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Therapeutic application of mesenchymal stem cells-derived extracellular vesicles in colorectal cancer

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Abstract: Colorectal cancer (CRC) is the third most common cancer and the leading cause of cancer death globally. Resistance to therapy is a challenge for CRC treatment. Mesenchymal stem cells (MSCs) have become one of the furthermost effective approaches for tumor treatment due to their specific feature; however, their therapeutic function is controversial. Recently, extracellular vesicles (EVs) derived from MSCs (MSCs-EVs) have attracted extensive research attention due to their promising role in CRC treatment. EVs are cell-derived vesicles that transfer different biomolecules between cells, contributing to intracellular communication. MSCs-EVs can suppress CRC by delivering therapeutic agents to tumor cells. Several studies indicate that MSCs-EVs can serve as a drug delivery system for the treatment of different cancers. Various methods are used to modify (engineer) MSCs-EVs for loading therapeutic agents. Modified MSCs-EVs have improved specificity, targeting ability, and immunogenicity compared to synthetic carriers. Furthermore, CRC-EVs participate in regulating different cells, such as immune cells, fibroblasts, and endothelial cells, promoting tumorigenesis. MSCs-EVs-based therapy indicates a high potential in the treatment of cancer; however, the majority of studies have been conducted in the pre-clinical, and their clinical applications need further scrutiny. In this review, we describe the biogenesis of EVs, focusing on the effect of MSCs-EVs on CRC cells and CRC-derived EVs on other cells. Furthermore, MSCs-EVs as a drug delivery system for cancers is also reviewed, and perspectives regarding the therapeutic application of MSCs-EVs are discussed.

Introduction

Colorectal cancer (CRC), a heterogeneous disease, develops malignant tumors in the inner walls of the colon and rectum in the form of polyps (Fanelli *et al.*, 2020). It is the third most prevalent malignant tumor and the third most lethal cancer worldwide. In 2018, 1.8 million new cases of CRC and 881,000 deaths were reported, accounting for approximately 10% of new cancer cases and deaths worldwide (Bray *et al.*, 2018). The number of new cases is estimated to increase to 2.5 million in 2035 (Xie *et al.*, 2020). For benign stage CRC, no treatment is needed, and in case of metastatic invasion, surgery can be used to eliminate lymph nodes and malignant tumors (Hashiguchi *et al.*, 2020). Chemotherapy is another treatment for CRC as a targeted therapy through the use of ramucirumab and bevacizumab to prevent some specific protein functions involved in CRC development (Bennouna *et al.*, 2019; Kanat and Ertas, 2019; Modest *et al.*, 2019). In addition, radiation therapy is another common treatment method for the treatment of CRC by applying high-energy radiation beams (Klement *et al.*, 2019). However, efficient therapy remains a challenge for clinicians. The evidence indicates that MSCs have a wide range of applications in the treatment of many diseases, including cancer (Fayazi *et al.*, 2021; You *et al.*, 2022). They are present within bone marrows and other tissues like dental pulp, umbilical cord blood, and adipose and help in homeostasis in healthy tissues in the regeneration and wound healing (Bernardo and Fibbe, 2013; Abdyazdani *et al.*, 2017; Mirershadi *et al.*, 2020). MSCs, as non-hematopoietic precursor cells, have several characteristics, such as their capability to differentiate to produce cells like osteocytes, adipocytes, neurocytes, and fibroblasts (Abdyazdani *et al.*, 2017; Wang *et al.*, 2018). Evidence indicates that MSCs can inhibit tumor cells through both direct contact and paracrine (François *et al.*, 2019; Li *et al.*, 2021). In recent years, nanomedicine has developed to improve the pharmacokinetics and pharmacological patterns of unstable anti-cancer drugs (Patra *et al.*, 2018). The

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nanocarrier-based approach has various properties, including enhancement of drug delivery efficacy to cancer cells and reduction of the side-effects and non-targeting effects (Hou *et al.*, 2022). In this regard, various studies indicate that extracellular vesicles (EVs) have numerous advantages over conventional synthetic carriers, making them suitable for drug delivery systems (Herrmann *et al.*, 2021). EVs are heterogeneous vesicles released from cells; they contain various biomolecules such as DNAs, proteins, and RNAs that contribute to intracellular communication. They are classified depending on the mechanism of generation and their size, into apoptotic bodies (2–5 μm), exosomes (30–150 nm), and microvesicles (100–1000 nm) (Basso and Bonetto, 2016; Gurung *et al.*, 2021). Among them, microvesicles and exosomes are released from living cells and are involved in many processes, including angiogenesis, proliferation, differentiation, and intercellular communication (Burrello *et al.*, 2016; Todorova *et al.*, 2017; Phan *et al.*, 2018). The therapeutic effects of EVs have increasingly been indicated in various diseases (Ahmadi *et al.*, 2018; Akbari *et al.*, 2020, Hassanpour *et al.* 2020b). MSCs-derived EVs (MSCs-EVs) have unique advantages as carriers for anti-cancer therapy (Weng *et al.*, 2021). Naseri *et al.* (2018) reported that MSCs-EVs could migrate to the tumor sites, just like the MSCs. MSCs-EVs are therapeutic tools in regenerative medicine (van Niel *et al.*, 2018) and advance an emerging strategy for CRC therapy due to their roles in metastasis and growth of cancer cells (Xing *et al.*, 2020). For example, in one study, these vesicles could be loaded with doxorubicin, where they could target CRC cells (Bagheri *et al.*, 2020). Therefore, MSCs-EVs can transfer therapeutic agents to tumor cells. The present study discussed EVs biogenesis, the effect of MSCs-EVs on CRC, and the effect of CRC-EVs on other cells, and reviewed targeted cancer therapy by modified MSCs-EVs, and perspectives regarding the application of MSCs-EVs in cancer therapy.

Extracellular vesicles

Many types of cells produce EVs to transfer biomolecules, communicating with other cells (Hessvik and Llorente, 2018). EVs are phospholipid membrane vesicles that contain bioactive molecules such as RNAs, DNA strands, proteins, signaling molecules, and lipids, therefore, can regulate the fate and behavior of target cells located near or away (Abels and Breakefield, 2016; Latifkar *et al.*, 2019). A growing body of evidence indicates that EVs can be found in various body fluids, including blood, milk, bile, saliva, cerebrospinal fluid (CSF), bronchoalveolar lavage fluid (BALF), and urine (Abels and Breakefield, 2016; Latifkar *et al.*, 2019). EVs are classically divided into three types such as exosomes, microvesicles, and apoptotic bodies, based on their size and origin (Raposo and Stahl, 2019) (Table 1, Fig. 1). Exosomes refer to 30–150 nm vesicles that are originated from endosomal compartments namely multivesicular bodies (MVBs) located in the cell cytoplasm, while microvesicles are 100–1000 nm vesicles originating from the plasma membrane a process resembling virus outward from infected cells (Abels and Breakefield, 2016; Latifkar *et al.*, 2019). Finally, apoptotic bodies are the largest EVs (200–5000 nm) generated when a cell goes through apoptosis (Abels and Breakefield, 2016; Latifkar *et al.*, 2019) (Fig. 1). Exosomes are initially generated from the inward budding of MVBs membrane where different molecules contribute to the sorting, loading, abscission, and formation of exosomes (Zhang *et al.*, 2019; Jafari *et al.*, 2020). If MVBs fuse with the plasma membrane, exosomes are released into the extracellular space; alternatively, MVBs may fuse with the lysosomes, and exosomes are degraded (Zhang *et al.*, 2019; Babaie, 2020) (Fig. 1). Some common molecules such as CD63, CD81, CD82, and Alix are present on the exosomal membrane and are known as exosomal markers (Kowal *et al.*, 2014; Zhang *et al.*, 2019; Feghhi *et al.*, 2021). Once released into the extracellular matrix, EVs can interact with target cells and participate in different cellular events. According to previous studies, three proposed mechanisms through which EVs reach target cells include receptor-ligand, endocytosis, and direct fusion with the cellular membrane (Mulcahy *et al.*, 2014; Gurung *et al.*, 2021). Thus, they are involved in several physiological and pathological processes (Hassanpour *et al.* 2020a, Soraya *et al.*, 2021). Regardless of the development in the field of EVs, additional research, considering the International Society for Extracellular Vesicles (ISEV) guidelines, is a necessary requirement for the progress of EVs terms, methodology, and study and also proposes more appropriate determination of the cargo and role of EVs. Different cell types release EVs containing numerous biomolecules with varying functions. In recent years, some databases were established to organize and present components of EVs from various sources such as Vesiclepedia (http://www.microvesicles.org), Exocarta (http://www.exocarta.org), and a Bioinformatics lab from China (http://bioinfo.life.hust.edu.cn).

Effect of mesenchymal stem cells derived extracellular vesicles on colorectal cancer

In the case of cancer, MSCs have been shown to act as a double-edged sword where they may promote and/or

TABLE 1

Type of extracellular vesicles (EVs)

Type of EVs	Origin	Size	Marker	Contents
Exosomes	MVB	30–150 nm	CD63, CD81, CD9, TSG101	Proteins, lipids, and nucleic acids
Microvesicles	Plasma membrane	100–1000 nm	Integrins, selectins, CD40	Proteins, lipids, and nucleic acids
Apoptotic bodies	Apoptotic cells	200–5000 nm	Annexin V, phosphatidylserine	Cell organelles, Nuclear fractions

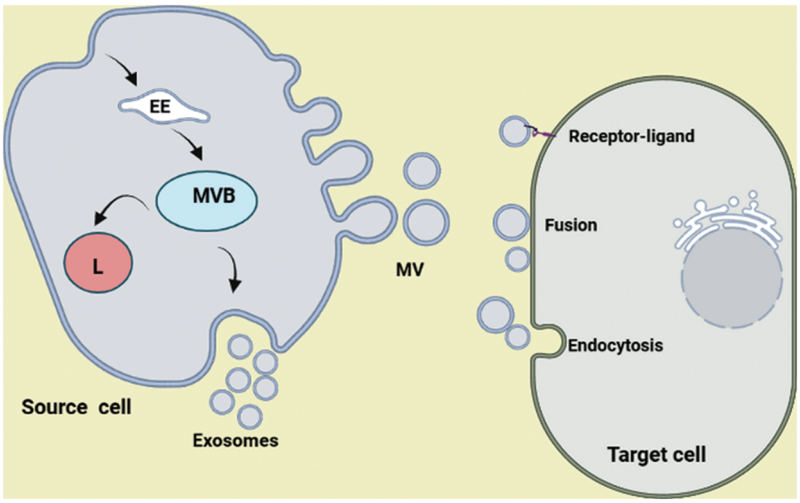

FIGURE 1. Biogenesis of extracellular vesicles (EVs). The release of EVs from cells occurs either through the inward budding of the membrane of multivesicular bodies (exosomes) or through the outward budding of the plasma membrane (microvesicles, MVs). When MVBs fuse with the plasma membrane, exosomes are released into the extracellular matrix. Once EVs are released, they can affect/reach target cells in three possible ways, including endocytosis, direct fusion, and receptor-ligand interaction. MVB: multivesicular body; L: lysosome; EE: early endosome.

suppress cancer progress (Tian *et al.*, 2020; Zhang *et al.*, 2022). EVs released by MSCs play a significant role in tumor development, proliferation, invasion, angiogenesis, and drug resistance. Nevertheless, contradictory findings have shown that MSCs-EVs can also inhibit tumors through different mechanisms, including intercellular signaling, and immune responses. Therefore, the association between MSCs-EVs and tumors is controversial. In this section, we discuss the distinct role of MSCs-EVs in CRC (Fig. 2). Luetzkendorf *et al.* (2010) produced tumor necrosis factor-related apoptosis-inducing ligand (*TRAIL*-MSCs) by third-generation lentiviral vector system and then co-cultured them with TRAIL-sensitive CRC-cell lines (HCT-15 and DLD-1) and resistant CRC-cell lines (SW480 and HCT-8). They found that these cells promoted apoptosis in CRC cells *in vitro*. In xenograft models, *TRAIL*-MSCs suppressed CRC-tumor growth by increasing apoptosis. When *TRAIL*-MSCs were administrated systemically, the growth of CRC was not affected, which may be due to pulmonary entrapment and a low rate of tumor absorption (Luetzkendorf *et al.*, 2010). Another study demonstrated that C-X-C motif chemokine receptor 4 overexpression-MSCs improved the homing ability of cells in the intestine. These cells recovered colitis-related tumors in the mice model through decreasing tumor load, pro-inflammatory cytokines, and signal transducer and activator of transcription 3 (STAT3) phosphorylation level (Zheng *et al.*, 2018). Conversely, Nishikawa *et al.* (2019) reported that MSCs can communicate with CRC cells through chemokine (C-C motif) ligand 3/4/5-CC chemokine receptor 5 signaling and increase the growth of CRC tumors *in vivo* (Nishikawa *et al.*, 2019). Similarly, de Boeck *et al.* (2013) showed that MSCs from bone marrow induced the survival, invasion, and growth of CRC cells by producing soluble NRG1 and activating human epidermal growth factor receptor 2 (HER2)/HER3-dependent phosphatidylinositol-3-kinase/protein kinase B (Akt) signaling pathway in CRC cells. Besides MSCs, EVs from MSCs have been shown to transfer miRNAs to CRC cells, inhibiting tumor growth. For instance, EVs derived from miR-16-5p-overexpression MSCs could deliver miR-16-5p to CRC cells and inhibit proliferation, migration, and invasion of cells and induce apoptosis through downregulating integrin subunit alpha 2 (ITGA2) expression (Xu *et al.*, 2019) (Fig. 2). Moreover, *in vivo* experiments showed that the MSCs-overexpressing miR-16-5p suppresses CRC growth. MSCs-EVs containing miR-4461 could decrease proliferation, migration, and invasion of CRC cells *in vitro* by inhibiting coat complex subunit beta 2 (COPB2) expression, proposing that miR-4461 may be a possible target for the diagnosis and treatment of CRC (Chen *et al.*, 2020). Li *et al.* (2021) showed that miR-3940-5p cargo of MSCs-EVs inhibited the invasion of cells and repressed the tumor growth and

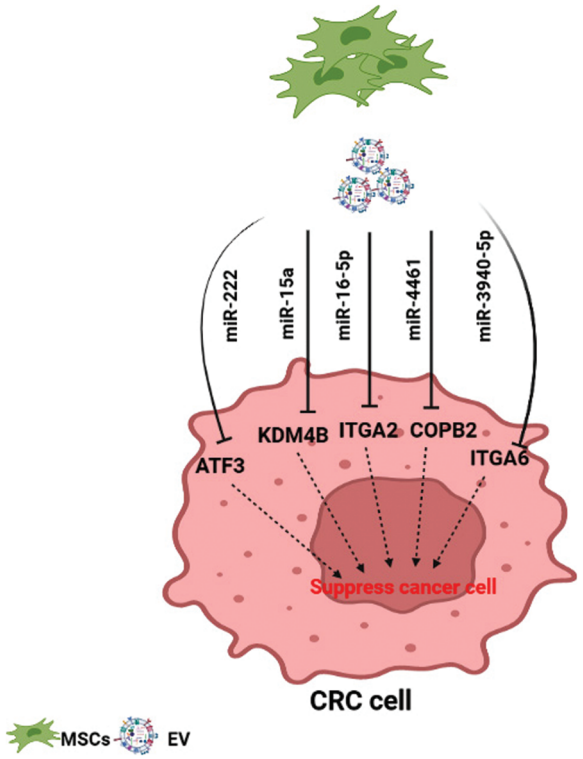

FIGURE 2. Therapeutic effect of mesenchymal stem cells derived extracellular vesicles (MSCs-EVs) on colorectal cancer (CRC) cells. Micro RNAs transferred by MSCs-EVs can target genes in CRC cells, inducing apoptosis and arrest in CRC cells, and suppressing tumorigenesis. (ITGA2: Integrin alpha 2; COPB2: COPI coat complex subunit beta 2; ITGA6: integrin alpha 6; ATF3: activating transcription factor 3; KDM4B: lysine demethylase 4B).

metastasis *in vivo*. MiR-3940-5p can directly target ITGA6 and suppress tumor cells. Recently, in an *in vivo* tumorigenesis experiment, researchers showed that MSCs-EVs overexpressing miR-15a could suppress the proliferation, migration, and invasion of cells. Further scrutiny indicated that these EVs increased the apoptosis of CRC cells through the down-regulation of lysine demethylase 4B (KDM4B) (Liu *et al.*, 2021). On the contrary, Li *et al.* (2021) found that EVs from MSCs contain miR-222 that could reach CRC cells and target activating transcription factor 3 (ATF3) binding and inhibits the activity of AKT1, increasing tumor invasion and immunosuppression of CRC cells. These results indicate that MSCs-EVs can inhibit tumorigenesis; however, some findings show that MSCs and their EVs may promote tumorigenesis. Therefore, the clinical application of MSCs-EVs in CRC treatment remains controversial. Despite the controversial results of MSCs-EVs therapy, MSCs-EVs can serve as a drug delivery system by delivering therapeutic agents to CRC cells.

Effect of CRC-EVs on other cells

Popēna *et al.* (2018) reported that CRC cell line-derived EVs regulate the immunophenotype and secretory profile in monocytes and inactive macrophages, inducing mixed M1 and M2 cytokine responses. THP-1 monocytes and M0 macrophages efficiently take up SW480 and SW620-derived EVs, and dynamin-dependent endocytic pathways may be involved. Interestingly, SW480 and SW620-derived EVs enhanced CD14 expression in M0 macrophages, while SW480-derived EVs reduced HLA-DR expression in M1 and M2 polarized macrophages. Furthermore, SW480-derived EVs significantly enhanced C-X-C motif chemokine ligand 10 (CXCL10) expression in M0 macrophages and monocytes. In contrast, SW620-derived EVs result in the secretion of CXCL10, interleukin (IL)-6, IL-10, and IL-23 in M0 macrophages. However, the addition of CRC cell line-derived EVs together with IFN-γ, LPS (M1), and IL-13, IL-4 (M2) stimuli during macrophage polarization had no additional influence on cytokine expression in M1 and M2 macrophages (Popēna *et al.*, 2018). Profilin-1 (PFN1) is a direct target of miR-375 and is positively regulated by HLA-F antisense RNA 1 (HLA-F-AS1) by binding to miR-375. Overexpression of HLA-F-AS1 suppressed miR-375 and enhanced the PFN1 expression pattern in CRC cells and CRC EVs, further increasing the M2 polarization of macrophages. In addition, macrophages treated with PFN1 in CRC EVs induced CRC *in vivo* and *in vitro* cell migration and proliferation. Therefore, application of HLA-F-AS1 in EVs may serve as a promising therapeutic strategy for CRC (Zhang *et al.*, 2021). Additionally, CRC small EVs can be specifically targeted to liver tissue and result in liver macrophage polarization toward an IL-6-secreting pro-inflammatory phenotype (Shao *et al.*, 2018). Also, EVs from CRC taken up by macrophages result in M2-like polarization and programmed death-ligand 1 (PD-L1) expression, then enhance $PD\text{-}L1^{+}CD206^{+}$ macrophage abundance and reduce T cell activity in the CRC tumor microenvironment. EVs-derived miR-21-5p and miR-200a are key signaling molecules that mediate the regulatory function of CRC on macrophages. CRC-derived miR-21-5p and miR-200a synergistically make macrophage M2-like polarization and PD-L1 expression by regulating the phosphatase and TENsin homolog deleted on chromosome 10/AKT and the suppressor of cytokine signaling 1/STAT1 pathways, resulting in reduced $CD8^{+}$ T cell activity and enhanced tumor growth. So, inhibiting the secretion of specific sEV-miRNAs from CRC and targeting PD-L1 in tumor-associated macrophages may serve as new means for CRC treatment as well as a sensitization method for anti-PD-L1 therapy in CRC (Yin *et al.*, 2022). CRC-EVs can regulate the CD8 T cells; in individuals with low body mass index (BMI)-CRC EVs, the rate of apoptosis in CD8 T cells was higher than in those with high BMI-CRC EVs. IL-10, IL-17A, granulysin, granzyme A, and perforin, for instance, were increased in the non-CRC EVs-treated CD8T cells (Abu *et al.*, 2020). On the other hand, Yamada *et al.* (2016) reported that EVs can impair T cell function. The CRC EVs alter the phenotype of the T cells to Treg-like cells by inactivating the stress-activated protein kinases signaling and stimulating the transforming growth factor-β/ suppressor of mothers against decapentaplegic (Smad) signaling. In addition, the CRC EVs-induced-Treg-like cells had a remarkable tumor-growth-enhancing function *in vivo* and *in vitro* (Yamada *et al.*, 2016). Fibroblast is one of the cells that influence by CRC EVs. CRC EVs are uptake by human fibroblasts that stimulate migration through the Rho-focal adhesion kinase signaling in co-incubated human fibroblasts. In addition, HT29 cell-derived EVs are more effective in activating human fibroblasts than cancer-associated fibroblasts (Clerici *et al.*, 2021). Suppressor of cytokine signaling 3 (SOCS3) is a direct target of miR-221-3p and the secreted miR-221-3p shuttled by CRC EVs has regulatory function on the STAT3/ vascular endothelial growth factor receptor-2 signaling axis by targeting SOCS3 in endothelial cells. CRC EVs increased endothelial cell migration, proliferation, and the formation of vessel-like structures. The proangiogenic effect of CRC EVs on the cells was recapitulated by miR-221-3p overexpression, indicating the importance of EVs-derived miR-221-3p in enhancing endothelial cell angiogenesis (Dokhanchi *et al.*, 2021).

Mesenchymal stem cells derived extracellular vesicles as a drug delivery system for cancer

MSCs-EVs can deliver therapeutic agents to tumor cells like pancreatic ductal adenocarcinoma (PDAC), CRC, hepatocellular carcinoma (HCC), breast cancer, and glioma. Generally, two methods are used to load therapeutic agents into MSCs-EVs (i) direct method, in which therapeutic agents are directly sorted into isolated EVs by different loading methods and (ii) the indirect method, in which EVs-producing cells (e.g., MSCs) are genetically manipulated to express distinct biomolecules (miRs, proteins) or co-cultured with therapeutic agents in which EVs derived from them would be contained with therapeutic agents (Tukmechi *et al.*, 2014; Patil *et al.*, 2020; Vahabi *et al.*, 2022) (Fig. 3). For example, Lou (2015) transfected MSCs with a miR-122 expression plasmid and then isolated EVs. After co-culturing with HCC cells *in vitro*, MSCs-EVs delivered miR-122 to the HCC cancer cells and augmented the sensitivity of HCC to sorafenib as the

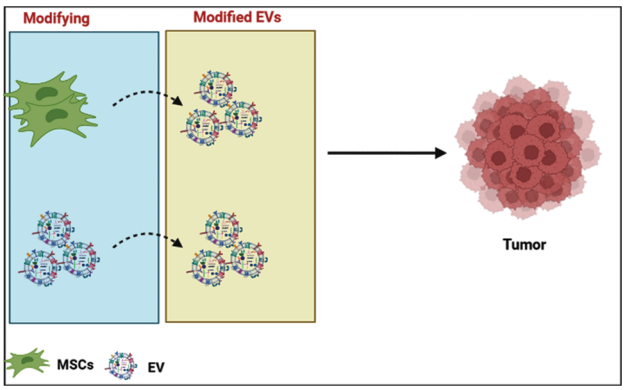

FIGURE 3. Application of mesenchymal stem cells derived extracellular vesicles (MSCs-EVs) for drug delivery system. In general, modified EVs can be produced through two approaches, including a direct method where EVs derived from MSCs are directly modified to load therapeutic agents and the indirect method in which MSCs are genetically or exogenously modified to load/express therapeutic agents. Therefore, EVs from these cells contain therapeutic agents.

chemotherapy drug. Moreover, intra-tumor administration of these EVs considerably stimulated the antitumor efficiency of sorafenib on HCC in animal models. They proposed that miR-122 could target genes coding for insulin-like growth factor1 receptor, A disintegrin and metalloproteinase 10, and cyclin G1, and therefore, induce cell cycle arrest and cell death, improving the sensitivity of tumor cells to chemotherapy (Lou, 2015). In another study, Lou *et al.* (2020) sorted miR-199a into EVs using miR-199a lentivirus infection to inhibit mTOR signaling in HCC cells and found that the sensitivity of HCC cells was augmented after cultivating with MSCs-EVs containing miR-199a. The administration of these EVs significantly increased the effect of doxorubicin on HCC *in vivo*. In one study, (Kanat and Ertas, 2019), anti-miR-142-3p oligonucleotides were incorporated into MSCs-EVs to be delivered to breast cancer cells to increase the expression of miR-142-3p and miR-150 in these cells (Naseri, 2018; Naseri, 2020). Results showed successful delivery of MSCs-EVs to cancer cells both *in vitro* and *in vivo* and up-regulated target RNA expression, improving the expression of *APC* and *P2X7R* genes. Furthermore, these EVs inhibited tumor growth and clone-formation abilities of the MCF7 cells. The MSCs-EVs exhibited a great bio-distribution capacity and successfully repressed tumor mass growth (Naseri, 2018; Naseri, 2020). Ding (2019) used MSCs-EVs to deliver miR-145-5p to PDAC cells. They observed that these EVs were efficiently distributed miR-145-5p to PDAC cells, suppressed growth and invasion, and increased apoptosis and cell cycle arrest associated with a low level of Smad3 mRNAs *in vitro*. Moreover, MSCs-EVs reduced the growth and invasion of cancer cells in the xerograph model. Seemingly, miR-145-5p inhibited Smad3 expression levels. MSCs-EVs can deliver various drugs, such as doxorubicin, paclitaxel, and magnolol, selectively to tumor cells. For instance, Gomari *et al.* (2018) demonstrated that doxorubicin carried by MSCs-EVs can significantly impede the growth of tumor cells in an animal breast cancer model. The surface of MSCs-EVs was modified to increase the targeting ability of EVs in glioma. Jia and co-workers linked neuropilin-1-targeted peptide to MSCs-EVs by click chemistry and then incorporated superparamagnetic iron oxide nanoparticles and curcumin into them. These MSCs-EVs successfully delivered the therapeutic agents to the targeting area and induced anti-cancer effects (Jia, 2018). Similarly, Zhaung *et al.* (2020) produced MSCs-EVs with modified surfaces and loaded them with superparamagnetic iron oxide nanoparticles. Proteins of cell-penetrating peptides (CPP) and TNF-α (CTNF-α)-anchored were linked to EVs containing superparamagnetic iron oxide nanoparticles. These EVs showed a targeting antitumor role and considerably suppressed tumor cell growth by inducing apoptosis by the TNFR I pathway, in both *in vitro* and *in vivo* mic melanoma subcutaneous cancer models. In clinical trials, exosomes derived from MSCs have been registered to load and deliver the KrasG12D siRNA to pancreatic adenocarcinoma cancer cells (gov Identifier: NCT03608631). The findings show that EVs can deliver therapeutic agents to cancer cells, inhibit tumor cell proliferation, and sensitize the tumor cells to chemotherapy. MSC-EV could induce various effects on cancer cells and tumor stromal cells; thus, it is necessary to limit the endogenous impact when used for drug delivery. In this context, one approach seems to inhibit/decrease the factor/s that support tumor cells. Another approach is to load EVs with relative inhibitors. For example, MSCs-EVs contain proangiogenic factors that may induce angiogenesis in tumor cells (Zhang *et al.*, 2022). So, by using a relative siRNA in EVs or down-regulating targeted gene/s in EVs-producing MSCs, it is possible to inhibit/decrease the supportive impact of MSCs-EVs in cancer.

Platelets-derived extracellular vesicles (p-EVs) for drug delivery

The number of platelets-derived EVs (platelet microparticles) in the blood rises on activation, shear stress, inflammation, and during apoptosis (Burnouf *et al.*, 2014; Melki *et al.*, 2017). These vesicles receive features from their parental cells: the expression of CD41, CD31, CD42, CD63 CD62, and CD61 platelet membrane surface antigens, which activate inherent interactions with the surrounding environment (Franco *et al.*, 2015; Chimen *et al.*, 2020), and physiological loading with complex functional components. These p-EVs bear cytokines, growth factors, chemokines, anticoagulant, pro-coagulant, anti-inflammatory, pro-inflammatory, and proangiogenic, antiangiogenic factors, lipids, and nucleic acids (mRNA and miRNA) (Melki *et al.*, 2017; Boilard, 2018). The structure and composition of p-EVs, as well as their implications in some pathologies, support their prospective therapeutic application in hemostasis, tissue regeneration, and immunomodulation, and as drug-delivery vehicles (Burnouf *et al.*, 2018; Kerris *et al.*, 2020; Wu and Zhou, 2020). These features resemble those of MSCs-EVs. Platelets have numerous advantages as an EV source. They are anucleated (opposite to MSCs), thus alleviating safety concerns related to possible teratogenic risks. Membranes with p-EVs express integrins that can be used to target recipient cells and tissues and may simplify the crossing of biological barriers (Burnouf *et al.*, 2014). Compared to MSCs-EVs, the production of clinical-grade allogenic platelets is already in place in many countries, including as a source of human platelet lysates

(Burnouf *et al.*, 2016), therefore providing a readily accessible national resource. The collection of platelet concentrates, and thus the production of EVs, can be done from autologous or allogeneic sources as reasoned appropriate, thereby creating greater opportunities for clinical applications in a given regulatory and clinical trials. Similar to MSCs, platelets have a high ability to produce EVs through several physiological and biophysical mechanisms *in vivo* and *in vitro* (Sung *et al.*, 2019; Wu and Zhou, 2020). EVs of platelets can be abundantly isolated from blood because it has long been thought to contribute to the majority (up to 70%–90%) of the pool of EVs (Berckmans *et al.*, 2019). Importantly, these EVs can be directly produced from collected platelet concentrates, in contrast to MSCs that require a phase of isolation and *ex vivo* incubation and expansion to prepare clinically relevant EVs doses. Therefore, in comparison to MSCs-EVs, bypassing the necessity for a GMP cell culture facility saves time required for facility design, qualification, validation, and operator training and decreases the capital and operational costs required to reach clinical phases and the market (Agrahari *et al.*, 2019; Burnouf *et al.*, 2019). The avoidance of such *ex vivo* processing also circumvents the preparation and regulatory issues in the quality control of growth medium supplements, including potential 'contamination' by the EVs present in fetal bovine serum or human platelet lysates (Agrahari *et al.*, 2019; Barro *et al.*, 2020). Major possible limiting issues in the use of platelets as a source of EVs include the dependence on blood donors or blood collection organizations for a robust source of the starting material and risks of pathogen contamination. Therefore, compared to producing and using MSCs-EVs, platelet concentrates are an established, licensed medicine in most countries and are listed as essential medicines by the World Health Organization (Johnson *et al.*, 2021). Platelet collection is under the supervision of national regulatory consultants. Medical devices for platelet collection are licensed by national regulatory authorities and can be used to prepare allogeneic or autologous platelet concentrates (Johnson *et al.*, 2021). However, there is a risk of contamination by blood-borne infectious agents resistant to existing pathogen-reduction processes. Most importantly, possible variability among platelet donors may affect the features and function of EVs (Johnson *et al.*, 2021). Also, isolation, purification, and characterization methods still lack standardization, and no guidelines for the application of platelets-derived-EVs based therapeutic exist, as is also the case for MSC-EVs (Lener *et al.*, 2015).

Future perspectives

Altogether, regardless of the discrepancies in the function of natural MSCs-EVs in CRC studies, their modification or loading and application as carriers for the delivery of therapeutic agents are promising in cancer therapy. MSCs are harmless and advantageous source cells for the production of EVs, and modification of their content may be a promising tool for cancer treatment (Rezaie *et al.*, 2022) (Fig. 3). Modified MSCs-EVs can deliver therapeutic agents to cancer cells effectively. MSCs-EVs represent very low immunogenicity with high biocompatibility, making them ideal for therapeutic goals. Finally, the content and surface of MSCs-EVs can be covalently or genetically modified (Rezaie *et al.*, 2022; Yang and Zhang, 2022). However, this field faces challenges, such as selecting an assured and suitable source of MSCs for delivering therapeutic agents is a serious step; consequently, various MSCs may yield different EVs with variations in size, cargo, and roles (Zhang *et al.*, 2022). EVs are heterogeneous regarding sizes or contents; therefore, the modifying process must not create more heterogeneity and membrane modification, which may negatively impact EVs loading and targeting potential. The side and unwanted effects of modifying EVs remain to be revealed in further studies (Théry *et al.*, 2018; Rezaie *et al.*, 2021). This field is proceeding and requests a deep understanding of the EVs kinetics and developments about modifying and loading methods of EBs to acquire better cancer treatment. The majority of studies were performed in a pre-clinical setting, and the results of clinical application of modified EVs remain a problem, as this field faces some challenges essential to be considered in clinical translation studies. The biology and role of EVs are not yet fully discovered. Several questions are associated with the biogenesis pathway and uptake, nomenclature, characterizations, and purification of EVs, which affect methods and programs that deal with their modifications and loading methods (Théry *et al.*, 2018). Large-scale production of EVs is another challenge and needs standardization for their isolation, purification, loading, and modification. Large-scale production of EVs, especially from MSCs, is very problematic because purification and incubation of human autologous MSCs are laborious and challenging *in vitro* in a short time. Similar to other EVs, MSCs-EVs may be cleaned by the liver, spleen, and lungs when intravenously injected; consequently, these are not effectively concentrated in the target tissue (Rani *et al.*, 2015).

Conclusion

CRC is the third most common cancer and the leading cause of death due to cancer worldwide. MSCs have become one of the furthermost effective tools for tumor treatment own to their unique properties; however, their therapeutic effects are controversial. MSCs-EVs are a promising tool for the treatment of CRC and other cancers. These vesicles also transfer certain RNAs and biomolecules that contribute to the inhibition of growth and development of CRC through different signaling pathways. On the other hand, CRC-EVs can target other cells and induce tumorigenesis. Modified MSCS-EVs offer a novel therapeutic avenue for the delivery of numerous synthetic and biological molecules to cancer cells. These vesicles represent very low immunogenicity with high biocompatibility, which makes them ideal for therapeutic objectives. However, this field of study is novel and has not yet reached adequate maturity to translate into clinical application, and more studies are desirable to recognize all therapeutic features of MSCs-EVs in CRC.

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Availability of Data and Materials: Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

Author Contribution: Study conception and design: R. Jafar; data collection: N. Mohedeseh, R. Yousef; draft manuscript preparation: R. Jafar, N. Mohedeseh. All authors reviewed the results and approved the final version of the manuscript.

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