MAbs against CD9 and CD98 cooperatively inhibit in vitro fertilization [46], but the gene-disrupted eggs have not been analyzed.

Epididymal protein CRISPI is a member of the cysteine-rich secretory proteins family. The *CRISP1*-deficient sperm presented a decreased level of protein tyrosine phosphorylation during capacitation, and an impaired ability to fertilize both zona-intact and zona-free eggs in vitro, but they exhibited normal fertility [47]. Testicular CRISP2 is also expected to be involved in sperm–egg fusion [48], but the gene-disrupted mice have not been investigated.

EQUATORIN/MN9 is a sperm-specific type I transmembrane protein and a widely distributed acrosomal protein in mammalian sperm [49, 50]. During the acrosome reaction, some amount of this protein translocates to the plasma membrane, covering the equatorial region. The mAb against this protein inhibited both in vitro and in vivo fertilization. In addition, the gamete interaction-related domain recognized by the MN9 antibody is post-translationally modified. The modified domain was identified near threonine 138, which was most likely to be O-glycosylated when analyzed by amino acid substitution, dephosphorylation, and O-glycosylation inhibitor assays. Immunoelectron-microscopic analysis showed EQUATORIN on the hybrid vesicles surrounded by amorphous substances at advanced stage of acrosome reaction. Thus, the established EQUATORIN-based progression model will be useful for analyzing not only the behavior of EQUATORIN, but also of other molecules of interest involved in the acrosome reaction.

Table 1 Predicted players in sperm–egg fusion in mammals

Gene name	Category of coding protein	Expression	Phenotypes of gametes in KO mice	References
ACE, testis-specific	Secreted protein, angiotensin-converting enzyme	Sperm	Failure of sperm transport into the oviduct and zona-binding	[30]
ACE3, testis-specific	Secreted protein, angiotensin-converting enzyme	Sperm	No fertilizing defect	[34]
Acrin1, MN7	Intra-acrosomal protein	Sperm	Unknown	[35]
Acrin2, MC41	Intra-acrosomal protein	Sperm	Unknown	[35]
ADAM1	Membrane protein	Sperm	Failure of sperm transport into the oviduct and zona-binding	[31]
ADAM2	Membrane protein	Sperm	Impaired binding of oviduct and zona with sperm	[32]
ADAM3	Membrane protein	Sperm	Impaired binding of oviduct and zona with sperm	[33]
Basigin, MC31, CE9, CD147	Membrane protein	Sperm	Unknown	[37]
E-cadherin	Membrane protein	Egg and sperm	Impaired membrane adhesion, but normal fusion	[42]
N-cadherin	Membrane protein	Egg and sperm	Unknown	[44]
Calmegin	ER chaperone	Sperm	Failure of sperm transport into the oviduct and zona-binding	[25, 26]
Calreticulin3	ER chaperone	Sperm	Failure of sperm transport into the oviduct and zona-binding	[27]
β-catenin	Cytoplasmic and nuclear protein	Egg and sperm	Impaired membrane adhesion, but normal fusion	[42]
CD81	Membrane protein, tetraspanin	Egg	Impaired fusion with sperm	[75–77]
CD9	Membrane protein, tetraspanin	Egg and sperm	Defective fusion with sperm	[57–59]
CD98	Membrane protein	Egg	Unknown	[46]
CrispI	Secreted protein	Sperm	Normal fertility, but impaired fertilization in vitro	[47]
Crisp2	Secreted protein	Sperm	Unknown	[48]
Equatorin, MN9	Membrane-anchored protein	Sperm	Unknown	[49, 50]
IGSF8	Membrane protein, IgSF	Egg	No fertilizing defect	[51]
Integrin α3	Membrane protein	Egg	Normal membrane adhesion and fusion	[21]
Integrin α6	Membrane protein	Egg	Normal membrane adhesion and fusion	[20]
Integrin α9	Membrane protein	Egg	Reduced fertilizing ability	[22]
Integrin αV	Membrane protein	Egg	Unknown	[21]
Integrin β1	Membrane protein	Egg	Normal membrane adhesion and fusion	[20]
Integrin β3	Membrane protein	Egg	Unknown	[21]
Izumo1	Membrane protein, IgSF	Sperm	Defective fusion with egg	[60]
Izumo2	Membrane protein, IgSF	Sperm	Unknown	[52]
Izumo3	Membrane protein, IgSF	Sperm	Unknown	[52]
Izumo4	Membrane protein, IgSF	Sperm	Unknown	[52]
PDILT	Protein disulfide isomerase	Sperm	Failure of sperm transport into the oviduct and zona-binding	[28]
PMIS2	Unidentified	Sperm	Failure of sperm transport into the oviduct and zona-binding	[29]
SPESP1	Equatorial segment protein	Sperm	Morphological defects of sperm	[53]
TMEM190	Membrane protein	Sperm	No fertilizing defect	[54]
Tpst2	Tyrosylprotein sulfotransferase	Sperm	Failure of sperm transport into the oviduct and zona-binding	[29]
Tssk6	Serine kinase	Sperm	Morphological defects of sperm	[55, 56]

An immunoglobulin superfamily member, IGSF8, tightly associates with CD9 on the egg surface and is undetectable on the surface of *CD9*-deficient eggs [51]. However, the IGSF8-deficient female mice showed no fertilization defect in vitro or in vivo [51], indicating that IGSF8 is dispensable in fertility.

A family of four genes (*Izumo1*, *2*, *3*, and *4*) is mostly expressed in the sperm with known and potential roles in sperm–egg fusion [52], but the gene-disrupted mice have not been investigated.

It is widely accepted that the equatorial segment of the acrosome-reacted sperm is important in initiating fusion with the egg plasma membrane during fertilization. A mouse line lacking sperm equatorial segment protein 1 (SPESP1) was generated [53]. The average number of pups that were fathered by $Spesp1^{+/-}$ and $Spesp1^{-/-}$ male mice was significantly lower than that of wild-type fathers. Fewer sperm were found to migrate into oviducts and fewer eggs were fertilized. The sperm produced in $Spesp1^{+/-}$ and $Spesp1^{-/-}$ male mice showed a lower fusing ability compared with the wild-type sperm. Moreover, scanning electron microscopy revealed that the membrane in the equatorial segment area, which usually forms an acrosomal sheath, disappears after acrosome reaction in *Spesp1*-deficient mice, suggesting that SPESP1 is necessary to produce the fully fusioncompetent sperm.

TMEM190, a small transmembrane protein containing the trefoil domain, was identified by proteomic analysis of mouse sperm [54]. Two structural features of TMEM190, trefoil domain and small transmembrane protein, are predicted to form a protein–protein complex required during fertilization. TMEM190 is an inner-acrosomal membrane protein of cauda epididymal sperm. During the acrosome reaction, TMEM190 partly relocated onto the surface of the equatorial segment, on which sperm–egg fusion occurs. Moreover, TMEM190 and IZUMO1 were co-localized in mouse sperm both before and after the acrosome reaction. Its role in fertilization is probably dispensable since TMEM190-deficient male mice were normally fertile.

TSSK6 is a member of the testis-specific serine kinase family of proteins and is expressed post-meiotically in male germ cells [55, 56]. The sperm produced by *Tssk6*-deficient mice present defects that prevent the successful fertilization of eggs in vitro and the fusion to zona-free eggs. *Tssk6*-deficient sperm fails to relocate IZUMO1 during the acrosome reaction. TSSK6 is involved in sperm–egg fusion through the regulation of actin polymerization and changes in IZUMO1 localization.

From these studies, in order to ensure the success of fertilization in mammals, overlapping functions of multiple proteins seem to be needed. In other words, there will be more than one way for a sperm and an egg to fuse, which may reduce the malfunction that occurs in sperm or eggs

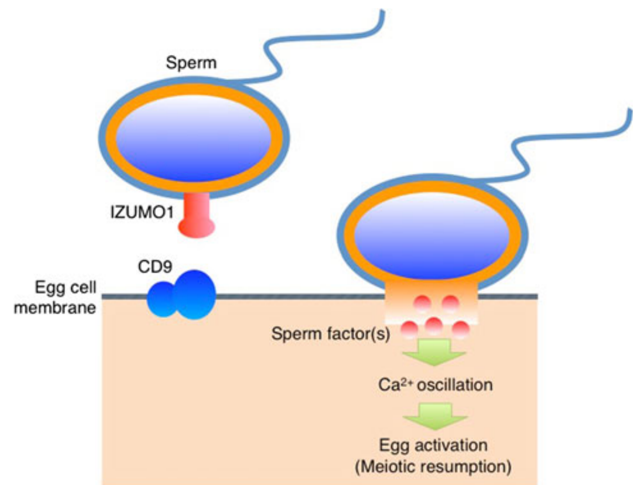

Fig. 2 Players identified in sperm–egg fusion. IZUMO1 is expressed on the sperm membrane, and *Izumo1*-deficient sperm show a defect in fusion with the egg cell membrane. CD9 is expressed on the egg cell membrane and functions in fusion with the sperm. Two membrane proteins, IZUMO1 and CD9, are essential for sperm–egg fusion in mice. Direct interaction between CD9 and IZUMO1 has not been identified, andunidentified sperm and egg factors may be involved in sperm–egg fusion. After sperm–egg membrane fusion occurs, a sperm factor triggers Ca^{2+} oscillations [95] and initiates egg activation in mammals

lacking a single gene. Exceptionally, CD9 on the egg membrane [57–59] and IZUMO1 on the sperm membrane [60] are factors proved to be essential for the sperm–egg fusion in gene disruption experiments (Fig. 2).

CD9 and its role in cellular function

CD9 gene encoding a 24-kDa protein is transcribed in all types of mammalian cells [61]. This protein is localized on the cell membranes and partly on endosomes, and it is expected to be involved in cell–cell adhesion, because CD9 associates with the integrin family [61]. CD9 is also known as a motility-related protein 1 (MRP-1), which plays a role in suppressing tumor metastasis [62]. As depicted in Fig. 3, CD9 has two extracellular loops, four transmembrane domains, and two short cytoplasmic domains. Its functional domain is expected to be positioned in a large extracellular loop (LEL), because CD9 associates with other membrane proteins via LEL in vitro [61]. In addition, due to its significantly higher levels in mesenchymal and embryonic stem cells versus fibroblastic cells, CD9 is useful as one of the cell surface markers for isolating undifferentiated cells from mixed cell populations in mice and humans [63].

In order to clarify in vivo roles of CD9, three laboratories independently generated $CD9^{-/-}$ mice [57–59]. All strains of the $CD9^{-/-}$ mice consistently showed severe female subfertility, whereas the $CD9^{-/-}$ male mice were fertile.

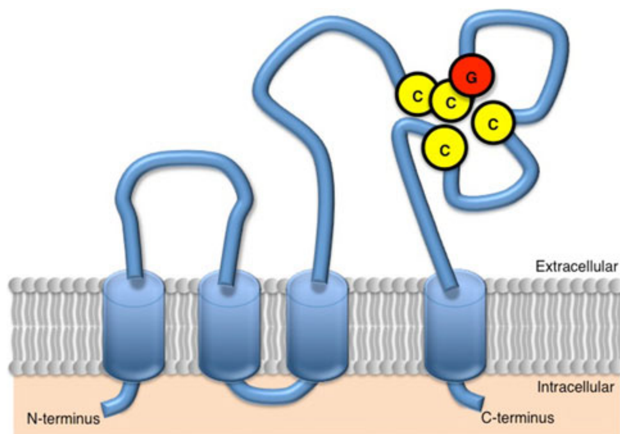

Fig. 3 Structural features of tetraspanin CD9. CD9 is a member of the tetraspan-membrane protein family, termed tetraspanin, and its molecular mass is 24 kDa. The structural features of CD9 include four transmembrane domains, two extracellular loops, short and large extracellular loops (SEL and LEL), and two short cytoplasmic tails. CD9 has cysteine–cysteine–glycine (CCG) residues (amino acids 152–154) as a tetraspanin-specific motif and two other cysteines within LEL

Moreover, the *CD9*-deficient eggs exhibited severely reduced fusion ability with sperm. Since these findings, CD9 has been studied as one of the crucial factors in sperm–egg fusion in mammals. A functionally essential domain of CD9 was predicted to be located within the LEL in sperm–egg fusion [64, 65]; however, even though CD9-binding proteins have been identified in non-gamete cells, LEL-binding, potentially fusion-related proteins have not been found yet.

Tetraspanin family

CD9 belongs to a membrane protein family, collectively termed "tetraspanin", which encompasses 35 members in mammals, such as CD9, CD37, CD53, CD63, CD81, CD82, and CD151 [61], 30 in nematodes [66, 67], and 30 in flies [68, 69]. The tetraspanin family is often thought to act as scaffolding proteins, anchoring multiple proteins to one area of the cell membrane in tissue formation [61] and even in infectious diseases [70]. Generally, pathogenic microorganisms, such as bacteria, viruses, parasites, or fungi, cause infectious diseases; the diseases can be spread, directly or indirectly, from one organism to another [71]. Members of this family are related to the onset of infectious and parasitic diseases; for instance, they are involved in cell–cell transmission of HIV-1 virus [72, 73]. After its primary infection into host cells, CD9, CD63, CD81, and CD82 are enriched at HIV-1 budding sites of HIV-1 virions. When tetraspanin-containing HIV-1 particles are next formed and released from host cells, they become 10-fold more infectious than the cell-free virus particles [73]. In mice, CD81 is also required for malarial parasites to commit to infection of the hepatocyte [74]. Malarial sporozoites, the cell form that infects new hosts, are transmitted into livers of the mammalian hosts through bites from infected mosquitoes, but the sporozoites failed to infect hepatocytes of $CD81^{-/-}$ mice, suggesting that CD81 is involved in the sporozoite entry into hepatocytes as a host factor. Furthermore, CD81 is involved in mammalian reproductive capacity. In other words, $CD81^{-/-}$ female mice were subfertile, because *CD81*-deficient eggs exhibited the impaired sperm fusion ability [75, 76]. In addition, CD81 is expressed on *CD9*-deficient eggs, and CD9 is also expressed on *CD81*-deficient eggs at the expression rate comparable with that of wild-type eggs, indicating that CD9 and CD81 independently work in sperm–egg fusion [77]. On the other hand, plants have more than 60 members of tetraspanin [78, 79], but their roles in membrane fusion-related events are unclear.

Tetraspanin-like proteins have also been identified in fungi, and their molecular masses (more than 200 kDa) are greater than those of tetraspanin identified in animals and plants (20–30 kDa) [80]. An appressorium is a specialized cell typical of fungal plant pathogens that is used to infect host plants. By analyzing a non-pathogenic mutant, *punchless*, isolated from the rice blast fungus *Magnaporthe grisea*, tetraspanin-like PLS1 (MgPLS1) has been shown to control the appressorial function, which is essential for the fungal penetration into host leaves [81]. Similarly, *Colletotrichum lindemuthianum* PLS1 (ClPLS1) is a functional homologue of MgPLS1, and the non-pathogenic *ClPLS1*-deficient mutant to bean leaves exhibits a defect in the formation and positioning of the penetration pore [82]. On the other hand, *MgPLS1* and *PaPls1* genes are functional orthologues, since MgPLS1 fully complements the germination defect of the *PaPls1*-null mutant in *Podospora anserine* [83]. Yet, MgPLS1 is required for the formation of the penetration peg originating at the pore of *M. grisea* melanized appressorium, while it is required for ascospore germination in *P. anserine* [83]. The phenotypes in both fungal species strongly differ. However, the appressorium and the ascospore share similar physiological features [83]. First, *P. anserina* ascospores and *M. grisea* appressoria both germinate through the differentiation of, respectively, a germination peg and a penetration peg after an induction. Second, they are heavily melanized cells. In both species, the melanin is deposited between the membrane and the cell wall as a dense continuous layer, and acts as a semi-permeable membrane-retaining substance affecting osmosis. Besides these shared characteristics, *P. anserina* ascospore germination and *M. grisea* appressorium peg formation rely on similar physiological processes such as the catabolism of lipids. This evidence suggests that the appressorium and the ascospore are physiologically comparable organs. Consequently, since the invasion of

pathogenic fungi into leaves is an event closely related to membrane fusion events, these studies indicate that tetraspanin-like PLS1s are involved in the membrane fusion-related event between fungus and plant.

Taken together, these results suggest that members of the tetraspanin family are closely related to membrane fusion-related events in multicellular organisms. Nonetheless, their fusogenic activity corresponding to fusogenic transmembrane proteins, such as syncytin, identified in human placenta [84], and virus envelope proteins [85], has not been identified, and their physiological activities are still unclear.

Tetraspanin as a major component of exosomes

In mammals, cell-cultured media contain nano-sized membrane vesicles, but these are not attractive to researchers, because they cannot be structurally distinguished from the debris of dead cells [86]. Recent studies have shown that the vesicles, termed exosomes, are derived from living cells, but not dead cells [87]. Furthermore, they have been proven to play a significant role in the mediation of adaptive immune reactions to pathogens and tumors through the enhancement of antigen-specific T cell responses [86, 88]. Besides immune cells, the exosomes are released from a wide range of normal and malignant mammalian cell types, and their diameter is estimated to range from 50 to 90 nm [88]. The protein composition of exosomes varies with the origin of cells, yet the exosomes commonly contain a ganglioside GM3, two kinds of heat shock proteins (HSP70 and HSP90), and tetraspanin [88]. The exosomes also contain transcripts, mRNA, and microRNA, which are thought to be shuttled from one cell to another, thereby influencing protein synthesis in recipient cells [89].

Exosome-like vesicles are released from mouse eggs

At least two reports suggest that CD9 contributes to the organization of the cell membrane in eggs. First, CD9 is transferred from the egg to the fertilizing sperm present in the perivitelline space, implying the involvement of a process similar to trogocytosis, which is a mechanism for the cell-to-cell contact-dependent transfer of membrane fragments from antigen-presenting cells to lymphocytes in immune responses to pathogens [90]. Secondly, CD9 deficiency alters the length and density of microvilli on the egg cell membrane [91]. However, treatment with fixatives often disturbs membrane organization and modifies the localization of membrane proteins. The above two studies exhibited the membrane localization of CD9 in eggs treated with paraformaldehyde.

On the other hand, the potential of enhanced green fluorescent protein-tagged CD9 (CD9-EGFP) was used as a reporter protein for studying sperm–egg fusion in living mouse eggs [92]. Interestingly, in eggs just before fertilization, CD9-EGFP was significantly accumulated within the perivitelline space that completely surrounded the eggs and lay between the egg cell membrane and the zona pellucida. In addition, when the eggs were carefully treated with fixatives, immunoelectron-microscopic analysis of wild-type eggs also revealed that CD9 was not only present in the perivitelline space, but also incorporated into vesicles of varying size (50–200 nm in diameter) without a sectional profile of a typical lipid bilayer [92]. Membrane vesicles were also previously detected via electron microscope within the perivitelline space of their eggs in opossums [93] and humans [94]. Moreover, the above study demonstrates that the vesicles identified in mouse eggs share CD9, GM3, and HSP90 with exosomes, and these components are absent in eggs lacking CD9 and are reproduced by CD9-EGFP, expression restricted to the eggs [92]. These results provide evidence as to the nature of CD9 in mouse eggs. First, CD9-incorporated exosome-like vesicles are produced in mouse eggs and are released outside the egg cell membrane just before fertilization. Secondly, CD9 is essential for the formation of the exosome-like vesicles (hereafter referred to as egg exosomes) in mouse eggs.

The finding of egg exosomes gives us the idea that they facilitate the sperm–egg fusion. As expected, CD9-containing egg exosomes rendered sperm capable of fusing with *CD9*-deficient eggs [92] (Fig. 4). Concretely, *CD9*-deficient eggs could not fuse with sperm, but the co-existence of wild-type eggs resulted in 60–70% of the *CD9*-deficient eggs fusing with at least one sperm [92]. Thus, sperm can fuse with *CD9*-deficient eggs with impaired microvilli via the egg exosomes released from wild-type eggs, which means that the egg exosomes, but not the egg microvilli, are essential for sperm–egg fusion.

Conclusions

The close relation between egg exosomes and sperm–egg fusion raises the question of how egg exosomes facilitate such fusion. According to a previous report, exosomes contain both functional mRNA and microRNA, which are shuttled from one cell to another, affecting the recipient cell's ability to produce protein [89]. Moreover, HIV-1 utilizes the exosome biogenesis pathway for the formation of infectious particles, and in macrophages, HIV-1 assembles into an intracellular plasma membrane domain-containing tetraspanin (i.e., CD9, CD81, CD53, or CD63) [73]. Thus, the exosomes play at least two roles in regulating cell function: first, shuttling proteins and RNAs (mRNAs and microRNAs) from one cell to another, and

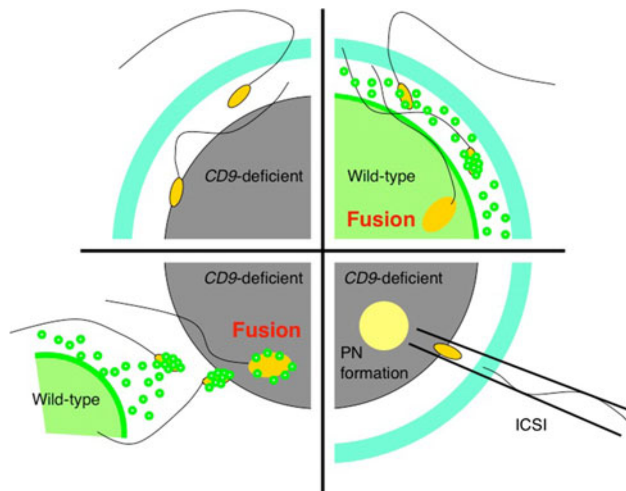

Fig. 4 Overview of the studies of *CD9*-deficient eggs. In wild-type eggs, CD9-containing egg exosomes are released from wild-type eggs before any interaction with the sperm (*upper right diagram*). Shortly after the sperm penetrates the perivitelline space, the egg exosomes are transferred on the acrosome-reacted sperm head. Then, a sperm fuses with the egg cell membrane. Interaction between the sperm and the exosomes is an essential step for sperm-fusing ability. In contrast, *CD9*-deficient eggs cannot release the egg exosomes, which are correlated with the formation of microvilli on the egg cell membrane (*upper left diagram*). The sperm cannot fuse to the cell membrane of the *CD9*-deficient egg. On the other hand, when the zona pellucida is removed from the eggs, the sperm is able to interact with the egg exosomes released from wild-type eggs and can fuse with the *CD9*-deficient egg (*lower left diagram*). By co-incubation with wild-type eggs, the sperm can fuse with a similar number of *CD9*-deficient and wild-type eggs. Intracytoplasmic sperm injection (ICSI) is an in vitro fertilization procedure in which a single sperm head is injected directly into an egg (*lower right diagram*). This procedure is most commonly used to overcome male infertility and fusion defects in *CD9*-deficient eggs

secondly, forming infectious particles. These two roles may be required for sperm–egg fusion in mammals. In conclusion, the studies of exosomes will present a useful strategy for regulating the cell-to-cell spread of specific viruses and fertilization ability.

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Conflict of interest The author declares that they have no conflict of interest.

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