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# Cell-cell fusion as an important mechanism of tumor metastasis (Review)

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**Abstract.** Cell-cell fusion is a dynamic biological phenomenon, which plays an important role in various physiological processes, such as tissue regeneration. Similarly, normal cells, particularly bone marrow-derived cells (BMDCs), may attempt to fuse with cancer cells to rescue them. The rescue may fail, but the fused cells end up gaining the motility traits of BMDCs and become metastatic due to the resulting genomic instability. In fact, cell-cell fusion was demonstrated to occur *in vivo* in cancer and was revealed to promote tumor metastasis. However, its existence and role may be underestimated, and has not been widely acknowledged. In the present review, the milestones in cell fusion research were highlighted, the evidence for cell-cell fusion *in vitro* and *in vivo* in cancer was evaluated, and the current understanding of the molecular mechanisms by which cell-cell fusion occurs was summarized, to emphasize their important role in tumor metastasis. The summary provided in the present review may promote further study into this process and result in novel discoveries of strategies for future treatment of tumor metastasis.

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**Contents**



## 1. Introduction

Tumors are hypothesized to originate as a result of and progress due to sequential genetic and epigenetic mutations of cells. Tumors originate gradually from the tumor stem cells (TSCs) that accrue several mutations (1-6). In recent years, it has been indicated that the origin of tumors or TSCs involves cell-cell fusion (1-6). It was originally hypothesized that tumor cells possessed characteristics indicative of aneuploidy and chromosomal disorders. Therefore, it was reasonable to hypothesize that these features of tumors may be associated with cell-cell fusion. It would be interesting to determine if the fusion of a cancerous cell with a normal healthy cell, such as a migrating bone marrow-derived cell (BMDCs), may give rise

to unique features in the resultant cell, such as increased tumor initiation, tumor metastasis and/or drug resistance capacity.

## 2. Milestones in cell-cell fusion research

Cell-cell fusion, also termed cell hybridization, refers to the process of the fusion of two or more cells into a single hybrid cell, with the formation of a single nucleus possessing genetic information from two or more lineages (7). At the beginning of the process, the membranes begin to fuse, followed by fusion of the cytoplasm and the nuclei, ultimately resulting in the formation of a single cell (8). In multicellular organisms, cell fusion is a basic developmental and physiological process. The fusion of a sperm and egg cell is one of the most classical examples of cell fusion. In 2002, Mohler *et al* (9) first identified that the eff-1 gene was essential for developmental cell fusion. In 2004, Shemer *et al* successfully demonstrated that the expression of EFF-1 protein leads to cell fusion, and that it could cause independent cell fusion in the absence of other proteins (10).

Cell-cell fusion can occur *in vivo* in an organism and *in vitro* in cell cultures, both spontaneously and artificially. In a laboratory, researchers can use an external agent, such as viral fusion agent (Sendai virus), chemical fusion agent (polyethylene glycol) or electric shock, to induce cell-cell fusion *in vitro* between the same or different cell types.

The major milestones in the study of cell-cell fusion are summarized in (Tables I and II; Fig. 1). In the 1930's, scientists observed the presence of multinucleated cells in smallpox, chickenpox, measles and other infectious diseases, and rabbit homotypic cell fusion *in vivo* in the formation of foreign body giant cells (11). In 1954, Enders and Peebles (12) reported that human multinucleated giant cells or syncytia were formed *in vitro* as a result of measles viral infection (12). In 1961, Barski (13) observed the somatic cell fusion phenomenon in tissue cultures. In 1962, Furusawa and Cutting (14) discovered that the hemagglutinating virus caused fusion of mouse Ehrlich ascites tumor cells *in vitro*. In 1965, Cascardo and Carzon (15) found and confirmed that the inactivated measles virus under the appropriate conditions could also induce human cell fusion *in vitro*. Harris and Watkins (16) reported the fusion of human HeLa cells and mouse Ehrlich ascites tumor cells *in vitro*. In 1968, Goldenberg (17) reported the fusion of human tumor and normal animal host cells *in vivo*. In 1970, a polykaryocyte was discovered (18) and Goldenberg *et al* (19) reported evidence of the fusion of transplanted human cancer cells with normal hamster cells *in vivo*. In 1984, Klein *et al* (20) reported the spontaneous fusion of mouse melanoma cells *in vitro*. In 1994, Lapidot *et al* (21) reported the generation of cancer stem cells from mouse cell fusion *in vivo*. In 1995, Gibson *et al* (22) observed spontaneous mouse heterotypic cell fusion *in vivo*, and in 2013, Goldenberg *et al* (23) reported cell-cell fusion of human lymphoma and rodent host cells *in vivo*.

In recent years, increasing evidence of cell-cell fusion and their underlying mechanisms have been reported (Table II; Fig. 1). In 2016, the interaction between the sperm protein Izumo sperm-egg fusion 1 and egg the protein IZUMO1 receptor, JUNO was revealed to mediate mouse fertilization (24). In 2017, cell-cell fusion was demonstrated to be mediated by cell division cycle 42 pseudogene 1-Fus2p and spectraplakin-EFF-1 interactions in yeast and *C. elegans*, respectively (25,26). In 2017, the myomaker, a myoblast fusion actor gene, was reported to be involved in cell-cell fusion, leading to Carey-Fineman-Ziter syndrome (27), and in 2017, lipid raft-associated stomatin was reported to form a molecular assembly that promoted membrane fusion (28).

## 3. Cell-cell fusion *in vitro* in cancer

A tumor is formed by the continuous proliferation of transformed cells, and may progress to become more carcinogenic through continuous evolution. Abnormal proliferation of non-physiological fusion cells in multicellular organisms may be one of the causes of tumor formation and progression. There is a considerable body of knowledge supporting the occurrence of spontaneous cell-cell fusion *in vitro* in cell cultures between cancerous and other cell types as demonstrated in Table III and Fig. 2. These studies have investigated the fusion of cancer cells with endothelial cells, BMDCs and epithelial cells.

Endothelial cells line the inner side of blood and lymphatic vessels, and cancer cells must cross this barrier to gain access to the circulation, and cross again to exit and metastasize. Fusions between cancerous and endothelial cells were revealed to occur *in vitro* in co-cultures of human breast cancer cells and endothelial cells (29). These observations demonstrated a novel type of cancer-endothelial cell interaction, which may be of fundamental importance in the process of metastasis (29). Song *et al* (30) demonstrated that the oral cancer cell line SCC9 could spontaneously fuse with co-cultured endothelial cells, and the resultant hybrid cells exhibited continuous division and proliferation following re-plating and thawing. Such hybrids express markers of both of the parental cells, and undergo nuclear fusion, resulting in the acquisition of novel properties, enhanced drug resistance and improved survival potential. The hybrid cells comprised a significant portion of the tumor composition as demonstrated by immunostaining and FISH analysis, even though the hybrid cells and SCC9 cells were inoculated with a ratio of 1:10,000 cells (30). These experimental findings provided further evidence supporting the hypothesis that cell fusion may be involved in cancer progression (31,32).

Human BMDCs, including embryonic stem cells (ESCs), hematopoietic stem cells (HSCs), mesenchymal stem cells (MSCs), macrophages and dendritic cells (DCs) have been reported to fuse *in vitro* with various types of cancer cells spontaneously (Table III; Fig. 2). Spontaneous *in vitro* formation of heterotypic hybrids was revealed to occur between human bone marrow-derived multipotent stromal cells and two different breast cancer cell lines, MDA-MB-231 and MA11 cells (33). The resultant fused cells formed of hepatocellular carcinoma cells and hESCs, expressed both cancer and stemness markers and exhibited increased drug resistance and enhanced tumorigenesis (34). MSCs and breast cancer cell fusion resulted in hybrids with enhanced migratory capacity, which promoted breast cancer metastasis (35,36). In 2019, it was demonstrated that actin cytoskeletal components served an important role in the cell fusion between breast cancer cells and MSCs (37). Cell fusion between lung cancer cells and MSCs provided a non-mutation-dependent mechanism that contributed to the aberrant gene expression patterns, and gave

Table I. Milestones in cell-cell fusion.

| Author(s), year | Cell-cell fusion | (Refs.) |
|---|---|---|
| Forkner, 1930 | Pulmonary tuberculosis, smallpox, varicella, measles, and rabbit homotypic cell fusion *in vivo* | (11) |
| Enders and Peebles, 1954 | Human multinucleated giant cells or syncytia were formed *in vitro* | (12) |
| Barski, 1961 | Somatic cell fusion was observed in tissue culture | (13) |
| Furusawa and Cutting, 1962 | A hemagglutinating virus induced mouse Ehrlick ascites tumor cell fusion *in vitro* | (14) |
| Cascardo and Karzon, 1965 | Inactivated virus induced human cell fusion *in vitro* | (15) |
| Harris and Watkins, 1965 | Cell fusion between human and mouse cells *in vitro* | (16) |
| Goldenberg, 1968 | Human tumor and normal animal cell fusion *in vivo* | (17) |
| Poste, 1970 | Polykaryocyte was found | (18) |
| Goldenberg *et al*, 1974 | Fusion between transplanted human cancer cells and normal hamster cells *in vivo* | (19) |
| Klein *et al*, 1984 | Spontaneous fusion between mouse melanoma cells *in vitro* | (20) |
| Lapidot *et al*, 1994 | Cancer stem cells generated by mouse cell fusion *in vivo* | (21) |
| Gibson *et al*, 1995 | Spontaneous mouse heterotypic cell fusion *in vivo* | (22) |
| Mohler *et al*, 2002 | The gene eff-1 was essential for developmental cell fusion | (9) |
| Goldenberg *et al*, 2013 | Cell fusion between human lymphoma and rodent cells *in vivo* | (23) |

Table II. More recent important discoveries in cell-cell fusion.

| Author(s), year | Species | Cell 1 | Cell 2 | Evidence | Function | Mechanism | (Refs.) |
|---|---|---|---|---|---|---|---|
| Kato *et al*, 2016 | Mouse | Egg | Sperm | Sperm-egg fusion assay | Fertilization | Sperm Izumo sperm-egg fusion 1 and egg IZUMO1 receptor, JUNO | (24) |
| Smith *et al*, 2017 | Yeast | Yeast | Yeast | Yeast mating assays | Fertilization | Cell division cycle 42 pseudogene 1-Fus2p interaction | (25) |
| Yang *et al*, 2017 | *C. elegans* | Seam cell | Hyp7 cell | Live cell imaging in *C. elegans* embryo and larvae | | Spectraplakin links EFF-1 to the actin cytoskeleton | (26) |
| Di Gioia *et al*, 2017 | Human and mouse | Human myoblast | Mouse C2C12 cells | Cell fusion assay *in vitro* and allelic comple mentation *in vivo* | Carey-Fineman-Ziter syndrome | Myomaker, myoblast fusion factor | (27) |
| Lee *et al*, 2017 | Human and hamster | Human embryo kidney 293T cells | Hamster ovary K1 cells | Western blot, immunofluores cence staining, flow cytometry | | Lipid raft-associated stomatin | (28) |

rise to highly malignant subpopulations with epithelial-mesenchymal transition (EMT) and TSC-like properties (38). Cell fusion between hMSCs and gastric cancer cells may contribute to the generation of tumorigenic hybrids, with EMT and TSC-like properties (39). The spontaneous *in vitro* fusion of mouse hMSCs and human SU3 glioma stem/progenitor cells is one of the driving factors for glioma neovascularization (40). Cell fusion between MSCs and lung cancer cells enhanced the metastatic capacity and characteristics of cancer stem cells by undergoing EMT (41). The hybrid cells that were formed of human liver cancer cells and mouse MSCs exhibited increased expression of E-cadherin, vimentin, twist, snail, and matrix metalloproteinase 2 and 9, were aneuploid, possessed enhanced invasive and migratory capacities and generated an increased number of metastatic liver and lung lesions (42). In 2020, prostate cancer cells were revealed to exhibit characteristics associated with neuroendocrine function and heterogeneity following fusion with bystander neural stem cells in the tumor

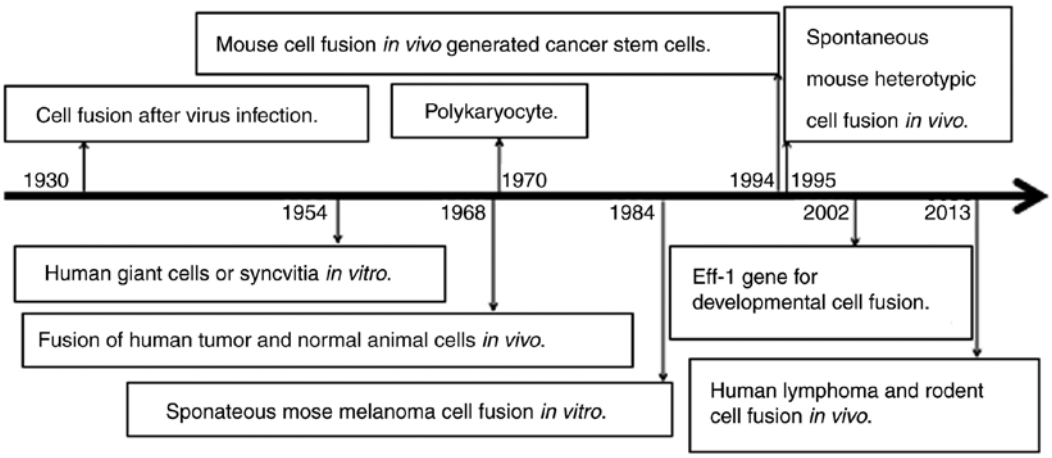


Figure 1. Milestones in the study of cell-cell fusion.

microenvironment (43). Macrophages serve an important role during the development of cancer, such as in breast cancer and melanoma (44). Macrophage-breast cancer cell hybrids become more proliferative and invasive as they undergo EMT and following increased activity of the Wnt/β-catenin signaling pathway (45), and may also acquire TSC properties (46). Fusion between cancer cells and macrophages generates metastatic hybrids with genetic and phenotypic characteristics of both maternal cells. Fusion hybrids of macrophages and melanoma cells exhibited upregulated expression of N-acety lglucosaminyltransferase V, β1-6 branching and were metastatic (47). Melanoma-peritumoral stromal cell fusion may assist in explaining the high rate of recurrence of melanomas in patients following removal of the primary tumors (48,49). Macrophage-cancer cell fusion was reported to generate a subpopulation of radiotherapy-resistant cells with enhanced DNA-repair capacity (50).

However, it is not always the case that fusion cells will exhibit increased tumorigenicity or TSC-like properties. He *et al* (51) reported in 2017 that hESCs and ovarian cancer cells can fuse *in vitro* spontaneously, and the fused cells interestingly exhibited epigenetic changes that led to inhibition of growth, which may provide a novel direction for the treatment of ovarian cancer. Although cell fusion between BMDCs and somatic cells may be the origin of TSCs, the hybrid cells that form as a result of the fusion of human HSCs and esophageal carcinoma cells did not generate esophageal TSCs (52,53). DC-cancer cell fusion vaccines are an attractive modality for the treatment of several types of cancers, such as prostate, liver, gastric, colorectal, lung and breast cancer (54-63). The cytotoxic T chemokine interferon-induced protein-10 was demonstrated to enhance the antitumor effects of DC/tumor cell fusion vaccines by alleviating the immunosuppressive tumor environment (64).

In addition, cancer cells can fuse with normal epithelial cells. The hybrid cells derived from the spontaneous fusion between the breast epithelial cell line M13SV1-EGFP-Neo and two breast cancer cell lines, HS578T-Hyg and MDA-MB-435-Hyg, both exhibited increased migratory capacity and increased drug resistance towards chemotherapeutic drugs, such as doxorubicin and paclitaxel. This finding further supported the hypothesis that cell fusion may give rise to drug resistant and metastatic cells (65). Human breast cancer cells and breast epithelial cell fusion was observed and verified using a Cre-loxP-based double fluorescence reporter system (35,66). The fusion between human breast epithelial cells and breast cancer cells gave rise to hybrid cells that possessed certain TSC or tumor initiating cell-like properties, indicating that cell fusion may be a mechanism underlying how tumor cells come to acquire a TSC phenotype (67). Additionally, the fusion of senescent human prostate epithelial cells and cancer cells was reported to promote tumor development in prostate cancer (68).

## 4. Cell-cell fusion *in vivo* in cancer

Tumor cells may fuse with several different types of cells, including stromal cells, epithelial cells and endothelial cells *in vivo*. Cell-cell fusion *in vivo* provides more convincing evidence of the involvement of this process in cancer development and progression than cell-cell fusion *in vitro*. However, providing direct evidence of cell-cell fusion at the DNA level is considerably more difficult, particularly for human cell-human cell fusions *in vivo*. There are >30 reports of cell-cell fusions *in vivo* between tumor cells and normal cells, in most of which, macrophages or other BMDCs are a component cell of the fusion (Table IV; Fig. 2) (50). These reports primarily revealed cell fusion between mouse-mouse cells or human-mouse cells, with only a few reports demonstrating fusion between human-human cells.

Mouse malignant cells were reported to fuse *in vivo* spontaneously with normal mouse cells. For example, spontaneous cell fusion *in vivo* was demonstrated between the mouse sarcoma cell line, MDW4, and normal mouse host cells, through the co-expression of their different major histocompatibility complex antigens in the fusion cells (69). In another example, mouse melanoma cells were revealed to fuse spontaneously *in vivo* with mouse host cells, and the fusion cells were indicated to serve an initiating mechanism for melanoma lung metastasis (70). A BALB/c nude mouse is an albino mouse with a tyrosine protein kinase homozygous mutation (c/c), which is a rate-limiting enzyme in the formation of melanin. Although the malignant melanoma cells transplanted into the mice were able to produce wild-type

Table III. Cell-cell fusion between cancer and other cells *in vitro*.

| Author(s), year | Species | Cancer | Other cell involved | Method | Mechanism | (Refs.) |
|---|---|---|---|---|---|---|
| Mortensen *et al*, 2004 | Human | Breast cancer | Endothelial cells | Cell culture, ICC, FISH. | None | (29) |
| Song *et al*, 2014 | Human | Oral cancer | Endothelial cells | Cell fusion assays, block assay, IHC, ICC, FC | TNF-α , VCAM-1/VLA-4 | (30) |
| Rappa *et al*, 2012 | Human | Breast cancer | Stromal cells | Viral vectors, wound-healing assay, invasion assays, implantation, gene expression, FM. | None | (33) |
| Dittmar *et al*, 2011 | Human | Breast cancer | Epithelial cells | Cell co-culture, short-tandem-repeat analysis, RT-qPCR, FC, cytotoxicity assay | None | (65) |
| Ozel *et al*, 2012 | Human | Breast cancer | Epithelial cells | FC, cell migration, WB | AKT, RAF-1-MAPKp42/44 | (66) |
| Mortensen *et al*, 2004 | Human | Breast cancer | Epithelial cells | A fluorescence double reporter vector, Cre transduction, blocking experiments | TNF-α, hypoxia | (29) |
| Bhatia *et al*, 2008 | Human | Prostate cancer | Epithelial cells | Retroviral vector, prospective cell-fusion, tumorigenicity assay, WT, IF, RT-qPCR | p16, p53, hTERT | (68) |
| He *et al*, 2016 | Human | Ovarian cancer | Embryonic stem cell | Fusion experiment, RT-PCR, WT, mouse model, cell growth | Suppressing p53 and PTEN | (51) |
| Wang *et al*, 2016 | Human | Hepatocellular carcinoma | Stem cells | Single-cell fusion technique, RT-qPCR, FC, tumorigenicity assay | Unknown | (34) |
| Noubissi *et al*, 2015 | Human | Breast cancer | MSCs | BiFC, coculture experiments, apoptotic and hypoxic treatment, annexin V apoptosis assay | Hypoxia-induced apoptosis stimulates fusion | (35) |
| Melzer *et al*, 2019 | Human | Breast cancer | MSCs | Cell culture, FC, cell cycle analysis, RT-qPCR, mass spectrometry | Actin cytoskeletal components | (37) |
| Wang *et al*, 2012 | Human | Esophageal carcinoma | Umbilical cord, MSCs | Xenograft assays, transfection, WT | DUSP6/MKP3 increased MAPK | (52) |
| Fan and Lu, 2014 | Human | Esophageal carcinoma | Bone hemopoietic stem cells | Cell fusion experiment | None | (53) |
| Xu *et al*, 2014 | Human | Lung cancer (HCC827) | MSCs | Co-culture, migration and invasion assays, FC and cell sorting of heterotypic hybrids, IF, RT-qPCR | EMT increased stemness of tumorigenic hybrids. | (38) |
| Sun *et al*, 2015 | Human | Glioma | BMSCs | Tube formation assay of the fused cells, ICC, IHC | None | (40) |
| Li *et al*, 2014 | Human | Liver cancer | BMSCs | Cell culture, chromosome analysis, cell invasion and migration assays, WT | None | (42) |
| Yin *et al*, 2020 | Human | Prostate cancer | Neural stem cell | Neural differentiation, Cell proliferation in 3-D, species-specific PCR, WB, IF | Tumor cell heterogeneity | (43) |
| Wang *et al*, 2017 | Human | Melanoma | Macrophages | Polyethylene glycol induced fusion | None | (44) |
| Ding *et al*, 2012 | Human | Breast cancer | Macrophages | IHC, cell tracker dye staining, PEG-mediated cell fusion, mammosphere formation assay, FC | None | (46) |

Table III. Continued.

| Author(s), year | Species | Cancer | Other cell involved | Method | Mechanism | (Refs.) |
|---|---|---|---|---|---|---|
| Chakraborty *et al*, 2001 | Human | Melanoma | Macrophages | | GnT-V and β1,6-branching enhanced in glycoproteins | (47) |
| Kemény *et al*, 2016; Kurgyis *et al*, 2016 | Human | Melanoma | Macrophages | Cell culture, detection of spontaneous cell fusion, fluorescent live cell imaging, IF | None | (48,49) |
| Xue *et al*, 2015 | Human | Gastric cancer | MSCs | The hybrids were generated by using PEG1500, RT-PCR, IF | None | (39) |
| Lindström *et al*, 2017 | Human | Breast cancer | Macrophages | Cell culture, cell fusion, radiation, clonogenic assay | None | (50) |
| Gauck *et al*, 2017 | Human | Breast cancer | Breast epithelial cell | Mammosphere-formation, karyotype analysis, cell morphology, cell migration | None | (67) |
| Liu *et al*, 2018 | Human | Cervical cancer | T effector cells | Cell fusion assay and RT-qPCR | microRNA-181 enhances HeV F-/G-mediated cell fusion | (94) |
| Song *et al*, 2012 | Human | Oral squamous carcinoma | Endothelial cells | Cell co-culture, RT-qPCR,, IHC, block and enhance assay | Wnt/β-catenin- syncytin-1 contributed to TNF-α-enhanced fusion | (32) |
| Yoo *et al*, 2010 | Human | Mel-DSP2 cell | CHO-DSP1 cell | Stable reporter fusion assay, syncytium morphology assay, confocal microscopy, immunoprecipitation, WT | VZV gB/gH-gL mediated cell-cell fusion | (55) |
| Kawada *et al*, 2003 | Human | Cervical cancer | 293T cells | Cell-cell fusion assays, pseudotyped virus entry assay, crystallization and structure determination | Hexamer-of-trimer interfaces enhance cell-cell fusion | (56) |
| Chakraborty *et al*, 2000 | Hamster | Melanoma | CHO-K1 Cre cells | Cell-cell fusion assay, quantitative cre reporter assay | gB modulated cell fusion via an ITIM-mediated Y881 phosphorylation | (70) |
| Melzer *et al*, 2018 | Mouse | Breast cancer | MSC | Cell-cell fusion assay | None | (36) |
| Zhang *et al*, 2019 | Mouse | Lung cancer | MSCs | Karyotyping, RT-qPCR, WB, cell proliferation, colony formation, DNA ploidy, wound healing, Transwell migration and invasion, xenograft, ICC | Cancer metastasis and cancer stem cell features | (41) |
| Hu *et al*, 2017 | Mouse | Hepatocellular carcinoma | Dendritic cells | IF, ELISA, FC, histology, IHC | MIP-10 alleviated immunosuppressive tumor environment | (64) |
| Zhang *et al*, 2019 | Murine | Breast cancer | Macrophage | Cell fusion, RT-qPCR, WB, CCK-8 assay | Cancer proliferation, migration and invasion | (45) |

FC, flow cytometry; FISH, fluorescent *in situ* hybridization; IF, immunofluorescence; FM, fluorescence microscopy; ICC, immunocytochemistry; TNF-α, tumor necrosis factor-α; WB, western blotting; CCK-8, Cell Counting Kit-8; RT-qPCR, reverse transcription-quantitative PCR; MSC, mesenchymal stem cell; BMSC, bone marrow-derived stem cell.

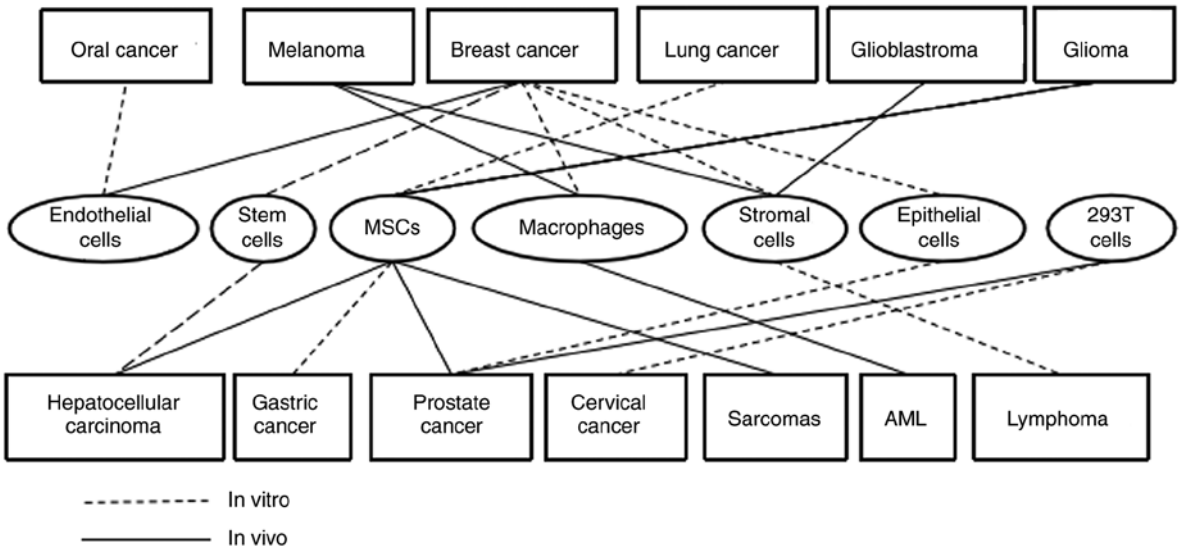


Figure 2. Cell-cell fusion between cancerous cells and other cells *in vitro* and *in vivo*. AML, acute myelogenous leukemia; MSC, mesenchymal stem cell.

tyrosine kinase (C/C), the resulting tumors produced little or no melanin and became pigment-free. Although metastases occurred frequently in these mice, the tumors were small, had no pigment in the lungs and could be tolerated by the mice. In one mouse, however, a tumor that produced melanin was generated near the site of the implant, in the tail dermis. The tail of the mouse was cut off and was observed to ascertain if there were any distant metastases. After 5 weeks, the mice began to die, and there was considerable pigment transfer in the lungs. DNA analysis revealed that the metastatic cells had a C/c phenotype, indicating that they were fused and derived from the fusion of the transplanted tumor and host cells. The DNA content of the cells derived from the metastatic foci increased by 30-40%, chemotaxis was enhanced *in vitro*, and the activity and expression of tyrosinase was increased. Additionally, it also produced large melanin granules and exhibited autophagy, which included the formation of melanosomes (70). Histopathological analysis of the site of origin indicated that the mice exhibited macrophage infiltration, which may support the possibility of fusion between melanoma tumor cells and macrophages. Recently, mouse bone marrow MSC and mouse prostate cancer cell fusion *in vivo* was reported, which may serve a role in promoting cancer progression (71). In 2019, mouse MSCs were revealed to fuse with glioma stem cells, and the hybrids exhibited enhanced angiogenic effects compared with the parental glioma cells both *in vivo* and *in vitro* (72).

Human lymphoma cells were reported to fuse *in vivo* with hamster stromal cells, and this was one of the first reports of *in vivo* cell-cell fusion of human tumor cells with a rodent host cell, indicating that the horizontal transfer of tumor DNA to adjacent stromal cells may be implicated in tumor heterogeneity and progression. The hybrid xenografts had a gene signature of B-cell malignancy (23). Synkaryons were formed in the solid tumor by spontaneous fusion between the malignant human breast epithelium and the surrounding normal mouse stroma. The transformed hybrid cells were tumorigenic with histopathological features of malignancy, indicating a novel mechanism for tumor progression (73), and the breast cancer progressed with cancer cell heterogeneity and generated invasive and metastatic breast cancer cells within the populations of non-metastatic cells in the primary tumor. In addition, the fusion of human acute leukemia cells with rodent macrophages may be a mechanism of gene transfer for cancer dissemination, and the fused cells may be used to identify, as of yet, unrecognized leukemogenic genes that are conserved in the hybrid cells and are able to perpetuate leukemia *in vivo* (74). Human breast cancer cells spontaneously fuse with mouse endothelial cells resulting in viable and actively dividing hybrid cells, which exhibit an enhanced capability to traverse the endothelial barrier and metastasize (29). Human breast cancer cells were also revealed to fuse with mouse MSCs spontaneously *in vivo,* and a significantly higher number of hybrids resided in the metastatic tumors compared with the primary tumors, supporting the possibility that hybrids can emerge from the primary tumors and become metastatic (75).

However, due to the lack of specific DNA markers of both fusion partner cells, the direct evidence of human-human cell fusion *in vivo* remains lacking. Human-human cell fusion *in vivo* was reported between human cancer cells and human BMDCs (76,77). Studies have demonstrated the presence of donor cell genes in recipient malignant cells after bone marrow transplantation (BMT), supporting the possibility of donor-recipient cell fusion *in vivo* (77). In a previous study, donor DNA was detected in the recipient tumors by continued genetic analysis of renal cell carcinoma specimens from allogeneic BMT patients who developed secondary malignancies (78). Donor DNA was analyzed by laser capture microdissection of the tumor cells followed by PCR. In another study, patients receiving radiotherapy and immunosuppression prior to transplantation increased the likelihood of recurrence of the tumors and the donor BMDCs were found in the tumors of the patients (76). Other researchers discovered that early papillary renal cell carcinoma originated from male to female HSC transplantation, and showed trisomy 17 characteristics, which is common in early stage renal cell carcinoma and other types of tumors; ~1% of trisomy 17 of the tumor cells also contained a Y chromosome. It is worth noting

Table IV. Cell-cell fusion between cancerous cells and other cells *in vivo*.

| Author(s), year | Species | Partner cell | Cancer cell | Method | Mechanisms | (Refs.) |
|---|---|---|---|---|---|---|
| Goldenberg *et al*, 2013 | Human | Stroma | Lymphoma | BMT, FISH, PCR, IHC | Tumor heterogeneity and progression | (23) |
| Pawelek and Chakraborty, 2008; Harkness *et al*, 2013 | Human | BMDCs | Renal cell carcinoma | BMT | Tumor metastasis and recurrence | (76,77) |
| Lazova *et al*, 2013; LaBerge *et al*, 2017 | Human | BMDCs | Melanoma | BMT, PCR, forensic genetic analyses of STR loci, allelic stutter | Tumor metastasis | (80,81) |
| Andersen *et al*, 2007 | Human | Osteoclasts | Myeloma | Cell culture, histology, TUNEL assay, microscopy, BrdU labelling | None | (82) |
| Chakraborty *et al*, 2004 | Human | Hematopoietic stem cells | Renal cell carcinoma | Allogeneic liver and BMT | Cancer progression | (78) |
| Clawson *et al*, 2015 | Human | Macrophages | Melanoma | Xenograft, IF, 3D confocal microscopy, live cell microscopy | Fusion cells at the periphery of primary tumors became metastasis initiating cells. | (83) |
| Kurgyis *et al*, 2016 | Human | Stromal cells | Melanoma | Laser-capture microdissection and DNA mutation | CXCR4, CD44 | (49) |
| Melzer *et al*, 2019 | Human | MSCs | Breast cancer | Cell culture, mouse experiments, FC, RT-PCR | Tumor heterogeneity | (84) |
| Martin-Padura *et al*, 2012 | Human and mouse | Macrophages (mouse) | Acute myeloid leukemia (Human) | Mouse and human leukemia transplants, IF, PCR, IHC, FACS | None | (74) |
| Goldenberg *et al*, 2012 | Human and hamster | Stromal cells (hamster) | Glioblastoma (Human) | Transplantation, FISH, PCR, IHC | None | (91) |
| Mortensen *et al*, 2004 | Human and mouse | Mouse endothelial cells | Human breast cancer | Cell culture, FISH, IHC | None | (29) |
| Chitwood *et al*, 2018 | Human and mouse | Mouse MSCs | Human breast cancer | Cell culture, RNA-Seq, Flox-luc mice, hematoxylin and eosin staining, IF, qPCR | Cancer proliferation and metastases | (75) |
| Luo *et al*, 2016 | Mouse | BMSCs | Prostate cancer | BMT, IHC, IF, RT-qPCR, FC | None | (71) |
| Chakraborty *et al*, 2000 | Mouse | Lung | Melanoma | Migration assay, FC of DNA content, histology, DNA sequencing, WT | Metastasis | (70) |
| Kerbel *et al*, 1983 | Mouse | MSCs | Sarcomas | Cell culture, chromosome analysis, serology, cytotoxic T-cell H-2 antigens typing | Metastasis | (69) |
| Jacobsen *et al*, 2006 | Mouse | Stroma | Breast cancer | Cell culture, IHC karyotyping, xenografts | None | (73) |
| Sun *et al*, 2019 | Mouse | BMSC | Glioma | A dual-color fluorescent protein tracer model, RT-qPCR, WB, ICC, IHC, tube formation, tumorigenicity | Angiogenic effect | (72) |

BMT, bone-marrow transplantation; FC, Flow cytometry; FISH, fluorescent *in situ* hybridization; IF, immunofluorescence; FM, Fluorescence microscopy; ICC, Immunocytochemistry; WB, western blotting; RT-qPCR, reverse transcription-quantitative PCR; MSC, mesenchymal stem cell; BMSC, bone marrow-derived stem cell; BMDC, bone marrow derived cell.

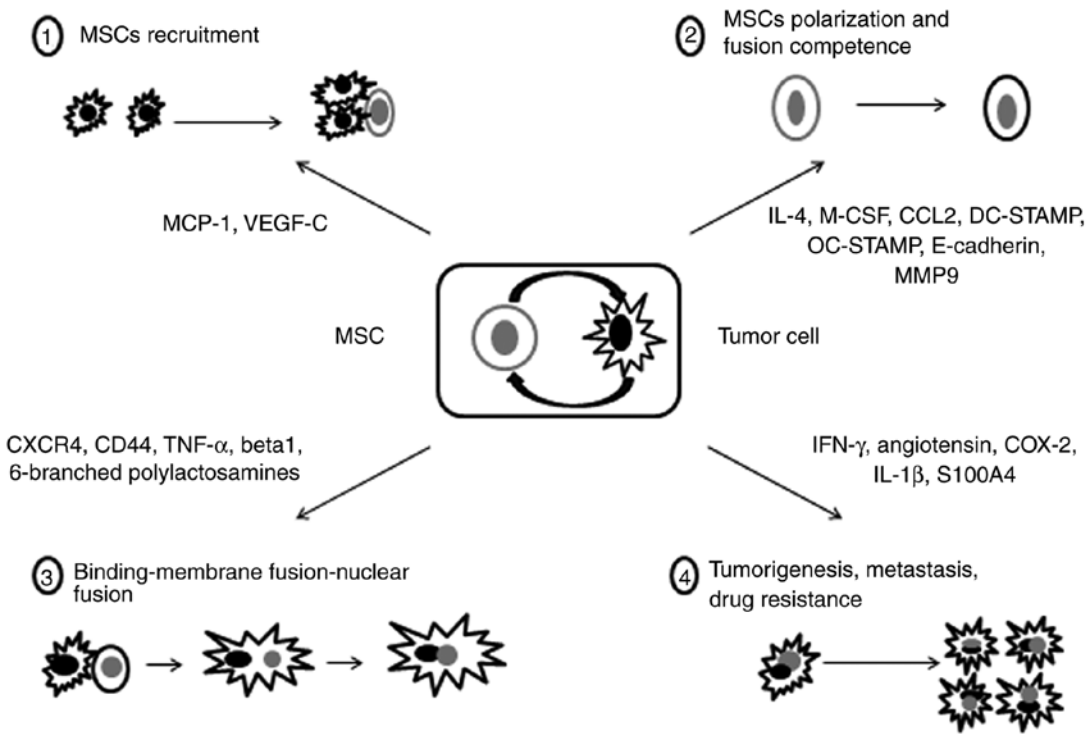


Figure 3. Cell-cell fusion steps and possible mechanisms in cancer. MSC, mesenchymal stem cell; TNF-α, tumor necrosis factor-α; IL, interleukin; IFN, interferon.

that Y chromosome-containing and chromosome 17 paired tumor cells clustered in the tumor during mitotic anaphase. In addition, tumor cells containing the Y chromosome appeared in ~10% of the tumor cells, indicating clonal growth of these cells. As aforementioned, HSCs were associated with tumor cells. However, it is possible that the tumor cells originated from the donor HSCs alone, that no fusion had occurred, and the Y chromosome was lost during tumor growth and proliferation (79). In another similar study, the tumor cells containing the Y chromosome were revealed in two patients with intestinal cancer and one patient with lung cancer who had previously received a male HSC transplant. The presence of XXY or XXXY chromosome phenotypes detected by XY fluorescence *in situ* hybridization analysis supported the notion that the tumors originated from a cancer cell-BMDC fusion (77). The first and second pieces of convincing evidence of human cell-human cell fusions *in vivo* came from the detection of a short tandem repeat of parental cell alleles (80,81). Both donor and recipient DNA were detected in single cells of melanoma lymph-node and brain metastases from sex mismatched BMT female cancer patients.

Potential human-human cell fusion was reported *in vivo* between malignant cells and macrophages. Potential fusion cells may originate through spontaneous fusion *in vivo* between human myeloma cells and human osteoclasts, as supported by the presence of chromosomal translocations specific for the myeloma cells in the osteoclast nuclei of patients with myeloma (82). Osteoclast-myeloma hybrids reflect a previously unrecognized mechanism of bone destruction. Transcriptional activation of both malignant and normal nuclei was observed in the tumor-associated osteoclasts derived from the patients with melanoma. In these osteoclasts, 30% of the nuclei were derived from the malignant cells. In a previous study, potential fusion cells of human melanoma cells and human macrophages were reported in the peripheral blood of patients with cutaneous melanomas, and they possessed the ability to form metastatic lesions when transplanted into mice (83). The researchers isolated and cultured the circulating tumor cells from the patients with melanoma and termed them fusions of macrophages with tumor cells (MTFs). They discovered that MTFs exhibited a macrophage-like appearance, but contained melanosomes. MTFs also expressed pan-macrophage and M2-macrophage markers (such as CD14 and CD68, as well as CD163, CD204 and CD206, respectively), melanocyte-specific markers (activated leukocyte cell adhesion molecule and melan-A), epithelial biomarkers (keratin 1 and epithelial cell adhesion molecule), the pro-carcinogenic cytokine macrophage migration inhibitory factor, and cancer stem cell markers [C-X-C motif chemokine receptor 4 (CXCR4) and CD44]. They also demonstrated that $5x10^5$ cultured human melanoma MTFs could induce the formation of metastatic tumors when subcutaneously injected into nude mice. The melanoma-derived BRAF (V600E) mutation was also detected in the micro-dissected peritumoral stromal cells of patients, indicating the occurrence of a potential *in vivo* fusion between human melanoma cells and human stromal cells (49). These potential hybrid cells display the phenotype of stromal cells and are therefore undetectable during routine histological assessments. In 2019, the *in vivo* fusion between human breast cancer cells and human MSCs was also found when co-injected in mice, and this fusion increased tumor heterogeneity (84).

## 5. Mechanisms of cell-cell fusion in cancer

Cell-cell fusion in cancer may involve several steps (Fig. 3). Here, MSCs are used as an example to illustrate the different

steps of fusion with a cancer cell. The first step of cell-cell fusion includes the recruitment of an MSC to the tumor microenvironment through tumor-secreted cytokines, such as C-C motif chemokine ligand 2 and VEGF-C (85-87). The MSC then undergoes polarization and acquires a competent phenotype, which is followed by the binding of the fusion partners, cell membrane and cytoplasm fusion and then nuclei fusion. During these processes, cytokines, such as β1,6-branched polylactosamines, CXCR4, CD44 and TNF-α serve important roles (88-91). The fused cells may promote tumorigenesis, metastasis and drug resistance by releasing cytokines, such as IFN-γ, angiotensin, COX-2, IL-1β and S100A4 (92,93).

Importantly, *in vitro* studies on virus-cell fusion and cell-cell fusion between cancer and other cells have provided a tool to understand the mechanisms of cell-cell fusion. Glycoprotein B (gB) of VZV was reported to serve a role in cell-cell fusion (94). Strict regulation of VZV gB/gH-gL-mediated cell-cell fusion between Mel-DSP2 cells and CHO-DSP1 cells through gBcyt and gHcyt was revealed to be required for effective viral propagation (55). The identification of the role of the gB lysine cluster in cell-cell fusion regulation revealed the molecular mechanisms that govern VZV syncytium formation during infection (55). Hexamer-of-trimers assembly of gB was important during fusion pore formation in both cell-cell fusion and virus-cell fusion systems (56). gB-modulated melanoma and CHO-K1 cell fusion was mediated via a T-cell immunoreceptor with Ig and ITIM domain-mediated Y881 phosphorylation-dependent mechanism, supporting a unique concept that intracellular signaling through this gBcyt motif regulates VZV syncytia formation and is essential for skin cancer pathogenesis (57). MicroRNA-181 was demonstrated to suppress ephrin receptors that negatively regulate henipavirus glycoprotein-mediated cell-cell fusion (95). CXCR4 was identified as a key fusion gene involved in cell-cell fusion. Hu *et al* (96) reported that urine-derived stem cells could fuse with different types of liver cells by upregulating CXCR4 expression during liver tissue recovery, following injury. Fusions of human melanoma cells and human macrophages were reported in the peripheral blood of patients with cutaneous melanomas, and they possessed the ability to form metastatic lesions when transplanted into mice, as cultured human melanoma fusion cells could induce metastatic tumors when subcutaneously injected into nude mice (83). In addition, other signaling pathways, such as the Wnt/β-catenin pathway may serve a role in cell-cell fusion in cancer. The Wnt/β-catenin pathway activation-dependent upregulation of syncytin-1 was found to be involved in TNF-α-induced cell-cell fusion between oral cancer cells and endothelial cells (54). However, additional *in vivo* studies are required to determine the roles and mechanisms of cell fusion in tumor progression.

## 6. Conclusions

Cell-cell fusion *in vitro* is a recognized biological process, which occurs not only under physiological conditions, but also during tumorigenesis and tumor metastasis. In the present review, the important pro-tumorigenic and pro-metastatic roles of cell-cell fusion were discussed. It is hypothesized that cell-cell fusion is an important mechanism that enables tumor metastasis, and may be one of the primary causes of tumor metastasis in the majority of different types of cancer. In fact, cell-cell fusion has been targeted for cancer therapy; VSV-G-mediated neural stem cell-glioma cell fusion was induced *in vivo* as a form of glioma therapy (97). However, further probing the molecular and cellular mechanisms of cell fusion in the context of tumor progression may pave the way for the development of novel techniques for the treatment of cancer.

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## Availability of data and materials

Not applicable.

## Authors' contributions

HWX designed and supervised the study. YFL, YYW, YYM and XQL reviewed the references. XCP and MZ wrote the manuscript. WQC, YZ, XWW and ZWM contributed to tables and figures. YYM, YX, LSZ, LMY and SZC revised the manuscript. HWX and XCP acquired funding. All authors read and approved the final manuscript.

## Ethics approval and consent to participate

Not applicable.

## Patient consent for publication

Not applicable.

## Competing interests

The authors declare that they have no competing interests.

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