Cancer cell fusion: a potential target to tackle drug-resistant and metastatic cancer cells

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Cell fusion is an integral, established phenomenon underlying various physiological processes in the cell cycle. Although research in cancer metastasis has hypothesised numerous molecular mechanisms and signalling pathways responsible for invasion and metastasis, the origin and progression of metastatic cells within primary tumours remains unclear. Recently, the role of cancer cell fusion in cancer metastasis and development of multidrug resistance (MDR) in tumours has gained prominence. However, evidence remains lacking to justify the role of cell fusion in cancer metastasis and drug resistance. Here, we highlight plausible mechanisms governing cell fusion with different cell types in the tumour microenvironment (TME), the clinical relevance of cancer cell fusion, its potential as a target for overcoming MDR and inhibiting metastasis, and putative modes of treatment.

Introduction to cell fusion

Cell fusion is a normal biological process (occurring during physiological processes such as fertilization and tissue regeneration) that contributes to the genotypic and phenotypic cellular diversity of daughter cells that renders them distinct from their parent cells [1]. The discovery of cell fusion as a potential contributor to tumour proliferation can be traced back to the early 1900s, wherein Otto Aichel proposed that spontaneous fusion between somatic cells could trigger chromosomal anomalies and cancer [2]. Later, it was re-established by Mekler [3] and Goldenberg *et al.* [4] that aberrant heterotypic cell fusion has a putative role in the generation of immature cells, now assumed to be either by retrodifferentiation or transdifferentiation into other cells of blastodermic origin. This anomaly was thought to contribute to the initiation of tumour development and plausible imbibition of the metastatic phenotype. Despite its relevance, the emergence of oncogenic mutation theory during the late 1980s eclipsed the fusion theory in cancer progression for a considerable period of time [5]. With recent research results involving the comparative evaluation of a genome in a primary tumour cell and the corresponding metastatic tumour cell, it was evident that mutations have a minimal role in metastasis [6]. Such results have renewed focus on the fusion theory to facilitate a better understanding of the migration of tumour cells from the primary tumour to secondary loci [7]. In general, most of the cancers, including breast, melanoma, sarcoma, glioblastoma, renal cell carcinoma, and ovarian carcinoma, exhibit cell fusion [8].

Cell–cell fusion has been proposed as a potential mechanism contributing to tumour heterogeneity. Hybrid cells resulting from homotypic or heterotypic cancer cell fusion are endowed with features, such as rapid tumour growth, formation of cancer stem cells, resistance to anticancer agents and metastasis, which facilitate tumour proliferation relative to nonhybrid cells [1]. Cell fusion results in the formation of polyploid giant cancer cells (PGCCs), which are larger than normal cells and show cancer stem cell (CSC)-like properties. PGCCs exhibit features of markers of both normal cells and stem cells and are tumorigenic [9]. Binuclear and multinucleate cells are commonly observed in various tumour cells, and are attributed to accidental cell fusion

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[10]. The plausible contribution of spontaneous cell fusion in different types of cancer in both tissue cultures and animal models has been documented. Although there is evidence of cell fusion in experimental animal tumour models and malignant cell lines (Table 1) [11], the documented evidence of cell fusion in human cancer is relatively little. The detection of cell fusion in tissue cultures and animal models is relatively easy compared with its detection in human cancers owing to the dearth of genetic tracing tools [2]. The earliest reported case of cell fusion involved a female patient with renal cell carcinoma, who was a recipient of her son's allogeneic bone marrow. Karyotyping of the cancer cells demonstrated the fusion of the son's transplanted bone marrow cells with

TABLE 1

Evidence for cell fusion events

Type of cell fusion	*In vitro/in vivo* model	Mechanism	Refs
Trophoblast fusion	Choriocarcinoma trophoblastic BeWo cell line	Syncytin-1 and syncytin-2; cAMP/PKA and PKC-dependent mechanisms	[84]
Cell fusion step of osteoclastogenesis	Murine monocytic cell line RAW264.7	RANKL, TNF-α, lipopolysaccharide, and peptidoglycan-induced cell fusion of osteoclasts through their own receptors; subsequent activation of signalling pathways involving PI3K, Src, ERK, and JNK molecules required for cell fusion; although DC-STAMP is considered to be requisite for cell fusion of osteoclasts, cell fusion-inducing factors other than DC-STAMP might also be necessary	[85]
Macrophage fusion	Filters implanted intraperitoneally in wild-type and MCP-1–null mice	IL-4 induces activation of at least two pathways, including the JAK/STAT pathway (JAK1/JAK3 and STAT6), leading to induction of E-cadherin, β-catenin, MCP-1, TNF-α, and MMP-9	[86]
Macrophage fusion (formation of multinucleated giant cells)		Local microenvironment-induced cytokines and signals derived from bacteria, parasites, or other foreign materials; close contact of plasma membranes via adhesive factors; fusion of membranes mediated by specific interaction of unknown ligands on surface of one cell with specific receptors on its fusion partners; fusion might also be regulated by signals promoted by membrane proteins and intracellular signalling components	[87]
Fusion of epithelial cells	Madin-Darby canine kidney (MDCK) GII, MCF7, 3T3, and wild-type and E-cadherin-expressing (LE) L cells	Self-contacts of normal epithelial cells rapidly eliminated by membrane fusion between two opposing plasma membranes of a single cell, most likely via an E-cadherin and actin–myosin network-dependent mechanism	[88]
Human hematopoietic progenitor cells and murine cardiomyocytes	HL-1 mouse cardiomyocytes and transplanted human peripheral blood CD34-positive cells; female SCID mice subjected to experimentally induced myocardial infarction; human CD34-positive cells injected directly into the myocardium adjacent to infarct area	VCAM-1/a4b1 interaction, IL-6 and TNF- α and hypoxia-mediated cell fusion	[89]
Cancer cell–endothelial cell fusion	Breast cancer cells (MCF-7, MDA-MB-231), HUVECs, and calf pulmonary artery endothelial (CPAE) cells; tumours from 165 female patients with ductal breast cancers without axillary lymph node involvement fixed in 10% buffered formalin and embedded in paraffin	Syncytin, an endogenous retroviral envelope protein, causes cell fusion; endothelial and cancer cells express alanine, serine, and cysteine selective transporter 2 (ASCT2), a D-type retroviral receptor for syncytin	[90]
Cancer cell–endothelial cell augmentation	Human A375 M melanoma and two clones 2/4 and 2/60 derived from human melanoma metastasis (Mel 665/2); tumour cells subcutaneously injected into female NCr nu/nu (nude) mice	Inflammatory cytokine IL-1 promoted tumour cell adhesion to cultured human endothelial cells *in vitro* and augmented experimental lung metastases through VCAM-1/VLA-4 signalling	[91]
Cancer cell–macrophage hybrid	Cloudman S91 melanoma clone PS-1-HGPRT-1/G418res implanted subcutaneously in tail of BALB/c nu/nu mice	Ingestion of apoptotic tumour cells by macrophages	[92]
Cancer cell–normal hamster cell fusion	Human cancer cell line GB-749; part of astrocytic glioma from brain of 44-year-old female injected as cell suspension into cheek pouch of male golden hamster	Rearrangement of host chromosomes and influence of tumour or host-borne fusing agents caused cell fusion	[4]
Cancer cell–stem cell fusion	Hepatocellular carcinoma cell (HepG2) and a hESC; HepG2 cells and fused cells subcutaneously injected into right scapula of male athymic nude mice (BALB/c nu/nu)	Artificially engineered laser-induced single-cell fusion technology	[35]
Cancer cell–cancer cell fusion	Endometrial carcinoma (polyps) from patients	Human endogenous retrovirus HERV-W envelope gene Syncytin-1-dependent cell fusion	[78]

the mother's early cancer cells [12]. In addition, stealth melanoma hybrids have been detected in lymph node metastases of patients with cancer [13]. Similarly, blood of patients with melanoma, pancreatic, or colorectal cancers showed circulating tumour cells expressing carcinoma and leukocytic markers, indicating bone marrow-derived cell (BMDC)–cancer cell fusion [14,15].

In this review, we provide a brief overview of the mechanisms of cell fusion and its implications in phenomena such as tumour proliferation, increased drug resistance, tumour metastasis, and possible drug targeting.

Mechanisms of cell fusion

The fusogenicity of tumour cells with host cells is mediated by several factors. Davies *et al.* [16] proposed that chronic inflammation along with cellular proliferation, which is rampant in the TME [17], are strong drivers of cell fusion. The authors reported that intestinal inflammation and epithelial proliferation augment the fusion between BMDCs and intestinal progenitors. In their study, a bone marrow transplant was carried out in a mouse model of inflammation and promoted intestinal epithelial cell proliferation. [16]. Johansson *et al.* [18] reported that, in response to chronic inflammation, cell fusion events between hematopoietic cells and Purkinje neurons increased by 10- to 100-fold. Similar observations were reported by Kemp *et al.* [19], wherein BMDCs fused with Purkinje neurons *in vivo* to form binucleate heterokaryons with the morphology, soma size, and ability to synthesize the neurotransmitter gamma-aminobutyric acid; synaptic innervation of healthy Purkinje cells from neighbouring cells could repair the pathological changes in Purkinje cell structure, confirmed by electrophysiology. Recently, Mohr *et al.* [20] demonstrated that the proinflammatory cytokine tumour necrosis factor-α (TNF-α) is a strong inducer of cell fusion in human M13SV1-Cre breast epithelial cells and human MDA-MB-435-pFDR1 cancer cells under both normoxic and hypoxic conditions. Song *et al.* also showed that inflammation in the TME facilitates cell fusion. Inflammatory cytokine TNF-α enhanced the fusion of human umbilical vein endothelium cells (HUVEC) and oral cancer cells up to threefold *in vitro*. Vascular cell adhesion molecule-1 (VCAM-1) is a receptor on the surface of activated endothelial and mesothelial cells. It interacts with very late activation antigen 4 (VLA-4), which is found in leucocytes. Increased levels of VCAM-1 in the inflammatory TME are associated with the development of malignant tumours. TNF-α was also found to trigger expression of VCAM-1 in endothelial cells. Prior treatment of cells with anti-VCAM-1 or anti-VLA-4 inhibited the TNF-α augmented fusion between oral squamous cell carcinoma cells and endothelial cells [21]. Weiler *et al.* [22] established the pivotal role of matrix metalloproteinase-9 (MMP-9) in the TNF-α-mediated cell fusion of human M13SV1-Cre breast epithelial cells and human MDA-MB-435-pFDR1 cancer cells. Noubissi *et al.* [23] established that hypoxia-induced apoptosis provided cues to promote cell fusion between mesenchymal stem/multipotent stromal cells (MSCs) and T47Ds breast cancer cells.

Recently, another form of cell–cell interaction was reported, entosis, wherein a cell-in-cell structure is generated by the cannibalistic behaviour of one living cell against another living cell [24]. This is followed by loss of cell–matrix adhesion, cell division, or degradation of the target cell in the newly formed hybrid mediated by the Rho-ROCK-actin/myosin pathway and resulting in polyploidy, leading to tumour progression and, eventually, aneuploidy [25,26]. Balvan *et al.* [27] demonstrated that polyploidy in prostate tumours was associated with overexpression of pluripotency genes (*Nanog*, *Sox2*, and *Pou5F1*), resulting in increased developmental plasticity and adaptability to changes in the tumour extracellular environment and possible resistance development. Interestingly, Melzer *et al.* [28] demonstrated that *in vivo* co-culture of primary human MSCs with human SK-OV-3 ovarian cancer cells stimulated tumour growth compared with parental human SK-OV-3 ovarian cancer cells and liver metastases. However, on formation of hybrid cells of MSC-ovarian cancer cells, there was a marked reduction in cancer cell proliferation compared with the parental SK-OV-3 cells and, as a consequence, failure to develop tumours in NOD/SCID mice. This suggested that entosis is either tumorigenic or tumour suppressive depending on the cancer cells. In addition, dysregulation of the fusogenic protein syncytin in tumour cells is also implicated in the stimulation of cell fusion [29] (discussed in detail later).

Cell fusion in tumour proliferation

Fusion between tumour cells and stem cells has been widely identified as one of the mechanisms responsible for the generation of CSCs [30]. The stem cell fusion model focusses on the contribution of BMDCs, such as MSCs, to cell fusion. MSCs in the mesenchymal stromal cell environment are reported to have an important role in cancer progression. Inflammation is present in the local TME, and involves cytokines and chemokines. An array of MSCs derived from bone marrow are recruited to the tumour site via chemokine mediation. These MSCs are responsible for promoting lung carcinoma proliferation. Fusion of MSCs with tumour cells can confer tumour cells with both mesenchymal and stem cell-like features.

Xu *et al.* demonstrated spontaneous cell fusion between lung cancer cells and bone marrow-derived MSCs resulting in hybrids having enhanced tumorigenicity in NOD/SCID mice, enhanced formation of pneumospheres, and mesenchymal properties, as exemplified by increased vimentin and fibronectin expression [31]. Schwitalla *et al.* carried out a fusion of breast stem cells (M13SV1-EGFP-Neo breast stem cells) with breast cancer cell lines (HS578T-Hyg and MDA-MB-435-Hyg). Based on proliferation studies, the hybrid cells showed proliferation rates up to 1.5-fold quicker than parental breast cancer cells and up to tenfold quicker compared with the breast stem cell lines [32]. Wang *et al.* demonstrated fusion between hepatocellular carcinoma cells (HepG2) and human embryonic stem cells (hESCs) resulting in the fused cell exhibiting cancer-like properties. These fused cells were also found to be more tumorigenic. Gene expression analysis of the fused cells revealed their similarity to the tumour-initiating cells (TIC) or tumour stem cells and increased expression of TIC markers, such as CD133, CD44, and ALDH1. The study highlighted an important plausible risk associated with the use of stem cells for cancer treatment [33].

Xue *et al.* explored MSC-mediated cell fusion in the tumorigenesis of gastric cancer. Human umbilical cord mesenchymal stem cells were fused with gastric cancer cells by using polyethylene glycol (PEG) 1500 as a fusion inducer. The hybrids obtained showed epithelial-mesenchymal transition (EMT) and stem-like

characteristics with a growth rate that was greater than that of the parental gastric cancer cells. The hybrid cells also showed a decrease in E-cadherin expression, increase in expression of mesenchymal markers, such as vimentin and N-cadherin, and enhanced expression of the CSC surface markers, CD44 and CD133. Hybrid cells also showed an ability to promote gastric tumour growth *in vivo* in a mouse model [34]. Wang *et al.* demonstrated fusion between human bone marrow-MSCs and multiple myeloma cells using PEG 1000. The fusion caused polyploidy with increased expression of *Nanog*, Sox2, and *Oct4*, and the development of drug resistance [35].

Using PEG, Zhou *et al.* showed the ability of cell fusion to trigger oncogenesis in a mouse model. The authors used rat intestinal epithelial cells (IEC-6), which resembled healthy intestinal epithelial cells with respect to phenotype and function. The authors revealed that, after injection of 2 million cells from a pool of fused IEC-6 cells, into the flanks of immunodeficient mice, 11 out of 18 injections resulted in tumour generation. The authors isolated and cloned the hybrid cells after the fusion and demonstrated that the cell fusion caused aneuploidy, DNA damage, phenotype heterogeneity, and tumorigenesis. All these events were mediated immediately or after only a few cell divisions after cell fusion [36].

The stroma in the TME surrounding tumour cells has a pivotal role in tumour growth and proliferation. Fibroblasts are a cardinal component of the stromal environment. Many fibroblasts undergo differentiation to form carcinoma-associated fibroblasts or myofibroblasts. These myofibroblasts are endowed with features of both fibroblasts and smooth muscle cells. Myofibroblasts are important players in carcinogenesis because they are both producers and recipients of tumour-promoting factors. Yu *et al.* demonstrated cell fusion between myofibroblasts and cancer cells in osteosarcoma [29]. Rappa *et al.* showed spontaneous *in vitro* formation of hybrids between human bone marrow-derived MSCs and two breast carcinoma cell lines MDA-MB-231 and MA11. Hybrids had a mesenchymal morphology, mixed gene expression, and increased DNA ploidy. Both the hybrids showed tumorigenic potential in immunodeficient mice. Homotypic hybrid formation was also observed in MA11 and MDA-MB-231 cells [37].

Huang *et al.* investigated the relation between hypoxia in the TME and cell fusion and demonstrated that hypoxia facilitated fusion between oral squamous carcinoma cells and human immortalized oral epithelial cells via induction of EMT. The presence of *N*-[*N*-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine t-butyl ester (DAPT), which is an EMT blocker, drastically decreased the rate of fusion between oral squamous carcinoma cells and human immortalized oral epithelial cells [38].

Wang *et al.* demonstrated that fusion of prostate cancer cells with stromal cells contributed to androgen-independent prostate cancer progression. The study of the resultant hybrids revealed that fusion of prostate cancer cells with stromal cells had two diametrically opposite roles in tumour progression. Most cancer-stromal cell hybrids showed difficulty in undergoing cell division and died. However, a few hybrids survived and showed genomic modification and androgen independence [39]. Mercapide *et al.* investigated the fusogenicity of glioma cells and found that fusion of glioma cells with fibroblasts resulted in reprogrammed hybrids with enhanced viability, which might contribute to tumour heterogeneity [40].

In addition, Sun *et al.* reported that cell fusion is a driving force responsible for tumour neovascularization. The authors isolated bone marrow-derived MSCs exhibiting expression of GFP from transgenic nude mice. SU3 glioma stem cells were transfected with red fluorescent protein (RFP). Fusion of bone marrow-derived MSCs with SU3 glioma stem cells resulted in hybrids capable of tube formation *in vitro* and also able to generate solid tumours and tumour vasculature *in vivo* [41].

Cell fusion in metastasis

Metastasis contributes to almost 90% of cancer deaths [42]. It is hypothesized that metastasis arises because of EMT, whereby mesenchymal cells with enhanced invasion and migration of the phenotypes are generated, leading to the development of resistance to apoptosis [43]. This cell–cell fusion results in the loss of epithelial traits, such as cell adhesion, which leads to E-cadherin overexpression and cell deformation with increased motility [44,45]. Gast *et al.* demonstrated fusion between macrophages and cancer cells and reported that hybrids had physiologically pertinent and functionally important features responsible for tumour evolution. The hybrids exhibited expression of CSF1R, which is involved in tumour progression. For example, this overexpression has been implicated in the proliferation of lung cancer. Circulating hybrid cells with features of both tumour cells and hematopoietic cells have been found in human tumour biopsies and peripheral blood, and also to outweigh the number of circulating tumour cells both in murine models and in humans. The increased levels of circulating hybrid cells compared with circulating tumour cells could be attributed to the circulating hybrid cells being spared from immune surveillance because of their leucocyte identity. This possibility warrants further investigation owing to its potential implications in cancer immunotherapy. The population of circulating hybrid cells is useful for identifying the tumour stage and prediction of clinical outcomes. Circulating hybrid cells have been reported to provide a direct correlation with tumour stage in patients with pancreatic cancer [46]. Fig. 1 illustrates the role of cell fusion in cancer cell proliferation and metastasis.

In a study in breast and colorectal cancer cell lines, data generated for the specific macrophage markers, CD163 and CD45, showed a positive correlation between advanced tumour stage and lower survival rates [47,48]. The study also revealed that, in the MCF-7 breast cancer cell line, CD163 and CD45 expression in hybrids cells was primarily the result of fusion between cancer cells and macrophages [48]. Ultimately, the authors concluded that CD163 expression in cancer cells could be considered a surrogate marker to confirm the evidence of cell fusion in solid tumours.

Another seminal study also demonstrated that cell fusion between myeloma cells and lymphocytes contributed to metastasis in the liver and spleen [2,49]. The cell fusion-resident cells of distant organs also led to the dissemination of the cancer cells in the TME, which ultimately led to the progression of a metastasis stage and eventually activation of oncogenes [2,50]. Radiotherapy has also been found to be the main contributor to tumour epithelial cell–macrophage fusion in TME [42,51]. In radiation-treated cancer, there is evidence that the survival of the patients is often compromised because of the exacerbated cell fusion of epithelial cells with macrophages. Hence, based on these observations,

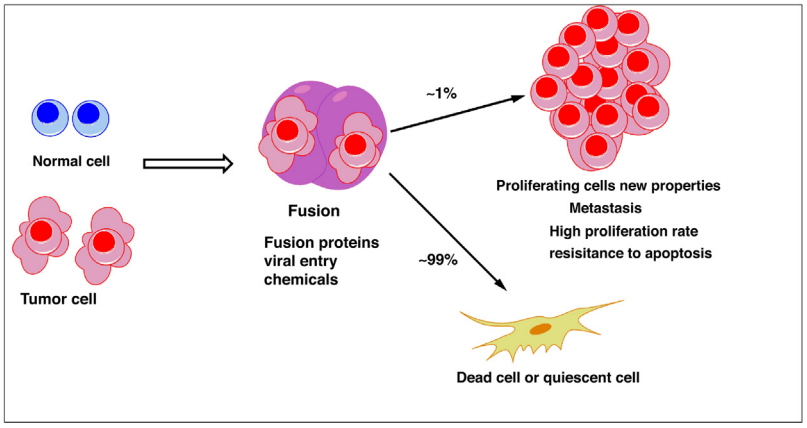

FIGURE 1

Overview of tumour cell fusion wherein the fusion of native cells and cancerous cells leads to polyploidy, with the combined attributes of native cells and cancerous cells.

irradiation of the brain could lead to the recurrence of tumours owing to cell fusion [42,52].

Cell fusion and drug resistance

Chemotherapy and radiation therapy are the major cancer treatments. Unfortunately, success of these treatments is limited because of the development of MDR. Several mechanisms are involved in the development of extrinsic MDR, such as loss of drug target, apoptosis suppression, increased DNA repair mechanisms, decreased uptake of drug, and increased efflux because of the overexpression of the ATP-binding cassette (ABC) transporter [53]. Cell fusion can mediate the consolidation of MDR by combining genes for the expression and/or overexpression of various proteins responsible for drug resistance and leading to formation of PGCCs with chemoresistant properties [54].

Yan *et al.* focused on cancer cell fusion in an *in vivo* environment and studied features of hybrid cells under the influence of chemotherapy. The study was the first to report tumour cell fusion *in vivo* and highlighted the fact that chemotherapy could lead to a poor prognosis by causing enrichment of hybrids with higher malignant potential. The researchers found that, in a mouse xenograft model, the tumours in mice treated with epirubicin showed a greater proportion of hybrid cells compared with the control group, which received no chemotherapy. The poor sensitivity of tumour cell hybrids to epirubicin contributed to a higher proportion of hybrid cells in the mice receiving chemotherapy [30]. Miller *et al.* reported the fusion of mitoxantrone-resistant tumour cells with 5-fluorouracil tumour cells, leading to the formation of hybrids that were resistant to both drugs [55]. These cells were also found to be resistant to melphalan, to which both tumours were sensitive. This suggests that cell fusion not only creates cross-resistance, but can also induce MDR in cancer cells. Duelli *et al.* also showed the development of resistance to apoptosis in primary cells when fused with drug-sensitive transformed cells. The authors found that this effect, although temporary, lasted for a few days, potentially resulting in the failure of chemotherapy, thereby allowing tumour cells to survive [56]. Yang *et al.* reported the formation of hybrids of breast cancer cells with doxorubicin-resistant cells, leading to the formation of a heterogenous population of cells. In this population, a few cells were found to be susceptible to the drug effects, whereas most showed drug resistance [57]. A study by Carloni *et al.*, found that cell fusion promoted chemoresistance in metastatic colon cancer. In this study, 5-fluorouracil and oxaliplatin-resistant cells (CT26) were transduced with RFP and GFP, respectively and injected into syngeneic mice. The cells isolated from the tumours formed in the mice were treated with 5-fluorouracil and oxaliplatin either together or separately, but showed resistance to both drugs. This again suggests the formation of a hybrid cell subpopulation with characteristics of resistance to both drugs via cell fusion [58]. Another seminal work supporting the role of cell fusion in drug resistance was carried out by Dittmar *et al.*; in their work, hybrid cells were derived from the fusion of breast epithelial cells with breast cancer cells. A few hybrid cells exhibited resistance to a variety of chemotherapeutics, such as etoposide, doxorubicin, and paclitaxel, whereas a few hybrids showed drug resistance to only etoposide [59].

A recent study by Wang *et al.* involving the fusion of stem cells with liver cancer cells showed the formation of highly tumorigenic and chemoresistant hybrid cells compared with the parent hepatocellular carcinoma cells. The fused cells showed significantly higher survival rates than HepG2 cells in the presence of doxorubicin, demonstrating increased drug resistance because of cell fusion. In this study, it was also shown that cell fusion led to overexpression of several proteins, including ALDH1A1, P-glycoprotein (ABCB1), and B cell lymphoma 2 (Bcl-2) [33]. It is well established that ABC transporters, such as ABCB1, have a vital role in the development of drug resistance in cancer. Nagler *et al.* showed the upregulation of ABCB1a and ABCB1b in hybrid cells formed by cell fusion of murine BMDCs with 67NR mouse mammary carcinoma cells. This resulted in marked resistance to chemotherapeutic drugs, such as doxorubicin, etoposide, 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG), and paclitaxel. The exchange of genetic information between two different cell population is suggested to be the origin of highly drug-resistant cancer cells [60]. Moreover, a recent study by Searles *et al.* also highlighted cell fusion as a mechanism of DNA exchange in cancer leading to chemoresistance in melanoma [1]. In this study, co-cultures of B16-GFP-Cre cells and

reporter target cells (MEF or BMDM) were grown and treated with paclitaxel. This resulted in the formation of B16xMEF and B16xDMDM hybrids that were more resistant to paclitaxel compared with the parent B16 cells. Initiation of the formation of such hybrids by cell fusion was suggested to be induced by chemotherapy [30]. Song *et al.* demonstrated spontaneous fusion between eGFP-labelled HUVECs and RFP-labelled SCC9. The resultant hybrids showed markers of both parental cells and enhanced resistance to cisplatin treatment. Injection of the hybrid cells in nude mice showed that the hybrids could survive and contribute to tumour growth *in vivo* [61].

CSCs have also shown resistance to radiation therapy and chemotherapy leading to relapse in cancer. Recurrence CSCs (rCSCs) have been suggested to be a specific CSC population responsible for re-initiating tumour growth after therapy and, thus, conferring drug resistance [62]. As documented by Rizvi *et al.*, CSCs could be fusion partners for cells with a bone marrow origin [63]. It has been suggested that rCSCs originate through the initial therapy itself, by selection to survive. The mechanism by which CSCs confer drug resistance include enhanced DNA repair mechanisms, overexpression of ABC transporter proteins, such as breast cancer resistance protein (ABCG2/BCRP), P-gp/MDR1/ABCB1. and multidrug resistance protein 1 (MRP1/ABCC1); and antiapoptotic proteins [64]. Other mechanisms involved in radiation resistance in CSCs include Notch signalling [65] and Wnt/β-catenin signalling [66]. However, given that cell fusion has been linked to the origin and promotion of rCSCs and their role in the drug resistance, further investigations with regards to the mechanisms involved are needed to pinpoint appropriate strategies to tackle such drug resistance.

Nevertheless, all these studies provide sufficient evidence for the role of cell fusion in the formation of chemoresistant cells from a heterogeneous cell population in tumours. Hence, targeting tumour heterogeneity before its inception could be a valuable tool to prevent cancer recurrence and improve cancer prognosis [67].

Strategies to tackle cell fusion-mediated cancer

Targeting transporter proteins

Several efforts are being taken towards overcoming MDR in cancer. As reiterated earlier, cell fusion leads to not only cross-resistance but also MDR ascribed to the overexpression of ABC transporter proteins. Thus, targeting ABC transporters could be an attractive target to control MDR in cancer hybrid cells overexpressing one or more ABC transporters.

An interesting study by Frank *et al.* pinpointed the role of ABCB5 P-gp in the regulation of membrane potential in progenitor cells and cell fusion [68]. Detection of cell fusion in human epidermal melanocytes (HEM) by flow cytometry revealed that specific blockade of ABCB5 by anti-ABCB5 P-gp mAb led to increased formation of fused cells compared with untreated cells. However, the study also revealed that blockade of ABCB1 by ABCB1 mAb did not exert any such effect. These findings outline the need for the careful design of selective inhibitors of ABCB1 to overcome MDR in cancer cells without influencing the cell fusion controlled by ABCB5.

Extensive studies have been undertaken to design potent and selective inhibitors of major ABC transporters (ABCB1, ABCG2, and ABCC1) to overcome drug resistance in cancer. These efforts have resulted in the identification of several selective and potent inhibitors of these transport proteins. Some of the most promising modulators of ABCB1, ABCG2, and ABCC1 are summarised in Fig. 2, which includes scaffolds such as N10-substituted acridones (ABCB1) [69,70], quinazolines (ABCG2) [71] and pyrrolopyrimidines (ABCC1) [72]. Although, these inhibitors are still in the preclinical stage, their potency demonstrates potential for overcoming MDR in cancer in the clinic.

Targeting the fusogenic protein, syncytin

Syncytin, a membrane protein derived from the envelope gene of an endogenous retrovirus of the HERV-W family, has an important role in the development of the human placenta by syncytial fusion of the cytitrophoblast with syncytiotrophoblasts. Syncytin-1, a profusogenic protein, has also been shown to be involved in cell fusion of several other types of cell, such as trophoblasts [73], macrophages [74], and breast cancer cells. Mortensen *et al.* found that expression of the syncytin-1 protein in breast cancer cell lines showed cell fusion with endothelial cells in culture [75]. It was observed that the use of syncytin-1 antisense oligonucleotides led to the downregulation of syncytin-1 expression and also inhibited breast cancer and endothelial cell fusion. In another study, syncytin-2 was shown to contribute to cancer cell–host cell fusion and the use of short hairpin (sh)RNA-directed downregulation led to a decrease in syncytin-1 and syncytin-2 expression [77]. Strick *et al.* reported that syncytin-1 was involved in cell fusion in endometrial carcinomas,

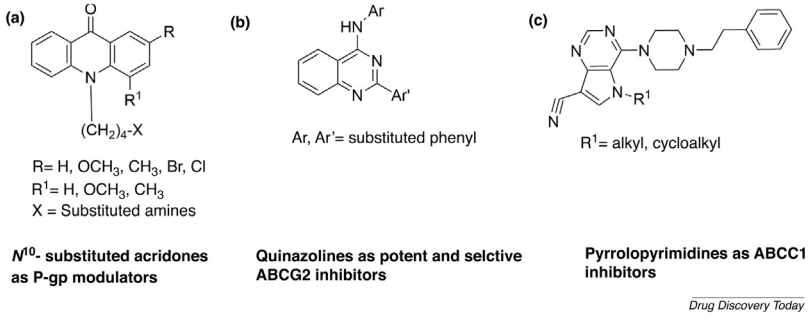

FIGURE 2

Molecules to tackle cell fusion-mediated cancer. Targeting ATP-binding cassette (ABC) transporter proteins could overcome multidrug resistance (MDR) in cancer; here, we highlight the classes of potent and selective inhibitors of ABC transporters.

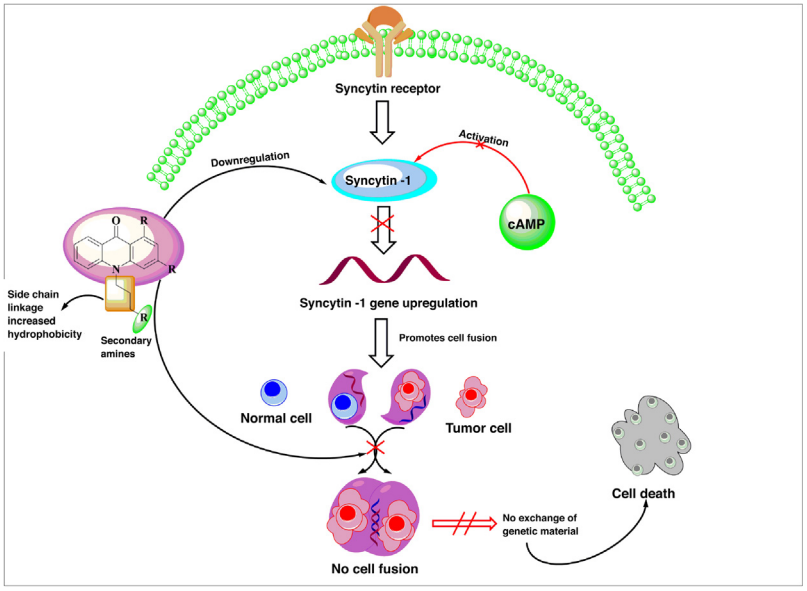

FIGURE 3

Strategies to tackle cell fusion-mediated cancer. (a) Inhibition of syncytin-1 would lead to decreased cell fusion. (b) Inhibition of cell fusion would not enable the exchange of genetic material and transfer of multidrug-resistant (MDR) genes. (c) Inhibition of ATP-binding cassette (ABC)t ransporters overexpressed as a result of cell fusion to overcome MDR.

where downregulation of syncytin-1 resulted in inhibition of cell fusion events [78]. Furthermore, a study by Yan *et al.*, using squamous cell carcinoma cells 9 (SCC-9) and HUVECs, revealed the role of syncytin-1 in a TNF-α-enhanced cancer-endothelial cell fusion [79]. Additionally, Sun *et al.* suggested the role of leukocytic syncytin-1 expression in the development of the acute myelogenous leukaemia and acute monocytic leukaemia phenotypes [80].

Although these data are preliminary and no clinical data are available, current evidence suggests the possibility of targeting syncytin to decrease cell fusion in cancer. Possible ways of targeting cell fusion and related targets are summarised in Fig. 3. However, a contrasting study by Larsson *et al.* reported that synyctin was expressed in 38% of patients with breast cancer and was correlated to the positive prognosis of recurrence-free survival of the patients studied [76]. This contradicts the fact that synyctin induces cell fusion, necessitating further studies to ascertain the precise role of syncytin in cell fusion and, consequently, cancer cell proliferation and metastasis.

Concluding remarks and future perspectives

Immunotherapy is an emerging concept in cancer treatment that involves the stimulation of cytotoxic T lymphocytes in patients with cancer through vaccination or *ex vivo* enlargement of tumour-selective cytotoxic T lymphocytes for cell transfer. Cell fusion has been explored for cancer immunotherapy, wherein hybrid cells are produced via fusion between competent antigen-presenting cells, such as dendritic cells (DCs), and tumour cells showing expression of pertinent tumour-related antigens. The resulting fusion hybrids are endowed with dual attributes of tumour-specific antigen expression and an ability to present to CD4 + T and CD8 + T cells for the generation of an immune response. The rationale behind using whole tumour cells as a source of antigens for vaccination is the multivalent stimulation of T cells resulting in a decreased possibility of immune response evasion by the tumour.

In vivo studies in animal models have established the potential of vaccination to protect against tumour challenge and cause regression of existing tumours, such as renal, colon, lung, breast, hepatic, and cervical carcinomas. Some issues need to be addressed for the successful clinical translation of cancer immunotherapy with fusion hybrids. These include optimization of dosing regimen, dose of fused cells to be injected, route of injection, and so on [81]. In addition, the *in vitro* culturing of DCs is tedious and lengthy; therefore, it will be necessary to optimize culture conditions for DC differentiation and maturation before their fusion with tumour cells [82].

The clinical success of vaccines based on fusion between DCs and tumour cells has been challenged by the immunosuppressive TME. Interferon-induced protein-10 (IP-10) is useful to augment the anti-tumour potential of DC–tumour cell fusion-based vaccines. It binds to the CXCR3 receptor on activated T cells and attracts T lymphocytes to the tumour. It is also known to inhibit tumour growth and neovascularization. Hu *et al.* used folate-modified chitosan nanoparticles as non-viral vectors for the expression plasmid of the mouse interferon-induced protein-10 (mIP-10) gene. The rationale behind the use of this expression plasmid was the short half-life of IP-10, which would stimulate expression of IP-10 *in vivo*. mIP-10 acts as a chemoattractant for cytotoxic T cells. The vaccine based on fusion when administered with the folate-modified chitosan nanoparticles containing the expression plasmid inhibited the growth of implanted hepatocellular carcinoma tumours and extended the survival of the mice. Myeloid-derived suppressor cells contribute to the creation of immunosuppressive TME. The combined therapy resulted in a decrease in myeloid-derived suppressor cells in spleen and augmented the tumour-selective interferon-γ response. The com-

bined therapy with nanoparticles and fusion vaccine also induced apoptosis in tumour-bearing mice [83].

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