

CELL FUSION: MECHANISMS AND BIOLOGICAL IMPLICATIONS

Abstract

The process through which two cells fuse their plasma membranes to generate a single cell with some genetic material from each parent cell while maintaining other characteristics is known as cell fusion. It happens in a number of physiological and pathological processes, including immune response, morphogenesis, tissue repair, and the development of cancer. Cell-cell fusions that take place naturally as cancer progresses demonstrate how this pathway has the capacity to result in the significant genetic changes that are seen in cancer. It is regulated by a number of proteins as well as signaling pathways, comprising glial cell missing-1, galectin-1, syncytin-1, and syncytin-2. Other proteins that regulate it include annexins, myomaker, and myomerger. Proteins (annexins, myomaker, myomerger, etc.) involved in cell fusing through the cyclical adenosine-dependent protein kinase A, mitogen-activated protein kinase, wntless/integrase-1, along with c-Jun N-terminal kinase signaling pathways include syncytin-1, syncytin-2, glial cell lacking 1, and galectin-1. The fusion of nuclei, cytoplasmic mixing, and membranes are all parts of cell fusion. It is an essential stage in the maturation of cells and the preservation of their unique functions while they grow in their native environments. We can gain a better understanding of the morphogenesis, immune response, tissue healing, and subsequent growth of malignant tumors by investigating the molecular pathways of cell fusion.

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Abbreviations used	Expansion
cAKA	Cyclic Adenosine-Dependent Protein Kinase-A
MAPK	Mitogen-Activated Protein Kinase
BMDCs	Bone Marrow-Derived Cells
HAP2/ GCS1	Hapless 2/Generative Cell-Specific Protein 1
T-SNARE	Target-Soluble N-Ethylmaleimide-Sensitive Factor Attachment Protein Receptor
V-SNARE	vesicular-SNARE
HERV	Human Endogenous Retrovirus
SU	Surface Subunit
TM	Transmembrane Subunit
Asct-2	Sodium-Dependent Type 2 Neutral Amino Acid Transporter
GCM1	Glial Cell Missing 1
β -HCG	β -Human Chorionic Gonadotropin
GRP78	Glucose-Regulatory Protein 78 kDa
Ig	Immunoglobulin
DC-STAMP	Dendritic Cell- Specific Transmembrane Protein
OC-STAMP	Osteoclast- Stimulating Transmembrane Protein
HA	Hemagglutinin
cAMP/PKA	Cyclic Adenosine-dependent Protein Kinase A
ZO-1	zona occludens-1
Wnt	wingless/integrase-1
PEG	Polyethylene Glycol
JNK	c-Jun N-Terminal Kinase
NIR	Near Infrared Laser

The cell fusion process involves the merging a number of uninucleate cells results in the formation of a multinucleate cell called syncytium. The cell fusion includes membranes fusion, cytoplasmic mixing and fusion of nuclei. It is a fundamental step for maturation of cells as well as the maintenance of their specific functions during growth. Also cell fusion occurs in various pathological and physiological processes like embryogenesis, morphogenesis and tissue repair, immunological response and also in cancer development. Various proteins and signaling pathways, including syncytin-1, syncytin-2, galectin-1, glial cell missing-1, and additional proteins such as annexins, myomaker, and myomerger, exert control over it.

I. INTRODUCTION

Cell fusion encompasses both physiological and pathological processes. Depending on the types of cells involved, cell fusion can be classified as homogeneous or heterogeneous. Furthermore, considering the contents of the cells when they merge, cell fusion can be categorized as complete fusion or hemifusion. The process involves the membrane fusion followed by cytoplasmic mixing and nuclear fusion which can leads to development of heterokaryon (multinuclear cells) or synkaryons (mononuclear cells). Daughter cells in synkaryons have all the chromosomes of the closely related cells and are created via recombination, nuclear membrane separation, and cell division. The hybrid cell transforms

into a polyploidy with more than two sets of chromosomes in its genome and adopts on a new phenotype. In numerous physiological or pathological stages, including those associated with growth, development, aging, stress, cancer, and other diseases, polyploidy cells can be seen. The heteromorphic cell fusion has a crucial effect on growth of tissues and disease progression.

II. TYPES OF CELL FUSION

Cell union can be of the homotypic or heterotypic type. Homotypic cell fusion happens when cells of the similar kind come together. The osteoclasts or myofibers fusion involves homotypic fusion. Heterotypic cell fusion take places among cells of dissimilar types for example fusion of the integration of bone marrow-derived cells (BMDCs) with the parenchymal organs.

III. THE PROGRESSION OF CELL FUSION

The fusion of membranes begins when separate membrane vesicles or compartments are brought close to together. Initiation requires overcoming two main forces: an attractive hydrophobic force between the hydrocarbon interiors of the bilayers and a repulsive hydration force brought on by water that is tightly linked to the hydrophilic lipid head groups, cell fusion includes both an integration of the internal components of the cells and also a morphological restoration of the cell membrane.

Under specific circumstances, proteins from fused cells are released, allowing them to communicate with their surroundings and the fusion target. It has been discovered that the group of proteins known as "fusogens" which are necessary to trigger cell fusion via several different methods. Cell fusions can be regulated by a particular fusogen or involve multiple proteins cooperating to accomplish or regulate the fusion process. For instance, myomaker and myomerger function as solitary fusogens in myoblast fusion, while HAP2/GCS1 becomes active during later stages of gamete fusion. In *Caenorhabditis elegans*, EFF-1 and AFF-1 operate independently to initiate auto-fusion.

The entire fusion process involves in three phases. The initial step starts with preparation of cells for fusion by cell-cell recognition followed by membrane approach mediated by fusogens and in final stage new cells formed by lipidic rearrangements (Fig:1).

The initial phase of cell fusion relies primarily on receiving extracellular signals designed for cell differentiation, making cell-cell recognition and interaction indispensable. When cells are fewer than 10 nm apart from one another, they begin to tightly adhere to one another, and then fusogens work in the last approach between membranes. The cell fusogens mediates in morphological changes includes dehydration, hemifusion, pore opening and spreading out cell membranes with the intention of the union of experiences. The final phase encompasses the merging of cell membranes into a single, contiguous structure that encompasses all cytoplasm and genetic material. However, on occasion, the process terminates abruptly before pore opening and extension transpire, resulting in the cell reaching with each other.

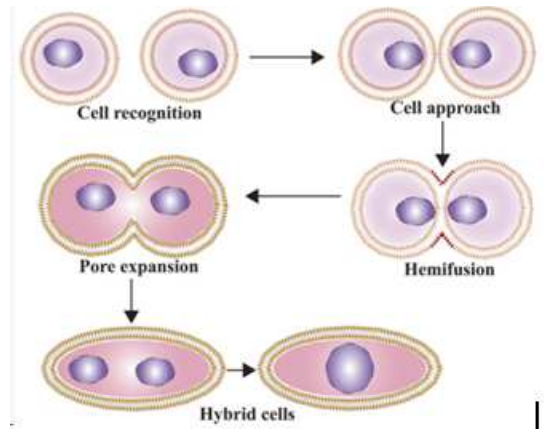


FIGURE 1: Cell fusion progression can be divided into 3 steps 1. preparation for fusion, 2. membrane approach under the accomplishment of fusogens, and 3. Constitution of new cells by means of lipidic rearrangements. (Source: Hao Zhang et al., 2021)

IV. CELL FUSION ASSOCIATED PROTEINS

Fusogenic proteins and actin-driven membrane protrusions play a crucial role in the initial phase of cell-cell fusion. For instance, the helix fusion process necessitates the presence of syncytin-1 and syncytin-2., T-SNARE (Target-soluble N-ethylmaleimide-sensitive factor attachment protein receptor) and V-SNARE (Vesicular-SNARE) are the components of the helical fusion mechanism that facilitate intracellular membrane fusion by forming -helical bundles that result in membrane fusion.

The membrane glycoprotein known as syncytin-1, which is encoded by the ENV gene of the HERV (human endogenous retrovirus) W family, was the initial fusion-promoting protein identified in syncytiotrophoblast cell formation. Syncytin-1 is expressed in the placenta and consists of surface subunit (SU) and transmembrane subunit (TM) components. The SU portion of syncytin-1 interacts with a type D retrovirus receptor called sodium-dependent type 2 neutral amino acid transporter (ASCT-2). This interaction induces a conformational change in the TM, leading to a close association of the phospholipid bilayers of the two trophoblast cells. Syncytin-1 primarily facilitates the fusion of mononuclear trophoblast cells into multinuclear syncytiotrophoblast cells. Additionally, syncytin-1 plays a role in myoblast fusion and osteoclast formation.

Syncytin-2, a endogenous retrovirus gene product and a fusion protein plays a role in arrangement of placental syncytiotrophoblast cells and placenta formation, is involved osteoclast and macrophage fusion.

GCM1 is a transcription factor containing zinc finger domains, and it is specifically found in embryos, with prominent expression in placental trophoblast cells. Its main role involves regulating the expression of syncytin-1 and facilitating the differentiation of mononuclear trophoblast cells into multinuclear syncytiotrophoblasts.

Galectin-1, a soluble lectin, plays a role in cell fusion and is associated with the expression of syncytin-2. Additionally, it has been linked to trophoblast cell fusion.

The fusion process involves various proteins specific to different cell types. For instance, during fertilization, oocyte tetrasomal proteins CD9 and CD81 play a crucial role. Glucose-regulatory protein 78 kDa (GRP78), an endoplasmic reticulum protein present on trophoblast cells' surface, contributes to enhancing cell fusion. Immunoglobulin-like cell adhesion molecules are essential for both cell-cell fusion and recognition in myoblasts. Proteins such as CD47, CD200, DC-STAMP (dendritic cell-specific transmembrane protein), and OC-STAMP (osteoclast-stimulating transmembrane protein) are vital for macrophage fusion in osteoclasts. Myomaker (Tmem8c), a muscle-specific protein located on the plasma membrane, is involved in myoblast fusion. Additionally, annexins, molecules with multiple Ca²⁺-binding sites, mediate the fusion reaction or induce a conformational change in fusogenic proteins.

V. CELL FUSION RELATED SIGNALING PATHWAYS

Syncytin-1 expression is regulated by a number of signaling pathways throughout embryonic development, including the wntless/integrase-1 (Wnt), cyclic Adenosine-dependent Protein Kinase A (cAMP/PKA), Mitogen-Activated Protein Kinase (MAPK), and c-Jun N-terminal kinase (JNK) signaling pathways. Syncytin-1 is increased as a result of the cAMP pathway being activated. TGF- β 1 and TGF- β 3 are major negative regulators of cell fusion.

VI. CELL FUSION DURING EMBRYONIC DEVELOPMENT

The growth of syncytiotrophoblasts in the placenta and the process of sperm/egg fertilization during sexual reproduction both involve cell fusion. Sperm's IZUMO proteins play a crucial role in fertilization through dynamic cell fusion. Failure to express these proteins leads to sterility due to the inability of sperm and egg to fuse. Placental formation, which occurs through cell fusion, consists of two distinct phases (Figure 2). In the initial phase, cytotrophoblast cells merge to form multinuclear syncytiotrophoblast cells. These cells then migrate and infiltrate the mother's uterus, serving as a conduit for the exchange of oxygen, nutrients, and metabolic waste between the embryo and the mother. In the second stage, cytotrophoblasts and syncytiotrophoblasts collaborate in tissue repair processes.

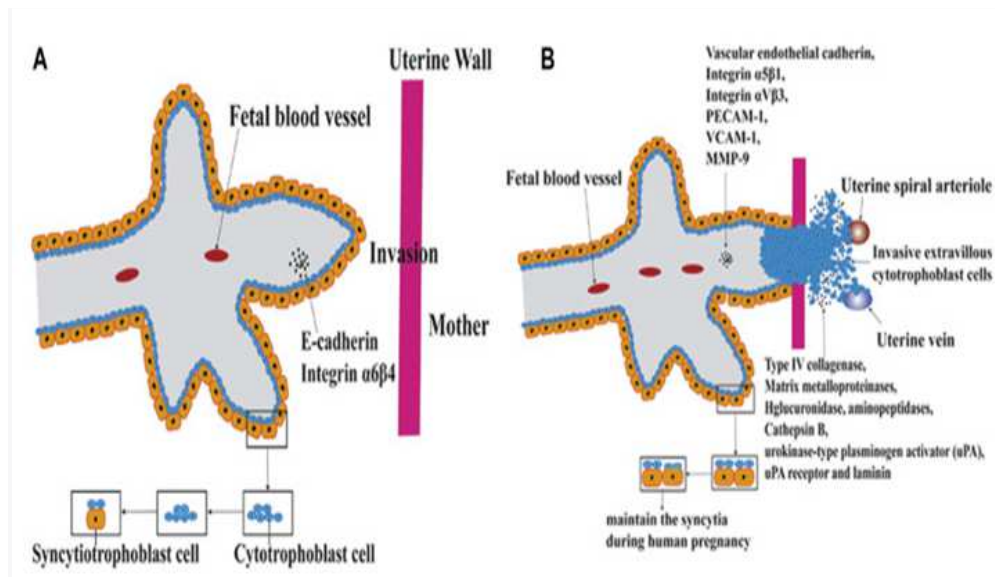


Figure 2: The arrangement of the placenta is accompanied by cell fusion and is separated into two phases according to the diverse functions in the fusion process. A. In the first phase, cytotrophoblasts and syncytiotrophoblasts fuse for tissue regeneration (B) In the second phase cytotrophoblasts and syncytiotrophoblasts combine for tissue regeneration. (Source: Hao Zhang et al., 2021)

VII. CELL FUSION TECHNIQUES

The various cell fusion procedures offer an effective tool for new discoveries in the realm of genetic and epigenetic studies of the genome.

1. **Electrical:** Cell fusion employs dielectrophoresis as a technique to bring two cells into contact using high-frequency alternating current. Once the cells are united, a pulsed voltage is applied. This voltage pulse induces permeability in the cell membranes, allowing them to merge and facilitate cell fusion. Subsequently, an alternating voltage is applied for a brief duration to ensure the stabilization of the fusion process, ultimately resulting in the complete fusion of cell membranes and cytoplasmic mixing.
2. **Polyethylene Glycol:** (PEG) is the simplest, However, it is the most toxic method for cell fusion. In this process, PEG serves as a dehydrating agent, inducing the fusion of plasma and intracellular membranes. PEG promotes cell agglutination through cell-to-cell contact. Unfortunately, the use of PEG can lead to uncontrolled cell fusions, resulting in the formation of large polykaryons. This form of cell fusion is commonly utilized in generating somatic cell hybrids and for nuclear transfer in mammalian cloning.
3. **Sendai Virus Induced:** Cell fusion proceeds through four distinct phases. The initial stage transpires within 10 minutes, during which virus adsorption takes place. The adsorbed virus can be inhibited by viral antibodies. In the subsequent 20 minutes, there is a pH-dependent phase where the addition of viral antiserum can still diminish the eventual fusion process. The viral envelope components remain observable on the cell surface during the antibody-resistant stage. Finally, in the fourth stage, cell fusion becomes evident, with fusion characteristics gradually diminishing.

- 4. Thermoplasmonics Induced:** The cell fusion technique relies on the utilization of a near-infrared laser (NIR) and a plasmonic nanoparticle. A minuscule plasmonic particle is subjected to exceedingly high temperatures within a confined region using a laser, primarily serving as an optical trap. This optical trapping effect takes place at the boundary between two membrane vesicles or two cells, resulting in prompt fusion, which is confirmed through both content and lipid mixing. Importantly, this method offers the advantage of enabling fusion with various types of cells and can occur in a wide range of buffer environments.

VIII. APPLICATIONS OF CELL FUSION

Biologists have started thinking about the possibility of therapeutic make use of cell fusion due to the lack of organs and tissue available for transplantation. Cell fusion finds applications in investigating cell division, gene expression, malignant changes, viral replication, genetic mapping, monoclonal antibody production via hybridoma formation, induced stem cell generation, and assessing protein movement through a technique referred to as heterokaryon fusion

IX. CONCLUSION

Cell fusion functions as a link between the discussed proteins and pathways. Nevertheless, several challenges must be addressed to harness cell fusion for therapeutic purposes. These challenges encompass the selection of suitable cells for reparative fusion, devising effective methods for introducing these cells into the target tissue, enhancing the occurrence of cell fusion, and ensuring that the resultant fusion products perform their intended functions. If these challenges can be overcome, cell fusion holds therapeutic potential.

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