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

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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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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 an euploidy 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

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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

Author(s), year	Cell-cell fusion	(Refs.)
Forkner, 1930	Pulmonary tuberculosis, smallpox, varicella, measles, and rabbit homotypic cell fusion <i>in vivo</i>	(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 <i>et al</i> , 2016	Mouse	Egg	Sperm	Sperm-egg fusion assay	Fertilization	Sperm Izumo sperm-egg fusion 1 and egg IZUMO1 receptor, JUNO	(24)
Smith <i>et al</i> , 2017	Yeast	Yeast	Yeast	Yeast mating assays	Fertilization	Cell division cycle 42 pseudogene 1-Fus2p interaction	(25)
Yang <i>et al</i> , 2017	C. elegans	Seam cell	Hyp7 cell	Live cell imaging in <i>C. elegans</i> embryo and larvae		Spectraplakin links EFF-1 to the actin cytoskeleton	(26)
Di Gioia <i>et al</i> , 2017	Human and mouse	Human myoblast	Mouse C2C12 cells	Cell fusion assay <i>in vitro</i> and allelic comple mentation <i>in vivo</i>	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

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 <i>et al</i> , 2014	Human	Oral cancer	Endothelial cells	Cell fusion assays, block assay, IHC, ICC, FC	TNF- α , VCAM-1/VLA-4	(30)
Rappa <i>et al</i> , 2012	Human	Breast cancer	Stromal cells	Viral vectors, wound-healing assay, invasion assays, implantation, gene expression, FM.	None	(33)
Dittmar <i>et al</i> , 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	(99)
Mortensen et al, 2004	Human	Breast cancer	Epithelial cells	A fluorescence double reporter vector, Cre transduction, blocking experiments	TNF- α , hypoxia	(29)
Bhatia <i>et al</i> , 2008	Human	Prostate cancer	Epithelial cells	Retroviral vector, prospective cell-fusion, tumorigenicity assay, WT, IF, RT-qPCR	p16, p53, hTERT	(68)
He <i>et al</i> , 2016	Human	Ovarian cancer	Embryonic stem cell	Fusion experiment, RT-PCR, WT, mouse model, cell growth	Suppressing p53 and PTEN	(51)
Wang <i>et al</i> , 2016	Human	Hepatocellular carcinoma	Stem cells	Single-cell fusion technique, RT-qPCR, FC, tumorigenicity assay	Unknown	(34)
Noubissi <i>et al</i> , 2015	Human	Breast cancer	MSCs	BiFC, coculture experiments, apoptotic and hypoxic treatment, annexin V apoptosis assay	Hypoxia-induced apoptosis stimulates fusion	(35)
Melzer <i>et al</i> , 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 <i>et al</i> , 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 <i>et al</i> , 2014	Human	Liver cancer	BMSCs	Cell culture, chromosome analysis, cell invasion and migration assays, WT	None	(42)
Yin <i>et al</i> , 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. Cell-cell fusion between cancer and other cells in vitro.

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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 <i>et al</i> , 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 <i>et al</i> , 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 <i>et al</i> , 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 <i>et al</i> , 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 <i>et al</i> , 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	(0)
Melzer et al, 2018	Mouse	Breast cancer	MSC	Cell-cell fusion assay	None	(36)
Zhang <i>et al</i> , 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 <i>et al</i> , 2017	Mouse	Hepatocellular carcinoma	Dendritic cells	IF, ELISA, FC, histology, IHC	MIP-10 alleviated immunosuppressive tumor environment	(64)
Zhang <i>et al</i> , 2019	Murine	Breast cancer	Macrophage	Cell fusion, RT-qPCR, WB, CCK-8 assay	Cancer proliferation, migration and invasion	(45)

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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 cancere	ous cells and other c	ells 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 <i>et al</i> , 2013	Human	BMDCs	Renal cell carcinoma	BMT	Tumor metastasis and recurrence	(76,77)
Lazova <i>et al</i> , 2013; LaBerge <i>et al</i> , 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 <i>et al</i> , 2004	Human	Hematopoietic stem cells	Renal cell carcinoma	Allogeneic liver and BMT	Cancer progression	(78)
Clawson <i>et al</i> , 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 <i>et al</i> , 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 MSCs	Human breast	Cell culture, RNA-Seq, Flox-luc mice, hematoxvlin and eosin staining TF oPCR	Cancer proliferation and	(75)
Luo <i>et al</i> , 2016	Mouse	BMSCs	Prostate cancer	BMT, IHC, IF, RT-qPCR, FC	None	(71)
Chakraborty <i>et al</i> , 2000	Mouse	Lung	Melanoma	Migration assay, FC of DNA content, histology, DNA sequencing, WT	Metastasis	(20)
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 <i>et al</i> , 2019	Mouse	BMSC	Glioma	A dual-color fluorescent protein tracer model, RT-qPCR, WB, ICC, IHC, tube formation,	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.

tumorigenicity



Figure 3. Cell-cell fusion steps and possible mechanisms in cancer. MSC, mesenchymal stem cell; TNF-a, tumor necrosis factor-a; 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 $\sim 10\%$ 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, 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 5x10⁵ 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 β 1,6-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-1 β 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-a-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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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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