REVIEW

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Tumor Microenvironment and Mechanisms of Cancer Metastasis: An Overlooked Fact: Cell Fusion

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ABSTRACT Cell fusion is a cellular mechanism in which cell membranes of two or more cells fuse to become a new hybrid cell. Cell fusion plays an important role in several physiological tasks such as fertilization, organogenesis, inflammatory response, and tissue repair. However, cell fusion aids in the development of a wide range of pathological conditions such as inflammation and cancer. Cell fusion of normal somatic cells is a tightly controlled process that is limited to only a few cell types in humans, resulting in terminally differentiated multinucleated cells incapable of proliferation. However, this tightly controlled process becomes dysregulated due to genetic alterations and leads to the development of cancer. In this review, the implications of cell fusion and its role in carcinogenesis are discussed in detail.

Keywords: Cell fusion; tumor microenvironment; carcinogenesis

In sexually reproducing species, life begins with the cell fusion of the oocyte and sperm. The syncytiotrophoblast is a massive cell formed by the union of a large number of cells, having a surface area of ~10 square meters, and is crucial to the genesis of life.[1] The syncytial multi-step union of myoblasts involving products from several genes forms the embryo's muscles as it develops further. Osteoclasts, multinucleated cells generated by the fusing of mononuclear progenitors, play a crucial role in the maintenance of adult bones.[2] Again, foreign body giant cells and Langerhans cells can be given as examples of physiological cells formed as a result of cell-cell fusion events; however, these cells do not undergo mitosis.

HOW DO CELLS FUSE?

Fusogens are proteins that are activated during cytoplasmic membrane fusion and cause direct cell fusion.[3] The fusogens produce unilateral or bilateral complexes that determine the area of fusion and overcome energy hurdles that would otherwise prevent the anti-fusion mechanism from functioning.[4] Only syncytins, which play a critical part in the creation of human placental syncytiotrophoblasts, are expressed in human cells out of the 4 families of fusogens involved in cell-cell fusion.[5]

In terms of cell membrane bridging and biological regulatory mechanisms, fusion processes differ significantly. Since this mechanism is one-sided, only one of the junctional membranes needs to be present in some fusions for the proteins mediating the fusion to occur. The bilateral process similarly necessitates the existence of identical or dissimilar fusogens in both membranes. The fusion protein machine's role in all fusion processes is to bring the lipid bilayers into contact as quickly as possible, stimulating the synthesis of energy-dense fusion intermediates, and transitioning from the pre-fusion to the post-fusion state. Fusion occurs when the continuity of each of

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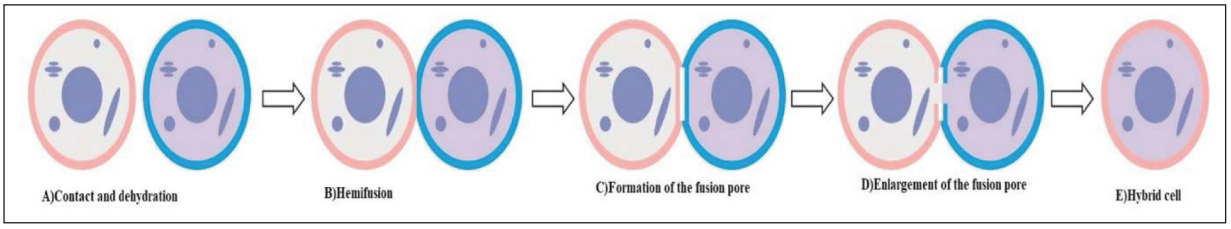

FIGURE 1: Stages of cell fusion.

the lipid bilayers is ruptured and is locally recombined.[6]

The establishment of stalk, hemifusion, and creation of the fusion pore are the 3 essential steps of the cell fusion model. Perfusion preparation is the initial stage as cell fusion requires perfusion preparation for accurate fusion. Although the expression of specific recognition or adhesion-related proteins during preparation is adequate and required for cell fusion, this does not imply that they are directly involved in the fusion process; rather, they help certain cells maintain their proximity.[5]

Although the cells are close enough (10 nm), the space between them narrows to 1 nm due to the activation of specific proteins and the removal of water molecules between them during dehydration. The plasma membrane begins to flex, leading to a fusion between the outer phospholipid layers, a process known as hemifusion. As a result, the inner cell layer coalesces even further and produces a fusion pore. The cytoplasm is extensively mingled as the fusion pores develop, forming a hybrid comprising their genomes and numerous organelles such as the mitochondria of the two-parent cells (Figure 1).[7]

CELL FUSION AND CARCINOGENESIS

In 1911, German physician, Otto Aichel proposed the involvement of cell fusion in the development of cancer. The most significant consequence of cell fusion is the production of tumor cell-normal cell hybrids (TN-hybrids) and subsequent proliferation, further leading to disease progression.[8]

The intratumoral heterogeneity in tumors is due to genomic instability that leads to tumorigenesis. Each of the 5 carcinogenesis hypotheses (mutation, genome instability, non-genotoxic, Darwinian cell selection, and tissue organization) proposes a distinct mechanism for explaining this phenomenon.[9]

The hybrid cells undergo a heterokaryon-to-synkaryon transition (HST), which involves the merging of the parental chromosomes, and is responsible for the genomic instability generated by cell-cell fusion events.[10] A multinucleated cell is called a heterokaryon, whereas a mononuclear cell is called a synkaryon. According to a stem cell-based tissue regeneration study, heterokaryons formed from cell fusion events, occurring between hepatic and bone marrow-derived dendritic cells, undergo ploidy reductions, resulting in daughter cells with half the amount of genetic information in each chromosome. Furthermore, this mechanism was not strictly regulated in individual cells, thereby resulting in a wide range of successful and failed bipolar, tripolar, and double mitoses.[11]

Unequal segregation of chromosomes has also been associated with ploidy reductions. Aneuploid karyotypes result from a gain or loss in the number of chromosomes. However, it is still unclear whether the ploidy reduction and HST mean the same. Since both processes are accompanied by cellular proliferation and nuclear membrane disintegration, an absence of these 2 might result in improper linking of the parent chromosomes as well as random sorting of daughter cells.[12] Both HST and ploidy reduction have been associated with chromosomal missegregation.[13]

Initiation of the HST process leads to several genomic aberrations induced by cell fusion. Chromosome rearrangements (e.g., substitutions, deletions, etc.) and damage (single and double chain breaks), loss of complete chromosomes, uneven chromosome segregation in daughter cells, and chromothripsis are

all associated with the evolution of cancer. Chromothripsis is a complex genomic rearrangement characterized by the breaking of chromosomes into various pieces and then reassembling in an uneven pattern, resulting in the loss of chromosomal fragments. This is analogous to the putative mutator phenotype theory that has been proposed to explain genomic instability for both the aneuploid hypothesis of carcinogenesis and tumor development.[10]

DETECTION OF CELL FUSION AND DARK MATTER

The TN-hybrid cells must be "visible", meaning they must express certain markers to be recognized in human tumors. The fusion of tumor cells and macrophages can be demonstrated using macrophage markers. However, cell fusion is not confined to tumor cells and macrophages but also occurs in other cells like tumor cells, fibroblasts, and bone marrow-derived cells. The presence of particular markers determines the presence of tumor cell-tumor cell (TT) hybrids or tumor cell-fibroblast/stem-like cell hybrid cells (also known as TN-hybrid cells) in the tumor microenvironment (TME).

These TT-and TN-hybrid cells are referred to be "invisible" or "dark matter hybrids" as they cannot be recognized due to a lack of specific markers.[14]

It is suggested that although dark matter accounts for 85% of all matter in the universe, its composition remains unknown. Dark matter is undetectable because it does not interact with observable electromagnetic energy such as light and can only be discovered indirectly through innate gravitational interactions.[15]

Dark matter is a concept for describing species that cannot be identified by biochemical assays, such as intrinsically disordered proteins, post-translational states, ion species, as well as infrequent, temporary, and weak interactions.[16] Dark matter in biology interacts with and performs tasks that can be sensed but cannot be directly measured and is similar to dark matter in gravitational physics. Non-coding DNA has been proposed as the genetic counterpart of dark matter in cosmology.[17] Although non-coding DNA influences genetic expressions, establishing its complete impact has been difficult due to the expensive computations necessary to interpret genomic and RNA expression data simultaneously.

Dark matter hybrids may be the unseen component of apparent tumor matter and might communicate and execute roles that aid tumor proliferation. Homotypic tumor cell fusion events may result in dark matter hybrids; TN hybrids are indistinguishable from unfused tumor cells, like TT hybrids. Moreover, the loss of ability to express certain markers by visible hybrid cells might induce the formation of dark matter hybrids.[14]

TUMOR MICROENVIRONMENT

The cellular environment inhabited by tumor cells is denoted as the TME, which consists of epithelial cells, stromal cells, immune cells, blood vessels, pericytes, adipocytes, mesenchymal stromal cells, cytokines, and extracellular matrix.

Interaction of tumor cells with the stroma actively modifies the TME, thus, allowing angiogenesis, cellular proliferation, invasion, metastasis, and treatment resistance.[18] TME interaction is also influenced by soluble substances, cytokines, microRNAs, and extracellular vesicles released by tumor or stromal cells.[19,20]

As cell-cell fusion events are governed by the influence of various genes and epigenetic factors, a spontaneous union between the 2 cells is rare. The incidence of cell fusion increases with aging, radiation exposure, inflammation, chemotherapy, and tissue damage.[21]

Several tumor cells having different genetic features and biological behaviors than the normal tissues produce a distinct environment, resulting in aberrant cell-cell fusions like TME, which is always devoid of oxygen and nutrients with a persistent low pH.[22] Thus, an unfavorable microenvironment enables tumor cells to speed up the cellular processes like cell fusion that leads to tumor progression. Pathological conditions like hypoxia and inflammation are both implicated in cell fusion regulation.[23] Similarly, a low pH level denotes a chronic inflammatory environment.

CELL FUSION AND METASTASIS

Fusogenic immune cells provide crucial biological abilities to tumor cells through cell fusion, namely the ability to metastasize and evade the immune system. Cell-cell fusion between tumor cells and macrophages yields hybrids with distinct migratory macrophage-like physical characteristics, such as circulation survival and immune system evasion.[24]

Cancer cells generate hybrids with phenotypic traits of macrophages and express the macrophage-specific marker CD163 during interaction with macrophages. Additionally, hybrid cells expressing CD163 acquire radioresistance and have better survival and colony formation abilities. In breast cancer, CD163 expression indicates advanced stages and a poor prognosis.[25] Recent evidence indicates that tumor-macrophage fusion provides new malignant abilities to hybrid cells that subsequently result in the acquisition of cancer stem cell properties.

In order to meet around 85% of their energy requirements, proliferating tumor cells use the glycolytic pathway to obtain energy by glucose degradation in the presence of oxygen, a phenomenon termed the "Warburg effect". Tumor cells use aerobic glycolysis to meet their energy needs and also provide substrates for protein and nucleic acid synthesis. The Warburg effect causes the fusion of cancer cells with macrophages, resulting in hybrids with high levels of structural autophagy, similar to macrophages under hypoxia and food restriction. Moreover, hypoxia increases tumor cell survival and proliferation due to the acquired features of fusion-derived macrophages.[26]

Metastasis is the most lethal attribute of tumor cells. The "wolf in sheep's clothes" hypothesis describes a putative link between cell fusion and metastasis. According to this hypothesis, a tumor cell becomes metastatic by fusing with normal circulating cells such as lymphocytes or macrophages.[27]

A previous study suggested that cell fusion is associated with the tissues where tumor cells have metastasized. Fusion of non-metastatic mouse plasmacytoma or myeloma cells with lymphocytes or splenic dendritic cells leads to metastatic hybrids that vary in target tissues depending on the normal cell type.[28] The "wolf in sheep's clothing" paradigm proposes that tumor cells are drawn to these tissues as they acquire macrophage tropism as macrophage hybrids because tissues with extensive metastasis are generally high in the macrophage population.[29]

Another hypothesis suggests that tumor cells fuse with a tissue's normal cell, gaining the "sheep's coat" and forming a tumor cell capable of growing in the new environment. This method is similar to the one hypothesized for stem cell differentiation, in which a stem cell differentiates from host tissue by fusing with an existing cell.[30]

CONCLUSION

The major processes through which cells undergo malignant transformation are suggestive of mutations and the accumulation of genetic defects. Cell fusion is a fast-paced phenotypic and functional evolution mechanism that generates new cells at a considerably faster rate than random mutagenesis. As a result, cell fusion is favorable for the expanding cancer cell population because it allows tumor cells to acquire new features that help them survive under specific selective pressures.

Tumor cells alter their genomes and exhibit malignant traits by fusing with numerous cell types in the TME. Tumor stem-like cells are produced by hybrids created by the fusion of macrophages and tumor cells. It is involved in tumor genesis as well as recurrence. The ability to survive, move, and metastasize is provided by circulatory mechanisms. Radioresistance and medication resistance are conferred by tumor cell fusion with other TME cells.

Cell fusion can also be used for therapeutic purposes. Cancer cell/dendritic cell fusion produces hybrid cells that can generate an anti-tumor immune response and could be employed to treat colorectal and kidney cancer.[31]

As a result, although the cell fusion process has been known for over a century, it has gone unnoticed due to the difficulty of detecting it. With technological advancements, our understanding of this issue is growing at a fast pace. However, questions about how the fusogenic cells regulate their biological func-

tions and identify other ambiguous fusogens remain unanswered.

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Conflict of Interest

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Authorship Contributions

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