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Unveiling the power of mitochondrial transfer in cancer progression: a perspective in ovarian cancer

Caixia Wang[1,2] and Chuan Xie[1,2*]

Abstract
Mitochondria are dynamic organelles integral to metabolic processes, coordination of essential biological pathways, and oncogenesis and tumor progression. Recent studies have revealed that mitochondria can be transferred between cells via multiple mechanisms, implicating their involvement in the pathogenesis and progression of ovarian cancer. This review provides a comprehensive analysis of intercellular mitochondrial transfer within the context of ovarian cancer and its tumor microenvironment. We also propose targeted pathways and therapeutic strategies that could be utilized to modulate diseases associated with mitochondrial transfer therapy. Finally, we examine recent advancements in this field and identify several unresolved questions.

Keywords Mitochondrial transfer, Ovarian Cancer, Tumor Microenvironment, Targeted therapy

Introduction

Mitochondria are double membrane-bound organelles. The outer membrane contains various protein channels that facilitate the exchange of small molecules, such as ions and metabolites [1]. Mitochondrial outer membrane permeabilization (MOMP) typically commits a cell to apoptosis upon induction [2]. The inner membrane forms invaginations known as cristae, where the oxidative phosphorylation (OXPHOS) complexes are located [3]. The mitochondrial matrix houses multiple copies of mitochondrial DNA (mtDNA) and ribosomes. mtDNA encodes 13 proteins essential for OXPHOS, as well as the 12 S and 16 S rRNAs and 22 tRNAs [4]. Mitochondria are multifunctional, encompassing processes such as adenosine triphosphate (ATP) synthesis, the maintenance of calcium (Ca^{2+}) homeostasis, and the induction of apoptosis, all of which are fundamental for cellular metabolism and homeostasis [5]. Mitochondria generate ATP for cells via OXPHOS, a process that concomitantly produces reactive oxygen species (ROS) as byproducts [6]. An overabundance of ROS can result in mitochondrial dysfunction and apoptosis [7]. Alterations in intracellular Ca^{2+} homeostasis prompt mitochondria to sequester Ca^{2+} from the cytoplasm to restore intracellular Ca^{2+} equilibrium [8]. In the endogenous apoptotic pathway, mitochondria release cytochrome c, which subsequently interacts with the apoptosis activator, protease 1, culminating in cellular apoptosis [9].

Mitochondrial dysfunction encompasses alterations in mitochondrial dynamics, including processes such as fission and fusion, which can precipitate cellular apoptosis and contribute to tumorigenesis [10]. Throughout tumor initiation and progression, mitochondria function

*Correspondence:
Chuan Xie
xiechuan85@163.com
[1]Department of Obstetrics and Gynecology, West China Second University Hospital, Sichuan University, Chengdu, Sichuan Province, P.R. China
[2]Key Laboratory of Birth Defects and Related Diseases of Women and Children, Sichuan University, Ministry of Education, Chengdu, Sichuan Province, China

as pivotal organelles for metabolic reprogramming. In the context of ovarian cancer, there is substantial evidence indicating that mitochondrial dysfunction, oxidative stress, and apoptosis signaling pathways are critically involved, underscoring the centrality of mitochondria in these processes [11]. Research has investigated the differences in mitochondrial function between normal ovaries and cancers. The findings demonstrated that individuals with ovarian cancer showed markedly reduced levels of cellular oxidative stress, mitochondrial mass, and mitochondrial biogenesis in their peripheral blood mononuclear cells. Furthermore, a notable decrease in mitochondrial membrane depolarization and mitochondrial swelling was observed in the ovarian cancer tissues. However, mitochondrial ROS levels did not significantly differ between normal and cancerous ovarian tissues [12]. Intercellular mitochondrial transfer can be conceptualized as an extension of intracellular mitochondrial dynamics or intercellular communication. This phenomenon has been documented both in vitro and in vivo under physiological and pathophysiological conditions across various cell types, including cancer cells [13, 14]. In a pioneering co-culture experiment, Spees et al. firstly demonstrated that human bone marrow mesenchymal stem cells (MSCs) could rescue cells with nonfunctional mitochondria through the transfer of mitochondria or mtDNA [15]. Subsequent research has observed intercellular mitochondrial transfer in the context of various diseases, including tissue regeneration [16], neurological disorders [17], and tumors [18].

The tumor microenvironment (TME), which includes stromal cells, the extracellular matrix, and exosome components, is characterized by hypoxia, elevated interstitial pressure, and inflammatory reactivity, and is closely linked to tumor growth, metastasis, and drug resistance [19]. In addition to malignant cells, the TME consists of mesenchymal cells, immune cells, adipocytes, among others [20]. Ovarian cancer is particularly noted for its propensity for intraperitoneal metastasis. During this metastatic process, cancer cells derive energy from the surrounding host cells, underscoring the critical importance of interactions among various cell types within the TME [21]. In this review, we examine the various aspects of intercellular mitochondrial transfer between ovarian cancer cells and the TME, and provide a comprehensive summary of the associated transfer routes, signaling pathways, and molecular mechanisms. From this vantage point, we suggest that this process and its intervention may serve as novel targets for therapeutic development.

The routes of intercellular mitochondrial transfer and the molecular mechanism implicated

Tunneling nanotubes

Tunneling nanotubes (TNTs) are slender, elongated, non-adherent structures that facilitate direct communication between distant cells. These protrusions contain filamentous actin and transport entire organelles [22]. In 2004, Rustom et al. were the first to observe TNTs, which consist of F-actin-containing channels that connect cells over considerable distances [23]. Subsequently, an increasing number of cell types, including myeloid cells [24], MSCs [25], and tumor cells [26], have been found to possess TNTs. This discovery underscores the ubiquity and prevalence of TNTs as a means of cellular communication. TNTs were initially identified in vitro and have recently been observed in vivo as well [27]. The intercellular connections formed by TNTs are characterized by their dynamic nature and heterogeneity. The morphology and composition of TNTs exhibit considerable variability, with lengths ranging from several micrometers to over 100 micrometers, and diameters spanning from tens of nanometers to several micrometers. The cytoskeleton within TNTs is composed of F-actin or microtubules [28]. TNTs not only play a role in the normal physiological activities of cells but also have a unique function in specific processes, such as immune cell differentiation and antigen presentation, Ca^{2+} signaling, and the transfer of organelles [24, 29–31]. Subcellular structures, notably mitochondria, are transported between cells via TNTs, which play a pivotal role in tumor networking and disease progression. In ovarian cancer, the presence of TNTs in ovarian cancer cells has been substantiated through two-photon excitation FLIM-FRET imaging. This technique has elucidated the structural composition of TNTs as lipid bilayers containing microtubules, which facilitate the transmission of ions and organelles between adjacent cells [32].

Two distinct mechanisms have been proposed for the dynamics and formation of TNTs, which may vary according to cell heterogeneity. The first mechanism involves the extension of filopodia-like protrusions towards another cell, resulting in the formation of either open or closed conduits, contingent upon whether the ends of the conduits fuse with the membrane of the target cell. The open conduits facilitate cell-cell contact through communicating cytoplasm, known as TNTs, whereas the closed conduits, analogous to an elongated synaptic contact, are referred to as cytonemes [24, 33]. The second mechanism entails the direct contact between two proximal cells, where the contact site is subsequently stretched and deformed as the cells move in opposite directions, thereby generating TNTs. The duration of cell contact is critical for the establishment of stable TNTs [33, 34]. Mechanistic studies have revealed

that the mammalian target of rapamycin (mTOR) signaling pathway and the cell division control protein 42 homolog (CDC42) play pivotal roles in regulating the protrusion and growth of nanotubes [35]. Furthermore, the activation of the MAPK signaling pathway is associated with the formation of TNTs. Research indicates that the epidermal growth factor receptor (EGFR) activates the MAPK cascade to promote the formation of TNTs between ovarian cancer cells, whereas inhibition of ERK and RSK can suppress this process [36]. Mitochondrial adaptors, including small mitochondrial Rho GTPases Miro1/2 and kinesin adaptor proteins TRAK1/2, as well as the myosin motor Myo19, play a pivotal role in facilitating mitochondrial movement [37–39]. Miro1/2 are integral to the regulation of mitochondrial transport by linking mitochondria to kinesin and dynein motor proteins. TRAK1/2 facilitate the anterograde movement of mitochondria, whereas Myo19 is implicated in mediating shorter-range mitochondrial movements [37]. Among these, Miro1 is a predominant protein involved in the intercellular transportation of mitochondria via TNTs (Fig. 1). Miro1, a calcium-sensitive adaptor protein, facilitates the connection between mitochondria and kinesin family motor protein 5 (KIF5). This interaction is inhibited when Ca^{2+} binds to the EF hand domains of Miro1 [40]. Beyond its well-documented role in neurological diseases, recent studies have demonstrated that Miro1 regulates the intercellular transfer of mitochondria from MSCs to epithelial cells via TNTs, thereby enhancing the therapeutic efficacy of MSCs [41].

Extracellular vesicles

Extracellular vesicles (EVs) constitute a heterogeneous group of membranous vesicles secreted by various cellular sources. Initially perceived as cellular waste disposal mechanisms, EVs are now recognized as pivotal mediators of intercellular communication. Based on their size, origin, and cargo, EVs can be classified into microvesicles (MVs), exosomes, apoptotic bodies (ApoBDs), and migrasomes [42, 43]. These vesicles are capable of transporting a diverse array of cargos, including nucleic acids, proteins, metabolites, and even organelles, thereby influencing recipient cells through long-distance transport mechanisms. The functional effects of these EVs are generally determined by the specific proteins or RNAs they contain.

MVs are formed by budding from the plasma membrane and have a diameter ranging from 50 to 1000 nm, closely resembling the parental cell membrane [44]. MVs derived from human brain endothelial cells have the capability to transfer mitochondria, thereby enhancing endothelial cell survival under ischemic conditions [45]. Exosomes, on the other hand, are nanovesicles that originate from endosomes and have a diameter ranging from approximately 40 to 160 nm [46]. The current study provides evidence that exosomes, although devoid of mitochondria, contain mtDNA that can be transferred between cells [45]. ApoBDs originate from the blebbing of apoptotic cells undergoing programmed cell death, with diameters ranging from 800 to 5000 nm [47]. These ApoBDs often contain organelles exhibiting healthy morphology, such as mitochondria, ribosomes, and the endoplasmic reticulum [48]. Migrasomes, which form in migrating cells, have diameters ranging from 500

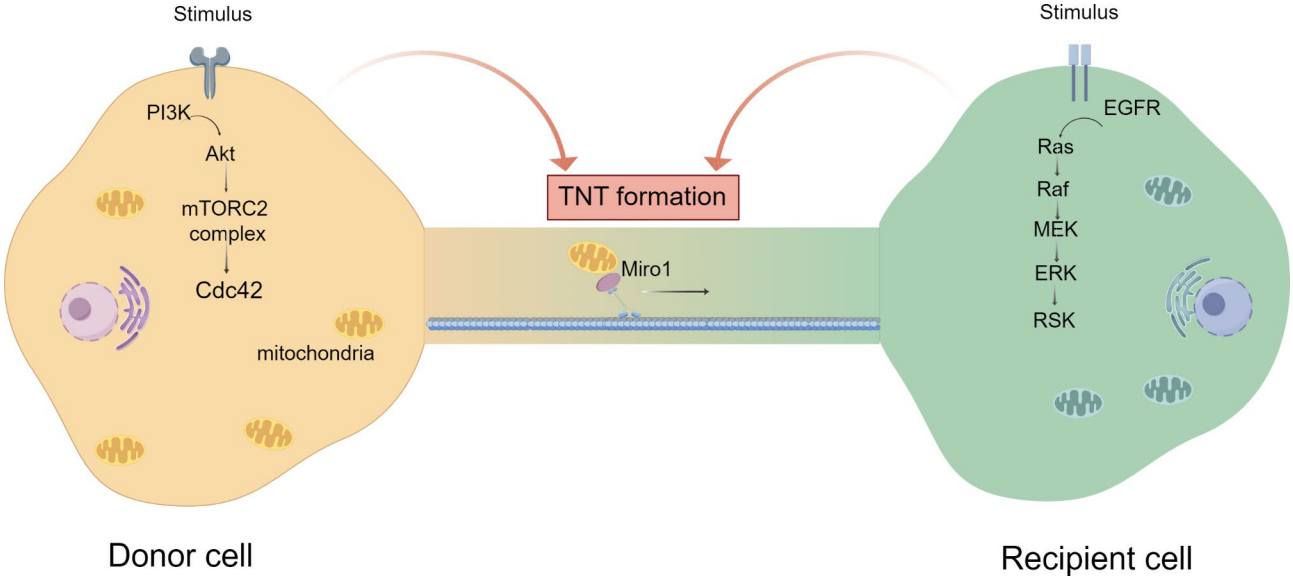

Fig. 1 TNTs-mediated intercellular mitochondrial transfer and signaling pathways. The mTOR/Cdc42 and EGFR/MEK signaling pathways are implicated in the formation of TNTs. Miro1 is primarily accountable for transporting mitochondria

to 3000 nm and contain multiple vesicles within their cavities [43]. Research has demonstrated that damaged mitochondria are transported into migrasomes and subsequently expelled from migrating cells in response to mild mitochondrial stress, as observed in mouse liver neutrophils [49].

Mechanistically, mitochondria-containing MVs typically express proteins associated with the endosomal sorting complex required for transport (ESCRT), such as tumor susceptibility gene 101 (TSG101) and arrestin domain-containing 1 (ARRDC1) [50, 51]. The MSCs utilize ARRDC1-mediated MVs (ARMMs) to export mitochondria across the plasma membrane, which are subsequently engulfed by macrophages and reutilized to enhance bioenergetic functions [50, 51]. Cluster of differentiation 38 (CD38) is a member of the nicotinamide adenine dinucleotide (NAD^+) glycohydrolase family, catalyzing the conversion of NAD^+ to cyclic ADP ribose (cADPR) [52]. Subsequently, cADPR induced the release of Ca^{2+} and facilitated the release of mitochondria-containing MVs [53, 54] (Fig. 2). The interaction between ovarian cancer cells and the TME mediated by EVs has been extensively investigated. The primary constituents of these EVs are microRNAs and proteins [55]. However, the role of mitochondria-rich EVs in ovarian cancer remains under exploration.

Cell fusion

Cell fusion entails the amalgamation of the membranes of two distinct cells, facilitating the exchange of organelles and cytosolic components while preserving the integrity of their nuclei [56]. This phenomenon is observed in various physiological processes, including placentation, myogenesis, and osteoclastogenesis, as well as in pathophysiological conditions such as cancer [57]. The process of cell fusion can be delineated into three sequential stages: competence, which involves cell induction and differentiation; commitment, characterized by cell determination, migration, and adhesion; and the final stage of cell fusion, which encompasses membrane merging and cytoplasmic integration [58].

It has been proposed that hypoxia and inflammation act as positive regulators of cell fusion. Hypoxia has been shown to enhance the cell fusion of ovarian cancer cells, thereby increasing their invasive potential [59]. Tumor necrosis factor alpha (TNFα) has been demonstrated to facilitate cell fusion between oral cancer cells and endothelial cells through the VCAM-1/VLA-4 pathway [60]. In addition, dysregulation of syncytin, a fusogenic protein, is implicated in the induction of cell fusion in tumor cells. Syncytin, a human endogenous retroviral (HERV) envelope protein, has been associated with both trophoblast and cancer cell fusions. Syncytin-1 and syncytin-2 mediate their fusion capabilities through binding to their respective receptors, ASCT-2 and MFSD-2 [61]. Both syncytin-1, syncytin-2, and their receptors

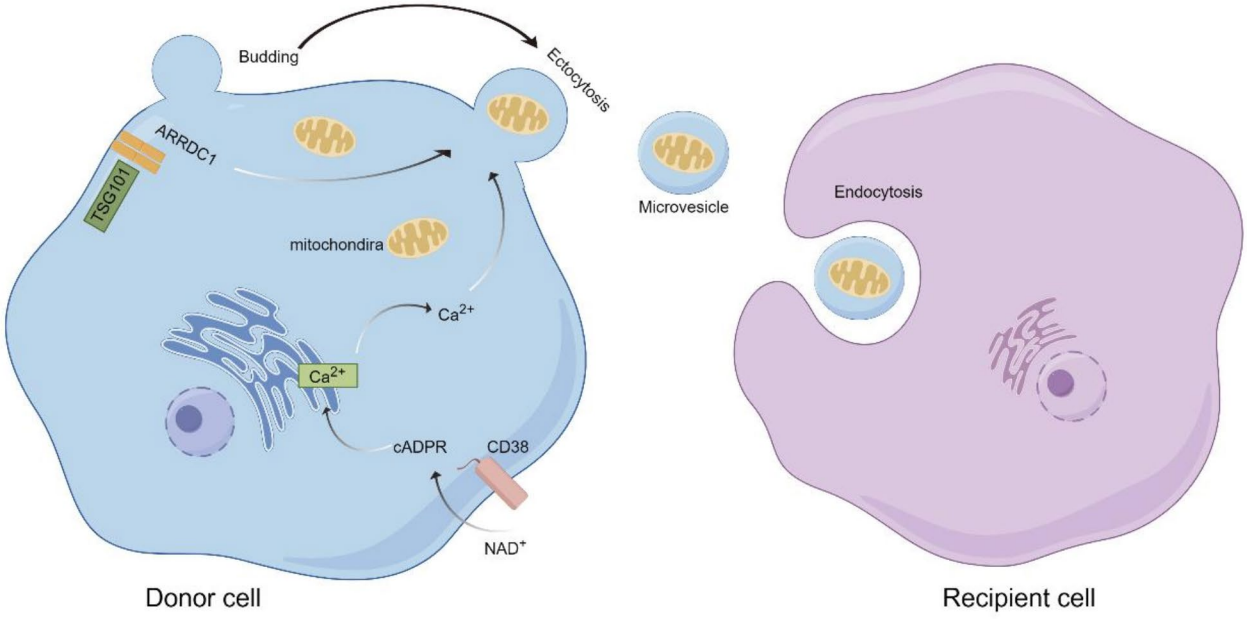

Fig. 2 Mitochondrial transfer via EVs and the molecular pathways implicated. CD38 catalyzes the NAD^+ to cADPR, and increases the release of Ca^{2+} from endoplasmic reticulum, thus promoting the release of mitochondria-containing MVs. ARRDC1 interact with TSG101 and unload mitochondria outward the plasma membrane

are markedly overexpressed in various cancers, indicating that cell fusion may significantly contribute to cancer initiation and progression [62, 63]. Additionally, TNFα has been shown to upregulate ASCT-2 expression via the PI3K/AKT signaling pathway in endothelial cells and to enhance syncytin-1 expression through the Wnt/β-catenin pathway in oral squamous cell carcinoma cells [64] (Fig. 3). Melzer et al. demonstrated that the co-culture of human umbilical cord derived-MSCs with ovarian cancer cells SKOV3 in vivo promotes tumor growth and liver metastasis. Upon the formation of hybrid cells between MSCs and ovarian cancer cells, the expression of syncytin-2 and MFSD-2 A was observed in both parental and hybrid cell populations. However, in comparison to the parental cancer cells, the hybrid cells exhibited a reduced proliferation capacity, an increased expression of E-cadherin, and a decreased expression of N-cadherin. These findings suggest that the hybrid cells undergo a mesenchymal-to-epithelial transition (MET) [65]. These studies indicate that cell fusion may exert dual effects, either promoting or suppressing tumor development. These variations suggest that the outcomes of cell fusion

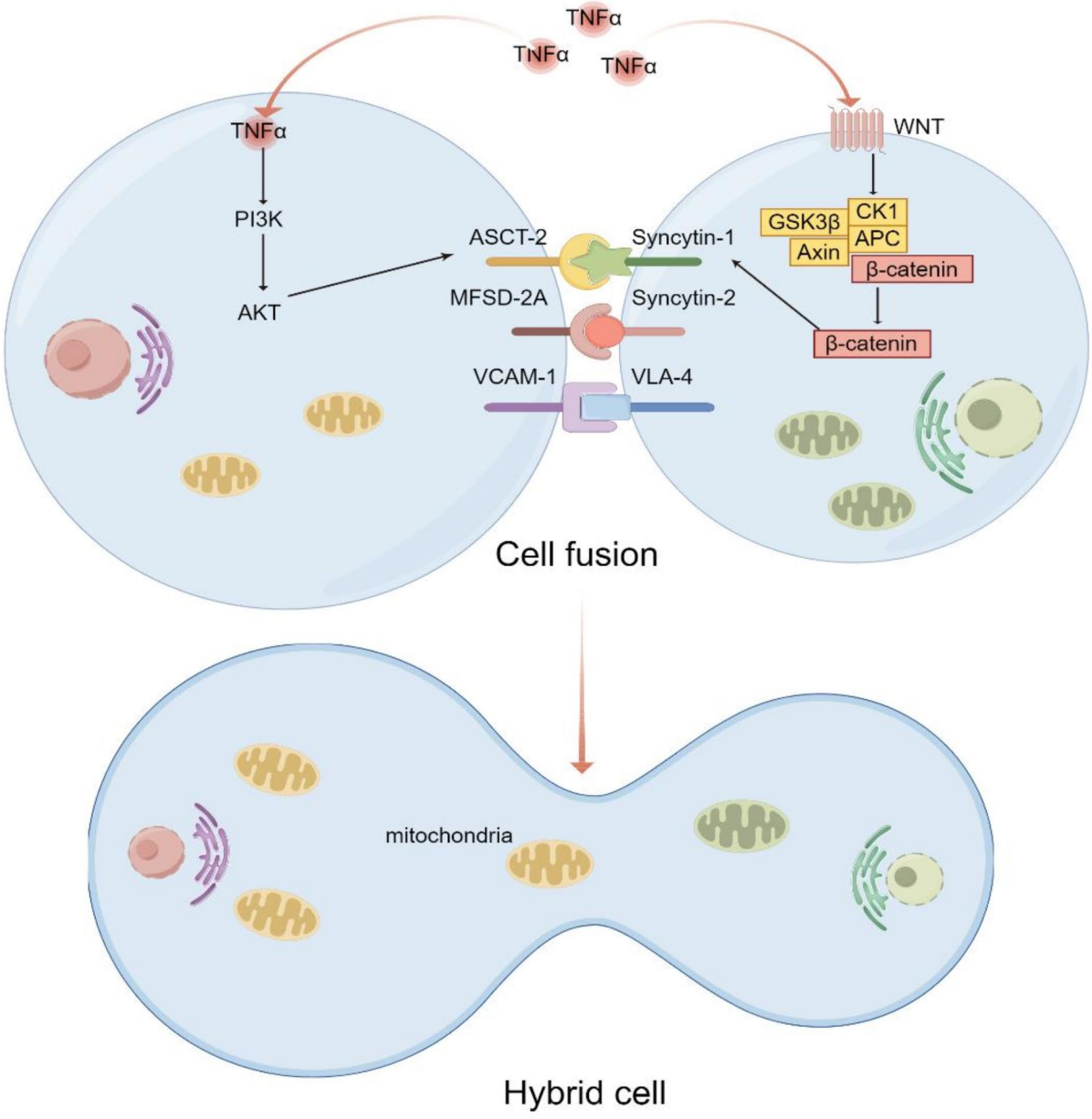

Fig. 3 Cell fusion-mediated intercellular mitochondrial transfer and signaling pathways. VCAM-1/VLA-4, syncytin-1/ASCT-2, and syncytin-2/ MFSD-2 mediate the process of cell fusion. TNFα can increase the expression of syncytin-1 and ASCT-2 via PI3K/AKT and Wnt/β-catenin signaling pathways respectively

may be contingent upon the specific cellular type or the TME. Furthermore, proteins such as cluster of differentiation 9 (CD9) and cluster of differentiation 47 (CD47) have been implicated in mediating cell fusion processes in cancer [66, 67].

Uptake of isolated mitochondria

Thierry AR et al. identified the presence of respiratory-competent free mitochondria in human blood, independent of EVs [68]. Conversely, Stier A provided evidence suggesting that free mitochondria are unlikely to be functional, as they exhibit no potential for OXPHOS in vitro [69]. Additionally, researchers have proposed a strategy for the artificial transfer of mitochondria to restore mitochondrial function in recipient cells. Studies have demonstrated that the transfer of human cardiac fibroblast-derived mitochondria to ovarian cancer cells did not affect cell proliferation; however, it significantly reduced cell migration and increased sensitivity to chemotherapy [70]. Aerobic glycolysis, also referred to as the Warburg effect, has been implicated in the biochemistry and metabolism of tumors [71]. Studies have demonstrated that isolated mitochondrial transplantation can enhance aerobic respiration, mitigate the Warburg effect, and activate apoptotic pathways. These findings indicate a potential therapeutic application in the treatment of ovarian cancer [70].

Endocytosis pathways can be classified into four types: clathrin/caveolae-mediated endocytosis, clathrin/caveolae-independent endocytosis, macropinocytosis, and phagocytosis [72]. It has been discovered that exogenous isolated mitochondria can be internalized into recipient cells via actin-dependent macropinocytosis rather than clathrin-mediated endocytosis [73, 74]. Macropinocytosis is a non-receptor-mediated, actin-driven process that involves membrane ruffling, macropinosome formation, and the subsequent internalization of extracellular material [75]. It has been demonstrated that several key regulators of actin polymerization, including small GTPases (such as Ras, Rac, Cdc42, Arf6, and Rab5), p21-activated kinase 1 (Pak1), and PI3K, play crucial roles in the formation of plasma membrane protrusions and in facilitating macropinocytic activity [76]. The detailed mechanism is shown in Fig. 4.

Gap junctions

Gap junctions (GJs) are composed of two connexons, each of which consists of six subunits known as connexins, forming the functional units that facilitate both electrical and metabolic communication between adjacent cells [77]. Extensive reviews have elucidated the critical role of GJs in the intercellular transfer of mitochondria. To date, at least twenty-one connexin isoforms have been identified within the human genome, with connexin 43 (Cx43) being the most extensively studied in the context of tumors [78]. Cx43 gap junction channels have been demonstrated to facilitate the intercellular transfer of mitochondria, potentially through mechanisms involving direct transfer, Ca^{2+}, or ROS exchange [79]. In models of ovarian follicles, Cx43 gap junctions have been implicated in mediating mitochondrial transfer [80]. However, multiple studies have reported low expression levels of Cx43 in ovarian cancer, and its overexpression has been associated with the inhibition of ovarian cancer cell proliferation [81, 82]. These findings imply that mitochondrial transfer in ovarian cancer cells may not be mediated by GJs.

The fate of mitochondrial transfer to recipient cells and related laboratory methodology

The fate of transferred mitochondria within recipient cells is intricate and multifaceted. Numerous studies have demonstrated that the acquisition of exogenous mitochondria by recipient cells results in the restoration of respiratory function, thereby enhancing cellular aggressiveness and resistance to chemotherapy [18, 83]. This indicates that the transferred mitochondria are capable of normal functionality within the recipient cell. One study has examined the long-term fate of foreign mitochondria in recipient cells and observed that these mitochondria eventually fuse with host lysosomes. Subsequently, the transferred mitochondria were progressively encapsulated in vesicles measuring 3–5 μm and excreted into EVs around day 8. These observations indicate that the transferred mitochondria may either be degraded by the host lysosome or expelled from the cells [84]. Mitochondria are dynamic organelles that continuously undergo fusion and fission processes to maintain functional complementarity and ensure mitochondrial quality control. The fate of the transferred mitochondria may vary, potentially influenced by mechanisms related to the quality control of mitochondria [85].

Currently, a range of experimental methodologies are available to investigate mitochondrial transfer between cells. MitoTracker dyes are widely utilized fluorescent probes that selectively stain mitochondria and can remain bound to these organelles even after cell death or fixation [86]. However, several limitations persist: Firstly, certain MitoTracker dyes depend on mitochondrial membrane potential, which can lead to non-specific staining in the cytoplasm. Secondly, MitoTracker dyes have the potential to disrupt mitochondrial networks, potentially causing cell death. Additionally, these dyes lack sufficient photostability to endure high-resolution, long-term stimulated emission depletion (STED) imaging [87, 88]. Recently, several alternative mitochondrial dyes have been introduced, complementing the classic MitoTracker dyes. MitoESq-635, a squaraine dye, has

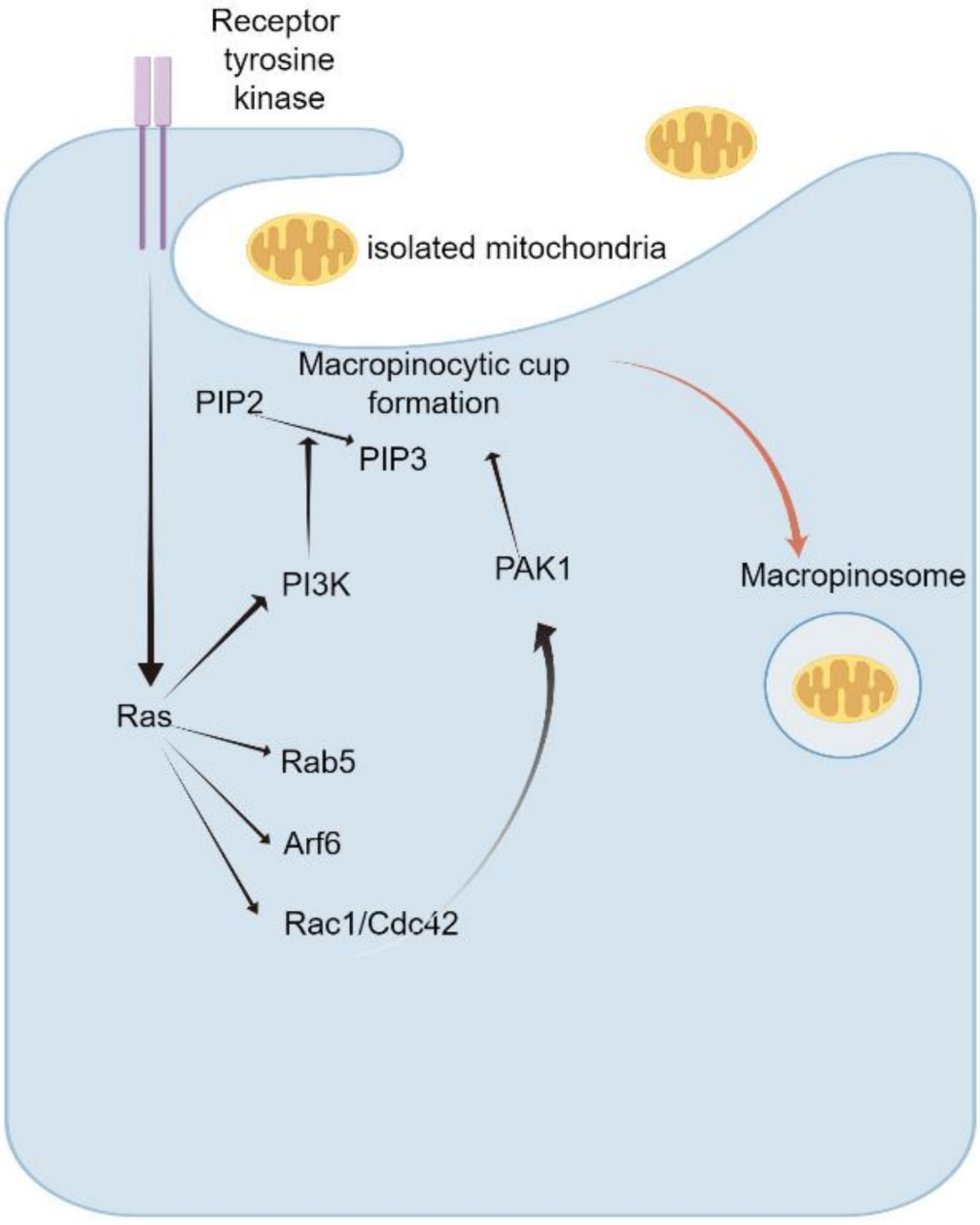

Fig. 4 Uptake of isolated mitochondria and implicated signaling pathways. Ras activates the subsequent cascades such as PI3K, Rab5, Arf6, and Rac1/Cdc42/PAK1, thereby mediating macropinocytic activity

been specifically designed for long-term live-cell STED imaging of mitochondria, enabling clear visualization of fusion and fission processes of mitochondria [89].

Plasmids and viral vectors have been employed to introduce fluorescent proteins tagged with a mitochondrial import sequence (e.g., mitoGFP, mitoRFP, mitoYFP, and mitoDsRed) into cells. These fluorescent proteins present a less toxic alternative to dyes and yield data that are less susceptible to confounding factors. However, these approaches also present limitations, including the induction of oxidative stress and apoptosis. Furthermore, accurately determining the precise localization of labeled donor mitochondria within or on the surface of recipient cells through microscopy remains challenging for image interpretation [90].

mtDNA polymorphisms have emerged as novel biomarkers for the detection of various diseases [91]. In co-cultured cells or tumor models, mtDNA polymorphisms

offer the most compelling evidence of acquired mtDNA within a recipient cell population, as determined through PCR amplification techniques. However, it is essential to identify stable polymorphisms in tumor cell lines [90]. Furthermore, assessing the respiratory capacity of recipient cells provides the most robust evidence for mitochondrial trafficking between cells [92]. Phenotypic changes and functional differences in recipient cells can also serve as indicators of intercellular mitochondrial transfer [83].

Cell types involved in intercellular mitochondrial transfer between ovarian cancer and tumor microenvironment

Between ovarian cancer cells

Intratumoral heterogeneity refers to the presence of cell subclones with varying phenotypes within a tumor, resulting in differences in cell proliferation, invasion, metastatic potential, and therapeutic response [93]. Certain subsets of cells engage in metabolic cooperation by sharing mitochondria, thereby synchronizing their response to drug therapy. Research has demonstrated that hypoxic conditions can induce the expression of HIF-1α and the formation of TNTs between ovarian cancer cells, facilitating mitochondrial transfer. Furthermore, inhibition of the mTOR pathway has been shown to suppress TNTs formation [94]. The unfolded protein response (UPR), triggered by factors such as hypoxia or chemotherapeutic agents, has been implicated in the promotion of polyploid giant cancer cells (PGCCs) through cell fusion in ovarian cancer cells [59]. Empirical evidence suggests that PGCCs in triple-negative breast cancer can generate mononuclear daughter cells filled with mitochondria and lipid droplets, thereby enhancing chemotherapy resistance through metabolic reprogramming [95]. Furthermore, the formation of TNTs has been observed at the tissue level in ovarian cancer. MitoTracker analysis further indicates that TNTs may facilitate intercellular mitochondrial transfer [96].

Ovarian cancer cells and immune cells

Immune suppression and immune evasion play a critical role in tumor progression. Tumor-associated macrophages (TAMs) are a crucial element of the TME and exist in two primary activation states: M1 TAMs, which secrete various pro-inflammatory factors and inhibit tumor growth, and M2 TAMs, which suppress the immune response and promote tumor growth and metastasis [97]. In a study by Cole JM et al., it was demonstrated that co-culturing M0, M1, and M2 macrophages with ovarian cancer cells induced morphological changes in the cancer cells and led to the formation of TNTs between ovarian cancer cells, and some of the structures were found to contain mitochondria. Subsequent research has suggested that the formation of the TNT structure may be facilitated by the EGFR/MAPK pathway [36]. A prior study demonstrated that the formation of heterotypic TNTs between macrophages and breast cancer cells promoted tumor cell invasion, a process also reliant on EGFR signaling [98]. However, in investigations related to ovarian cancer, TNTs have been observed to establish connections exclusively between tumor cells, rather than with macrophages.

Ovarian cancer cells and endothelial cells

As tumors expand and oxygen levels decline, they secrete soluble vascular endothelial growth factor (VEGF), which diffuses across the cellular space and is absorbed by the vascular endothelium. Research has demonstrated that ovarian cancer cells can form heterotypic TNTs with endothelial cells, thereby facilitating the transport of VEGF and HIF-1α [99]. In co-culture experiments, endothelial cells were paired with ovarian cancer cell lines (SKOV3 and OVCAR3) as well as breast cancer cell lines (MCF7 and MDA). The results indicated that the mitochondria of endothelial cells were transferred to all four cancer cell lines via TNTs. Further investigations have shown that breast cancer cells MCF7 acquire chemotherapy-resistant phenotype; however, this experiment was not conducted on ovarian cancer cell lines [100].

Ovarian cancer cells and mesothelial cells

Advanced ovarian cancer is frequently characterized by substantial ascites and intraperitoneal implantation. Ovarian cancer cells predominantly metastasize to the coelomic-lining mesothelial cells, with adhesion to these cells playing a crucial role in their dissemination. Recent research on ovarian cancer and mesothelial cells has primarily focused on the induction of apoptosis and the mesothelial-to-mesenchymal transition of mesothelial cells [101, 102]. When the volume of ascites exceeds 2 L, there is a significant increase in intraperitoneal pressure, rising from the normal level of 5 mmHg to as high as 22 mmHg, which enhances the adhesion of ovarian cancer cells to the peritoneum and promotes the formation of TNTs between ovarian cancer cells and peritoneal mesothelial cells, facilitating the transport of mitochondria [103]. Notably, TNTs were absent in co-cultures of mesothelial and tumor cells in the absence of external pressure. Additionally, when ovarian cancer cells were co-cultured with omental adipocytes, only a few membrane protrusions were observed [36]. Future research should investigate the interactions between ovarian cancer cells and adipocytes under conditions of external pressure.

Ovarian cancer cells and carcinoma-associated MSCs

Carcinoma-associated mesenchymal stem cells (CA-MSCs) represent pivotal stromal progenitor cells within the TME, playing a crucial role in promoting tumor cell

proliferation, enhancing cancer stemness, and increasing resistance to chemotherapy [104]. During the metastasis of ovarian cancer cells, MSCs undergo an epigenomic MET, resulting in the formation of CA-MSCs, which possess pro-tumorigenic properties and directly interact with cancer cells, thereby acting as drivers or facilitators of metastasis [105]. Empirical evidence indicates that CA-MSCs form heterocellular units with cancer cells, thereby fostering ovarian cancer metastasis and heterogeneity through the direct transfer of mitochondria. This mechanism of mitochondrial donation from CA-MSCs to cancer cells with low mitochondrial levels may contribute to cancer cell survival and metastatic potential. Further investigation revealed that knockdown of MIRO1 in CA-MSCs effectively precluded mitochondrial transfer to cancer cells [106]. A summary of the aforementioned studies on mitochondrial transfer in ovarian cancer is provided in Table 1.

Therapeutic strategies for intercellular mitochondrial transfer in ovarian cancer

TNTs inhibitors

Recent studies indicate that intercellular mitochondrial transfer between ovarian cancer cells and the TME predominantly occurs via TNTs. Targeting TNTs is emerging as a novel strategy for cancer treatment. These studies have shown that the formation of TNTs is induced under conditions of hypoxia or external stress, with the primary signaling pathways involved being the mTOR and EGFR/MAPK pathways. In vitro experiments have demonstrated that inhibitors of the mTOR pathway, such as everolimus and metformin, effectively suppress the formation of TNTs [94]. The ERK inhibitor SCH772984 has been shown to inhibit the formation of TNTs between ovarian cancer cells [36]. Additionally, a Miro1 reducer has been demonstrated to decrease Miro1 protein levels in a dose-dependent manner, an effect that is blocked by the proteasome inhibitor MG132 [107]. These studies primarily investigated the formation of TNTs and the proteins involved in mediating intercellular mitochondrial transfer. However, specific biomarkers of TNTs in cancer have yet to be identified. Future research should focus on a comprehensive exploration of TNTs as potential therapeutic targets.

EVs inhibitors

A substantial body of research on EVs focuses on liquid biopsies; however, some studies are investigating EV inhibitors as a research tool. Catalano et al. provided a comprehensive summary of several commonly used EV inhibitors, categorizing them based on their mechanisms of action. Certain inhibitors specifically target the trafficking pathways of EVs, including calpeptin, manumycin A, and Y27632. Calpeptin is known to inhibit calpain activity, thereby affecting cytoskeletal dynamics essential for vesicle transport. Manumycin A interferes with Rho GTPase signaling pathways, which are critical for cell motility and membrane trafficking processes essential for EV release. Y27632 specifically inhibits Rho-associated protein kinase (ROCK), thereby modulating actin cytoskeleton organization and subsequently influencing the secretion of both MVs and exosomes. Other inhibitors primarily affect lipid metabolism within cells that produce EVs. Notable examples include pantethine, an intermediate in coenzyme A synthesis; imipramine, a tricyclic antidepressant that alters cellular lipid profiles; and GW4869, a compound known for its inhibition of neutral sphingomyelinase 2 activity, which results in reduced exosome production [108]. Moreover, recent finding indicates that heparin may play an inhibitory role regarding the uptake mechanisms associated with EVs in ovarian cancer models [109].

Table 1 Mitochondrial transfer studies in ovarian cancer

Donor cells	Recipient cells	Mechanism	Triggers	Cellular effect	Reference
ovarian cancer cells	ovarian cancer cells	TNTs	hypoxia	ND	[94]
ovarian cancer cells	ovarian cancer cells	TNTs	ND	ND	[96]
ovarian cancer cells	ovarian cancer cells	cell fusion	UPR	invasion	[59]
ovarian cancer cells	ovarian cancer cells	TNTs	macrophage-conditioned media	ND	[36]
endothelial cells	ovarian cancer cells	TNTs	ND	ND	[100]
mesothelial cells	ovarian cancer cells	TNTs	ascites-induced compression	ND	[103]
CA-MSCs	ovarian cancer cells	ND	hypoxia	increased proliferation, chemoresistance, metabolic fitness	[106]
MSCs	ovarian cancer cells	cell fusion	ND	reduced proliferation	[65]
human cardiac fibroblast cells	ovarian cancer cells	isolated mitochondrial transplantation	ND	chemotherapeutic sensitivity	[70]

ND: not defined; UPR: unfolded protein response; CA-MSCs: carcinoma-associated mesenchymal stem cells; MSCs: mesenchymal stem cells

Cell fusion inhibitors

Hypoxia, TNFα, and associated signaling pathways are intricately linked to the process of cell fusion. In the context of breast cancer, TNFα-induced cell fusion is inhibited by minocycline through the targeting of the NF-κB pathway [110]. Emerging evidence indicates the potential for targeting syncytins to mitigate cell fusion in cancer. Strick et al. demonstrated that syncytin-1 is implicated in cell fusion in endometrial cancer cells, contingent upon steroid hormones or cAMP activation. Furthermore, posttranscriptional silencing of syncytin-1 gene expression, or the application of TGF-β1 and TGF-β3, can effectively inhibit cell fusion [111]. In another study, the knockdown of syncytin-2 or its receptor MFSD-2 A in MDA-MB-231 cells inhibits intercellular membrane fusion [112]. Furthermore, anti-VLA-4 or anti-VCAM-1 treatment can also impede the process of cell fusion [60].

Exogeneous mitochondrial transfer enhancer

Previous studies have demonstrated that the ingestion of exogenous mitochondria can mitigate the malignant biological behavior of cancer cells; however, the efficiency of this transfer process remains suboptimal. In recent years, various methodologies have been developed to facilitate the introduction of isolated exogenous mitochondria into cells. Notably, in the context of acute myocardial infarction, mitochondria complexed with transactivator of transcription dextran (TAT-dextran) have exhibited significantly enhanced cellular uptake [113]. Additionally, centrifugation has been reported as a straightforward and effective method for mitochondrial transfer across various cell types, including cancer cell lines and MSCs [114]. This system demonstrates potential adaptability for both adherent and suspension cells. Methodologies employing anti-mitochondrial import receptor TOM22 magnetic beads, in conjunction with a magnetic plate, facilitate direct mitochondrial transfer [115]. However, there is a concern regarding the retention of magnetic beads within cells for up to four days post-transplantation, and the precise impact of these beads on cellular function remains undetermined. Additionally, photothermal nanoblades, which utilize laser pulse-induced light energy, produce highly localized, shaped, and explosive cavitation bubbles that transiently disrupt cell membranes. This technique allows for the perforation of cell membranes and the transfer of mitochondria into cells [116].

Conclusions and future expectations

The significance of intercellular mitochondrial transfer in the progression and chemoresistance of ovarian cancer is increasingly being recognized. Recent research has demonstrated that mitochondria, the organelles responsible for cellular energy production, can be transferred between adjacent cells via mechanisms such as TNTs or cell fusion. This mitochondrial transfer may play a pivotal role in tumor biology by modulating metabolic pathways, enhancing cell survival under stress conditions, and contributing to chemotherapy resistance. Nonetheless, several questions remain unanswered concerning the energy cooperation and tumor heterogeneity underlying intercellular mitochondrial transfer.

As is well established, defective mitochondrial function plays a crucial role in tumorigenesis and progression, characterized by increased aerobic glycolysis. Tumors exhibit two distinct metabolic phenotypes: highly glycolytic and OXPHOS-dependent phenotypes [117]. The balance between glycolytic and oxidative energy metabolism is a dynamic process during the initiation and progression of cancer. Tumor cells with a highly glycolytic phenotype primarily depend on glycolysis for energy production and demonstrate increased aggressiveness. However, purely glycolytic ρ^0 tumor cells are unable to form tumors due to the absence of mitochondrial electron transport, unless they acquire mitochondria from adjacent cells [118, 119]. Previous research has demonstrated that ovarian cancer cells internalize isolated mitochondria, thereby attenuating the Warburg effect and enhancing apoptosis [70]. Furthermore, a substantial body of literature suggests that ovarian cancer cells acquire mitochondria from the TME, which facilitates the restoration of respiratory function and contributes to increased aggressiveness and resistance to chemotherapeutic agents [36, 100, 103, 106]. The duality of intercellular mitochondrial transfer appears to be intricately linked to energy metabolism in cancer [120]. Moreover, these findings predominantly arise from in vitro cytological experiments, the role of mitochondrial transfer in vivo warrants further investigation [120].

The directionality of intercellular mitochondrial transfer, whether unidirectional or bidirectional, appears to be contingent upon cell type and disease context [14]. In bidirectional scenarios, the delineation between donor and recipient cells remains ambiguous. The mutual exchange of mitochondria theoretically influences both cell types. Current research predominantly addresses the transfer of mitochondria from the TME to ovarian cancer cells; however, the reverse transfer, from ovarian cancer cells to the TME, and its subsequent effects on the TME have not been adequately explored. Furthermore, the competition among various cell types may significantly influence mitochondrial transfer within tumor tissue, which is a complex environment characterized by cellular diversity. Consequently, co-culture studies involving multiple cell types should be conducted to elucidate this phenomenon more comprehensively in future research. Overall, ongoing investigations into intercellular mitochondrial communication are promising, as they not only

enhance our understanding of ovarian cancer biology but also have the potential to inform the development of novel therapeutic strategies.

Abbreviations

MOMP	Mitochondrial outer membrane permeabilization
OXPHOS	Oxidative phosphorylation
mtDNA	mitochondrial DNA
ATP	Adenosine triphosphate
Ca^{2+}	Calcium
ROS	Reactive oxygen species
MSCs	Mesenchymal stem cells
TME	Tumor microenvironment
TNTs	Tunneling nanotubes
mTOR	Mammalian target of rapamycin
CDC42	Cell division control protein 42 homolog
EGFR	Epidermal growth factor receptor
KIF5	Kinesin family motor protein 5
EVs	Extracellular vesicles
MVs	Microvesicles
ApoBDs	Apoptotic bodies
ESCRT	Endosomal sorting complex required for transport
TSG101	Tumor susceptibility gene 101
ARRDC1	Arrestin domain-containing 1
ARMMs	ARRDC1-mediated MVs
CD38	Cluster of differentiation 38
NAD^+	Nicotinamide adenine dinucleotide
cADPR	Cyclic ADP ribose
TNFα	Tumor necrosis factor alpha
HERV	Human endogenous retroviral
MET	Mesenchymal-to-epithelial transition
CD9	Cluster of differentiation 9
CD47	Cluster of differentiation 47
Pak1	p21-activated kinase 1
GJs	Gap junctions
Cx43	Connexin 43
UPR	Unfolded protein response
PGCCs	Polyploid giant cancer cells
TAMs	Tumor-associated macrophages
VEGF	Vascular endothelial growth factor
CA-MSCs	Carcinoma-associated mesenchymal stem cells
ROCK	Rho-associated protein kinase
STED	Stimulated emission depletion

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References

1. Gupta A, Becker T. Mechanisms and pathways of mitochondrial outer membrane protein biogenesis. Biochim Biophys Acta Bioenerg. 2021;1862(1):148323.
2. Bock FJ, Tait SWG. Mitochondria as multifaceted regulators of cell death. Nat Rev Mol Cell Biol. 2020;21(2):85–100.
3. Andrieux P, Chevillard C, Cunha-Neto E, Nunes JPS. Mitochondria as a Cellular hub in infection and inflammation. Int J Mol Sci 2021, 22(21).
4. Gustafsson CM, Falkenberg M, Larsson NG. Maintenance and expression of mammalian mitochondrial DNA. Annu Rev Biochem. 2016;85:133–60.
5. Monzel AS, Enriquez JA, Picard M. Multifaceted mitochondria: moving mitochondrial science beyond function and dysfunction. Nat Metab. 2023;5(4):546–62.
6. Annesley SJ, Fisher PR. Mitochondria in Health and Disease. *Cells* 2019, 8(7).
7. Chen XY, Ren HH, Wang D, Chen Y, Qu CJ, Pan ZH, Liu XN, Hao WJ, Xu WJ, Wang KJ et al. Isoliquiritigenin Induces Mitochondrial Dysfunction and Apoptosis by Inhibiting mitoNEET in a Reactive Oxygen Species-Dependent Manner in A375 Human Melanoma Cells. *Oxid Med Cell Longev* 2019, 2019:9817576.
8. Dou JP, Wu Q, Fu CH, Zhang DY, Yu J, Meng XW, Liang P. Amplified intracellular ca(2+) for synergistic anti-tumor therapy of microwave ablation and chemotherapy. J Nanobiotechnol. 2019;17(1):118.
9. Tian X, Li S, Zeng Q, Huang W, Liu X, Song S. Relationship between loss of desiccation tolerance and programmed cell death (PCD) in mung bean (Vigna radiata) seeds. PLoS ONE. 2019;14(7):e0218513.
10. Mendelsohn DH, Schnabel K, Mamilos A, Sossalla S, Pabel S, Duerr GD, Keller K, Schmitt VH, Barsch F, Walter N et al. Structural Analysis of Mitochondrial dynamics-from cardiomyocytes to osteoblasts: a critical review. Int J Mol Sci 2022, 23(9).
11. Shen L, Zhan X. Mitochondrial Dysfunction Pathway Alterations Offer Potential Biomarkers and Therapeutic Targets for Ovarian Cancer. *Oxid Med Cell Longev* 2022, 2022:5634724.
12. Charoenkwan K, Apaijai N, Sriwichaiin S, Chattipakorn N, Chattipakorn SC. Alterations in mitochondria isolated from peripheral blood mononuclear cells and tumors of patients with epithelial ovarian cancers. Sci Rep. 2024;14(1):15.
13. Shanmughapriya S, Langford D, Natarajaseenivasan K. Inter and intracellular mitochondrial trafficking in health and disease. Ageing Res Rev. 2020;62:101128.
14. Zampieri LX, Silva-Almeida C, Rondeau JD, Sonveaux P. Mitochondrial transfer in Cancer: a Comprehensive Review. Int J Mol Sci 2021, 22(6).
15. Spees JL, Olson SD, Whitney MJ, Prockop DJ. Mitochondrial transfer between cells can rescue aerobic respiration. Proc Natl Acad Sci U S A. 2006;103(5):1283–8.
16. Yao X, Ma Y, Zhou W, Liao Y, Jiang Z, Lin J, He Q, Wu H, Wei W, Wang X, et al. In-cytoplasm mitochondrial transplantation for mesenchymal stem cells engineering and tissue regeneration. Bioeng Transl Med. 2022;7(1):e10250.
17. Jain R, Begum N, Tryphena KP, Singh SB, Srivastava S, Rai SN, Vamanu E, Khatri DK. Inter and intracellular mitochondrial transfer: future of mitochondrial transplant therapy in Parkinson's disease. Biomed Pharmacother. 2023;159:114268.
18. Saha T, Dash C, Jayabalan R, Khiste S, Kulkarni A, Kurmi K, Mondal J, Majumder PK, Bardia A, Jang HL, et al. Intercellular nanotubes mediate mitochondrial trafficking between cancer and immune cells. Nat Nanotechnol. 2022;17(1):98–106.
19. Luo Z, Wang Q, Lau WB, Lau B, Xu L, Zhao L, Yang H, Feng M, Xuan Y, Yang Y, et al. Tumor microenvironment: the culprit for ovarian cancer metastasis? Cancer Lett. 2016;377(2):174–82.
20. Schoutrop E, Moyano-Galceran L, Lheureux S, Mattsson J, Lehti K, Dahlstrand H, Magalhaes I. Molecular, cellular and systemic aspects of epithelial ovarian cancer and its tumor microenvironment. Semin Cancer Biol. 2022;86(Pt 3):207–23.
21. Martinez-Outschoorn UE, Sotgia F, Lisanti MP. Power surge: supporting cells fuel cancer cell mitochondria. Cell Metab. 2012;15(1):4–5.
22. Kimura S, Hase K, Ohno H. The molecular basis of induction and formation of tunneling nanotubes. Cell Tissue Res. 2013;352(1):67–76.
23. Rustom A, Saffrich R, Markovic I, Walther P, Gerdes HH. Nanotubular highways for intercellular organelle transport. Science. 2004;303(5660):1007–10.

24. Dupont M, Souriant S, Lugo-Villarino G, Maridonneau-Parini I, Verollet C. Tunneling nanotubes: intimate communication between myeloid cells. Front Immunol. 2018;9:43.
25. Luchetti F, Carloni S, Nasoni MG, Reiter RJ, Balduini W. Tunneling nanotubes and mesenchymal stem cells: new insights into the role of melatonin in neuronal recovery. J Pineal Res. 2022;73(1):e12800.
26. Roehlecke C, Schmidt MHH. Tunneling nanotubes and Tumor microtubes in Cancer. Cancers (Basel) 2020, 12(4).
27. Chinnery HR, Pearlman E, McMenamin PG. Cutting edge: membrane nanotubes in vivo: a feature of MHC class II + cells in the mouse cornea. J Immunol. 2008;180(9):5779–83.
28. Austefjord MW, Gerdes HH, Wang X. Tunneling nanotubes: diversity in morphology and structure. Commun Integr Biol. 2014;7(1):e27934.
29. Jahnke R, Matthiesen S, Zaeck LM, Finke S, Knittler MR. Chlamydia trachomatis cell-to-cell spread through tunneling nanotubes. Microbiol Spectr. 2022;10(6):e0281722.
30. Smith IF, Shuai J, Parker I. Active generation and propagation of Ca2 + signals within tunneling membrane nanotubes. Biophys J. 2011;100(8):L37–39.
31. Barutta F, Bellini S, Kimura S, Hase K, Corbetta B, Corbelli A, Fiordaliso F, Bruno S, Biancone L, Barreca A, et al. Protective effect of the tunneling nanotube-TNFAIP2/M-sec system on podocyte autophagy in diabetic nephropathy. Autophagy. 2023;19(2):505–24.
32. Wang S, Li Y, Zhao Y, Lin F, Qu J, Liu L. Investigating tunneling nanotubes in ovarian cancer based on two-photon excitation FLIM-FRET. Biomed Opt Express. 2021;12(4):1962–73.
33. Sherer NM, Mothes W. Cytonemes and tunneling nanotubules in cell-cell communication and viral pathogenesis. Trends Cell Biol. 2008;18(9):414–20.
34. Lou E, Fujisawa S, Morozov A, Barlas A, Romin Y, Dogan Y, Gholami S, Moreira AL, Manova-Todorova K, Moore MA. Tunneling nanotubes provide a unique conduit for intercellular transfer of cellular contents in human malignant pleural mesothelioma. PLoS ONE. 2012;7(3):e33093.
35. Walters HE, Cox LS. Intercellular Transfer of Mitochondria between Senescent Cells through Cytoskeleton-Supported Intercellular Bridges Requires mTOR and CDC42 Signalling. *Oxid Med Cell Longev* 2021, 2021:6697861.
36. Cole JM, Dahl R, Cowden Dahl KD. MAPK signaling is required for generation of Tunneling Nanotube-Like structures in Ovarian Cancer cells. Cancers (Basel) 2021, 13(2).
37. Lopez-Domenech G, Covill-Cooke C, Ivankovic D, Halff EF, Sheehan DF, Norkett R, Birsa N, Kittler JT. Miro proteins coordinate microtubule- and actin-dependent mitochondrial transport and distribution. EMBO J. 2018;37(3):321–36.
38. Loss O, Stephenson FA. Developmental changes in trak-mediated mitochondrial transport in neurons. Mol Cell Neurosci. 2017;80:134–47.
39. Quintero OA, DiVito MM, Adikes RC, Kortan MB, Case LB, Lier AJ, Panaretos NS, Slater SQ, Rengarajan M, Feliu M, et al. Human Myo19 is a novel myosin that associates with mitochondria. Curr Biol. 2009;19(23):2008–13.
40. Macaskill AF, Rinholm JE, Twelvetrees AE, Arancibia-Carcamo IL, Muir J, Fransson A, Aspenstrom P, Attwell D, Kittler JT. Miro1 is a calcium sensor for glutamate receptor-dependent localization of mitochondria at synapses. Neuron. 2009;61(4):541–55.
41. Ahmad T, Mukherjee S, Pattnaik B, Kumar M, Singh S, Kumar M, Rehman R, Tiwari BK, Jha KA, Barhanpurkar AP, et al. Miro1 regulates intercellular mitochondrial transport & enhances mesenchymal stem cell rescue efficacy. EMBO J. 2014;33(9):994–1010.
42. Witwer KW. Minimal information for studies of extracellular vesicles 2023: relevance to cell and gene therapies. Cytotherapy; 2024.
43. Jiang Y, Liu X, Ye J, Ma Y, Mao J, Feng D, Wang X. Migrasomes, a new mode of intercellular communication. Cell Commun Signal. 2023;21(1):105.
44. Mager SELA, Breakefield I, Wood XO. Extracellular vesicles: biology and emerging therapeutic opportunities. Nat Rev Drug Discov. 2013;12(5):347–57.
45. D'Souza A, Burch A, Dave KM, Sreeram A, Reynolds MJ, Dobbins DX, Kamte YS, Zhao W, Sabatelle C, Joy GM, et al. Microvesicles transfer mitochondria and increase mitochondrial function in brain endothelial cells. J Control Release. 2021;338:505–26.
46. Kalluri R, LeBleu VS. The biology, function, and biomedical applications of exosomes. Science 2020, 367(6478).
47. Xu X, Lai Y, Hua ZC. Apoptosis and apoptotic body: disease message and therapeutic target potentials. Biosci Rep 2019, 39(1).
48. Gregory CD, Rimmer MP. Extracellular vesicles arising from apoptosis: forms, functions, and applications. J Pathol. 2023;260(5):592–608.
49. Jiao H, Jiang D, Hu X, Du W, Ji L, Yang Y, Li X, Sho T, Wang X, Li Y, et al. Mitocytosis, a migrasome-mediated mitochondrial quality-control process. Cell. 2021;184(11):2896–e29102813.
50. Nabhan JF, Hu R, Oh RS, Cohen SN, Lu Q. Formation and release of arrestin domain-containing protein 1-mediated microvesicles (ARMMs) at plasma membrane by recruitment of TSG101 protein. Proc Natl Acad Sci U S A. 2012;109(11):4146–51.
51. Phinney DG, Di Giuseppe M, Njah J, Sala E, Shiva S, St Croix CM, Stolz DB, Watkins SC, Di YP, Leikauf GD, et al. Mesenchymal stem cells use extracellular vesicles to outsource mitophagy and shuttle microRNAs. Nat Commun. 2015;6:8472.
52. De Flora A, Franco L, Guida L, Bruzzone S, Zocchi E. Ectocellular CD38-catalyzed synthesis and intracellular ca(2+)-mobilizing activity of cyclic ADP-ribose. Cell Biochem Biophys. 1998;28(1):45–62.
53. Hayakawa K, Esposito E, Wang X, Terasaki Y, Liu Y, Xing C, Ji X, Lo EH. Transfer of mitochondria from astrocytes to neurons after stroke. Nature. 2016;535(7613):551–5.
54. Suh J, Kim NK, Shim W, Lee SH, Kim HJ, Moon E, Sesaki H, Jang JH, Kim JE, Lee YS. Mitochondrial fragmentation and donut formation enhance mitochondrial secretion to promote osteogenesis. Cell Metab. 2023;35(2):345–60. e347.
55. Croft PK, Sharma S, Godbole N, Rice GE, Salomon C. Ovarian-Cancer-Associated Extracellular vesicles: Microenvironmental Regulation and potential clinical applications. Cells 2021, 10(9).
56. Torralba D, Baixauli F, Sanchez-Madrid F. Mitochondria Know No boundaries: mechanisms and functions of intercellular mitochondrial transfer. Front Cell Dev Biol. 2016;4:107.
57. Sieler M, Dittmar T. Cell Fusion and Syncytia formation in Cancer. Results Probl Cell Differ. 2024;71:433–65.
58. Melzer C, von der Ohe J, Hass R. In vivo Cell Fusion between Mesenchymal Stroma/Stem-Like cells and breast Cancer cells. Cancers (Basel) 2019, 11(2).
59. Yart L, Bastida-Ruiz D, Allard M, Dietrich PY, Petignat P, Cohen M. Linking unfolded protein response to ovarian cancer cell fusion. BMC Cancer. 2022;22(1):622.
60. Song K, Zhu F, Zhang HZ, Shang ZJ. Tumor necrosis factor-alpha enhanced fusions between oral squamous cell carcinoma cells and endothelial cells via VCAM-1/VLA-4 pathway. Exp Cell Res. 2012;318(14):1707–15.
61. Larsen JM, Christensen IJ, Nielsen HJ, Hansen U, Bjerregaard B, Talts JF, Larsson LI. Syncytin immunoreactivity in colorectal cancer: potential prognostic impact. Cancer Lett. 2009;280(1):44–9.
62. Menendez L, Benigno BB, McDonald JF. L1 and HERV-W retrotransposons are hypomethylated in human ovarian carcinomas. Mol Cancer. 2004;3:12.
63. Strissel PL, Ruebner M, Thiel F, Wachter D, Ekici AB, Wolf F, Thieme F, Ruprecht K, Beckmann MW, Strick R. Reactivation of codogenic endogenous retroviral (ERV) envelope genes in human endometrial carcinoma and prestages: emergence of new molecular targets. Oncotarget. 2012;3(10):1204–19.
64. Yan TL, Wang M, Xu Z, Huang CM, Zhou XC, Jiang EH, Zhao XP, Song Y, Song K, Shao Z, et al. Up-regulation of syncytin-1 contributes to TNF-alpha-enhanced fusion between OSCC and HUVECs partly via Wnt/beta-catenin-dependent pathway. Sci Rep. 2017;7:40983.
65. Melzer C, von der Ohe J, Hass R. MSC stimulate ovarian tumor growth during intercellular communication but reduce tumorigenicity after fusion with ovarian cancer cells. Cell Commun Signal. 2018;16(1):67.
66. Fei F, Li C, Wang X, Du J, Liu K, Li B, Yao P, Li Y, Zhang S. Syncytin 1, CD9, and CD47 regulating cell fusion to form PGCCs associated with cAMP/PKA and JNK signaling pathway. Cancer Med. 2019;8(6):3047–58.
67. Zhang H, Ma H, Yang X, Fan L, Tian S, Niu R, Yan M, Zheng M, Zhang S. Cell Fusion-Related proteins and Signaling pathways, and their roles in the Development and Progression of Cancer. Front Cell Dev Biol. 2021;9:809668.
68. Al Amir Dache Z, Otandault A, Tanos R, Pastor B, Meddeb R, Sanchez C, Arena G, Lasorsa L, Bennett A, Grange T, et al. Blood contains circulating cell-free respiratory competent mitochondria. FASEB J. 2020;34(3):3616–30.
69. Stier A. Human blood contains circulating cell-free mitochondria, but are they really functional? Am J Physiol Endocrinol Metab. 2021;320(5):E859–63.
70. Celik A, Orfany A, Dearling J, Del Nido PJ, McCully JD, Bakar-Ates F. Mitochondrial transplantation: effects on chemotherapy in prostate and ovarian cancer cells in vitro and in vivo. Biomed Pharmacother. 2023;161:114524.
71. Paul S, Ghosh S, Kumar S. Tumor glycolysis, an essential sweet tooth of tumor cells. Semin Cancer Biol. 2022;86(Pt 3):1216–30.
72. Malefyt AP, Walton SP, Chan C. Endocytosis pathways for nucleic acid therapeutics. Nano Life. 2012;2(3):1241005.

73. Kitani T, Kami D, Matoba S, Gojo S. Internalization of isolated functional mitochondria: involvement of macropinocytosis. J Cell Mol Med. 2014;18(8):1694–703.
74. Kesner EE, Saada-Reich A, Lorberboum-Galski H. Characteristics of mitochondrial Transformation into Human cells. Sci Rep. 2016;6:26057.
75. Lim JP, Gleeson PA. Macropinocytosis: an endocytic pathway for internalising large gulps. Immunol Cell Biol. 2011;89(8):836–43.
76. Xiao F, Li J, Huang K, Li X, Xiong Y, Wu M, Wu L, Kuang W, Lv S, Wu L, et al. Macropinocytosis: mechanism and targeted therapy in cancers. Am J Cancer Res. 2021;11(1):14–30.
77. Herve JC, Derangeon M. Gap-junction-mediated cell-to-cell communication. Cell Tissue Res. 2013;352(1):21–31.
78. Bonacquisti EE, Nguyen J. Connexin 43 (Cx43) in cancer: implications for therapeutic approaches via gap junctions. Cancer Lett. 2019;442:439–44.
79. Qin Y, Jiang X, Yang Q, Zhao J, Zhou Q, Zhou Y. The functions, methods, and mobility of mitochondrial transfer between cells. Front Oncol. 2021;11:672781.
80. Norris RP. Transfer of mitochondria and endosomes between cells by gap junction internalization. Traffic. 2021;22(6):174–9.
81. Toler CR, Taylor DD, Gercel-Taylor C. Loss of communication in ovarian cancer. Am J Obstet Gynecol. 2006;194(5):e27–31.
82. Qiu X, Cheng JC, Klausen C, Chang HM, Fan Q, Leung PC. EGF-Induced Connexin43 negatively regulates cell proliferation in human ovarian Cancer. J Cell Physiol. 2016;231(1):111–9.
83. Goliwas KF, Libring S, Berestesky E, Gholizadeh S, Schwager SC, Frost AR, Gaborski TR, Zhang J, Reinhart-King CA. Mitochondrial transfer from cancer-associated fibroblasts increases migration in aggressive breast cancer. J Cell Sci 2023, 136(14).
84. Jiang D, Chen FX, Zhou H, Lu YY, Tan H, Yu SJ, Yuan J, Liu H, Meng W, Jin ZB. Bioenergetic Crosstalk between Mesenchymal Stem cells and various ocular cells through the intercellular trafficking of Mitochondria. Theranostics. 2020;10(16):7260–72.
85. Ashrafi G, Schwarz TL. The pathways of mitophagy for quality control and clearance of mitochondria. Cell Death Differ. 2013;20(1):31–42.
86. Chazotte B. Labeling mitochondria with MitoTracker dyes. Cold Spring Harb Protoc. 2011;2011(8):990–2.
87. Neikirk K, Marshall AG, Kula B, Smith N, LeBlanc S, Hinton A Jr. MitoTracker: a useful tool in need of better alternatives. Eur J Cell Biol. 2023;102(4):151371.
88. Niehorster T, Loschberger A, Gregor I, Kramer B, Rahn HJ, Patting M, Koberling F, Enderlein J, Sauer M. Multi-target spectrally resolved fluorescence lifetime imaging microscopy. Nat Methods. 2016;13(3):257–62.
89. Yang X, Yang Z, Wu Z, He Y, Shan C, Chai P, Ma C, Tian M, Teng J, Jin D, et al. Mitochondrial dynamics quantitatively revealed by STED nanoscopy with an enhanced squaraine variant probe. Nat Commun. 2020;11(1):3699.
90. Berridge MV, Herst PM, Rowe MR, Schneider R, McConnell MJ. Mitochondrial transfer between cells: methodological constraints in cell culture and animal models. Anal Biochem. 2018;552:75–80.
91. Bussard KM, Siracusa LD. Understanding mitochondrial polymorphisms in Cancer. Cancer Res. 2017;77(22):6051–9.
92. Peruzzotti-Jametti L, Bernstock JD, Willis CM, Manferrari G, Rogall R, Fernandez-Vizarra E, Williamson JC, Braga A, van den Bosch A, Leonardi T, et al. Neural stem cells traffic functional mitochondria via extracellular vesicles. PLoS Biol. 2021;19(4):e3001166.
93. Pu T, Zhang C, Su B, Li L, Fu J. Research progress in intratumoral heterogeneity and clinical significance of ovarian cancer. Med (Baltim). 2024;103(4):e36074.
94. Desir S, Dickson EL, Vogel RI, Thayanithy V, Wong P, Teoh D, Geller MA, Steer CJ, Subramanian S, Lou E. Tunneling nanotube formation is stimulated by hypoxia in ovarian cancer cells. Oncotarget. 2016;7(28):43150–61.
95. Sirois I, Aguilar-Mahecha A, Lafleur J, Fowler E, Vu V, Scriver M, Buchanan M, Chabot C, Ramanathan A, Balachandran B, et al. A unique morphological phenotype in Chemoresistant Triple-negative breast Cancer reveals metabolic reprogramming and PLIN4 expression as a molecular vulnerability. Mol Cancer Res. 2019;17(12):2492–507.
96. Thayanithy V, Dickson EL, Steer C, Subramanian S, Lou E. Tumor-stromal cross talk: direct cell-to-cell transfer of oncogenic microRNAs via tunneling nanotubes. Transl Res. 2014;164(5):359–65.
97. Shapouri-Moghaddam A, Mohammadian S, Vazini H, Taghadosi M, Esmaeili SA, Mardani F, Seifi B, Mohammadi A, Afshari JT, Sahebkar A. Macrophage plasticity, polarization, and function in health and disease. J Cell Physiol. 2018;233(9):6425–40.
98. Hanna SJ, McCoy-Simandle K, Leung E, Genna A, Condeelis J, Cox D. Tunneling nanotubes, a novel mode of tumor cell-macrophage communication in tumor cell invasion. J Cell Sci 2019, 132(3).
99. Lou E, Zhai E, Sarkari A, Desir S, Wong P, Iizuka Y, Yang J, Subramanian S, McCarthy J, Bazzaro M, et al. Cellular and Molecular networking within the ecosystem of Cancer Cell Communication via Tunneling nanotubes. Front Cell Dev Biol. 2018;6:95.
100. Pasquier J, Guerrouahen BS, Al Thawadi H, Ghiabi P, Maleki M, Abu-Kaoud N, Jacob A, Mirshahi M, Galas L, Rafii S, et al. Preferential transfer of mitochondria from endothelial to cancer cells through tunneling nanotubes modulates chemoresistance. J Transl Med. 2013;11:94.
101. Pascual-Anton L, Cardenes B, de la Sainz R, Gonzalez-Cortijo L, Lopez-Cabrera M, Cabanas C, Sandoval P. Mesothelial-to-mesenchymal transition and Exosomes in Peritoneal Metastasis of Ovarian Cancer. Int J Mol Sci 2021, 22(21).
102. Gao L, Nie X, Gou R, Hu Y, Dong H, Li X, Lin B. Exosomal ANXA2 derived from ovarian cancer cells regulates epithelial-mesenchymal plasticity of human peritoneal mesothelial cells. J Cell Mol Med. 2021;25(23):10916–29.
103. Asem M, Young A, Oyama C, ClaureDeLaZerda A, Liu Y, Ravosa MJ, Gupta V, Jewell A, Khabele D, Stack MS. Ascites-induced compression alters the peritoneal microenvironment and promotes metastatic success in ovarian cancer. Sci Rep. 2020;10(1):11913.
104. Coffman LG, Pearson AT, Frisbie LG, Freeman Z, Christie E, Bowtell DD, Buckanovich RJ. Ovarian Carcinoma-Associated Mesenchymal stem cells arise from tissue-specific normal stroma. Stem Cells. 2019;37(2):257–69.
105. Fan H, Atiya HI, Wang Y, Pisanic TR, Wang TH, Shih IM, Foy KK, Frisbie L, Buckanovich RJ, Chomiak AA, et al. Epigenomic reprogramming toward mesenchymal-epithelial transition in Ovarian-Cancer-Associated Mesenchymal stem cells drives Metastasis. Cell Rep. 2020;33(10):108473.
106. Frisbie L, Pressimone C, Dyer E, Baruwal R, Garcia G, St Croix C, Watkins S, Calderone M, Gorecki G, Javed Z, et al. Carcinoma-associated mesenchymal stem cells promote ovarian cancer heterogeneity and metastasis through mitochondrial transfer. Cell Rep. 2024;43(8):114551.
107. Hsieh CH, Li L, Vanhauwaert R, Nguyen KT, Davis MD, Bu G, Wszolek ZK, Wang X. Miro1 Marks Parkinson's Disease Subset and Miro1 Reducer rescues neuron loss in Parkinson's models. Cell Metab. 2019;30(6):1131–e11401137.
108. Catalano M, O'Driscoll L. Inhibiting extracellular vesicles formation and release: a review of EV inhibitors. J Extracell Vesicles. 2020;9(1):1703244.
109. Samuel P, Mulcahy LA, Furlong F, McCarthy HO, Brooks SA, Fabbri M, Pink RC, Carter DRF. Cisplatin induces the release of extracellular vesicles from ovarian cancer cells that can induce invasiveness and drug resistance in bystander cells. *Philos Trans R Soc Lond B Biol Sci* 2018, 373(1737).
110. Weiler J, Dittmar T. Minocycline impairs TNF-alpha-induced cell fusion of M13SV1-Cre cells with MDA-MB-435-pFDR1 cells by suppressing NF-kappaB transcriptional activity and its induction of target-gene expression of fusion-relevant factors. Cell Commun Signal. 2019;17(1):71.
111. Strick R, Ackermann S, Langbein M, Swiatek J, Schubert SW, Hashemolhosseini S, Koscheck T, Fasching PA, Schild RL, Beckmann MW, et al. Proliferation and cell-cell fusion of endometrial carcinoma are induced by the human endogenous retroviral Syncytin-1 and regulated by TGF-beta. J Mol Med (Berl). 2007;85(1):23–38.
112. Zhang C, Schekman R. Syncytin-mediated open-ended membrane tubular connections facilitate the intercellular transfer of cargos including Cas9 protein. Elife 2023, 12.
113. Maeda H, Kami D, Maeda R, Murata Y, Jo JI, Kitani T, Tabata Y, Matoba S, Gojo S. TAT-dextran-mediated mitochondrial transfer enhances recovery from models of reperfusion injury in cultured cardiomyocytes. J Cell Mol Med. 2020;24(9):5007–20.
114. Kim MJ, Hwang JW, Yun CK, Lee Y, Choi YS. Delivery of exogenous mitochondria via centrifugation enhances cellular metabolic function. Sci Rep. 2018;8(1):3330.
115. Macheiner T, Fengler VH, Agreiter M, Eisenberg T, Madeo F, Kolb D, Huppertz B, Ackbar R, Sargsyan K. Magnetomitotransfer: an efficient way for direct mitochondria transfer into cultured human cells. Sci Rep. 2016;6:35571.
116. Wu TH, Sagullo E, Case D, Zheng X, Li Y, Hong JS, TeSlaa T, Patananan AN, McCaffery JM, Niazi K, et al. Mitochondrial transfer by Photothermal Nanoblade restores Metabolite Profile in mammalian cells. Cell Metab. 2016;23(5):921–9.
117. Sancho P, Barneda D, Heeschen C. Hallmarks of cancer stem cell metabolism. Br J Cancer. 2016;114(12):1305–12.
118. Guerra F, Arbini AA, Moro L. Mitochondria and cancer chemoresistance. Biochim Biophys Acta Bioenerg. 2017;1858(8):686–99.

119. King MP, Attardi G. Human cells lacking mtDNA: repopulation with exogenous mitochondria by complementation. Science. 1989;246(4929):500–3.
120. Herst PM, Dawson RH, Berridge MV. Intercellular Communication in Tumor Biology: a role for mitochondrial transfer. Front Oncol. 2018;8:344.

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