BBA - Reviews on Cancer 1871 (2019) 192–198

Contents lists available at ScienceDirect

BBA - Reviews on Cancer

journal homepage: www.elsevier.com/locate/bbacan

Review

Role of mesenchymal stromal cell-derived extracellular vesicles in tumour microenvironment

Adamo A., Dal Collo G., Bazzoni R., Krampera M.*

Stem Cell Research Laboratory, Section of Hematology, Department of Medicine, University of Verona, Verona, Italy

A B S T R A C T

Stromal cells, deriving from mesenchymal stromal cells (MSCs), are crucial component of tumour microenvironment and represent key regulators of tumour processes. MSCs can be recruited to the tumour environment and interact with many cellular elements, thus influencing tumour biology. Cell-to-cell communication is in part mediated by the release of extracellular vesicle (EVs). EVs can induce significant molecular changes in recipient cells, delivering bioactive molecules. In this review, we describe the MSC-derived EVs content and discuss their role in different processes related to cancer biology. Furthermore, we summarize chemical or biological EVs modifications aiming to develop more efficient antitumor therapies.

1. Introduction

Tumour initiation and progression require the generation of a supportive niche that constitute a favourable microenvironment promoting tumour cell viability, proliferation, and invasion. Different cell types contribute to the tumour cell niche including stromal cells, endothelial cells and immune cells. Stromal cells represent the main cell component with both supportive and immunoregulatory functions; they derived from multipotent cells of mesodermal origin, called mesenchymal stromal cells (MSCs), which virtually reside in all tissues [1]. Therefore, stromal cells can be recruited to the tumour environment and establish dynamic interactions with tumour cells and other cellular elements [2–4]. The role of MSCs in tumour microenvironment has been increasingly studied in the last years. MSCs have shown to enhance or suppress tumour progression and metastasis both *in vitro* and *in vivo* depending not only on tumour model and stage considered, but also on the dose and time of cell treatment [5,6].

Recently, a new mechanism of intercellular communication within the tumour microenvironment has been recognized. This mechanism involves the release of membrane-derived vesicles, called extracellular vesicles (EVs). EVs include exosomes, the smallest EV fraction arising from intracellular endosomes, and microvesicles (MVs) that are generated by budding from the plasma membrane. EVs influence various biological processes by both activating directly into target cell surface receptors through protein and bioactive lipid ligands, and delivering different effectors, including transcription factors, oncogenes, mRNAs, and non-coding regulatory RNAs (lncRNAs), thus inducing functional changes in recipient cells [7]. The pattern of proteins, lipids, and nucleic acids contained in EVs depends on their cell origin and may be influenced by different environmental conditions. EVs are released by either tumour cells or their surrounding cells and are considered as important mediators of the cell cross-talk in the tumour microenvironment. Tumour-derived EVs are involved in the immune response regulation, thus creating an immunosuppressive niche and triggering immune escape mechanisms [8]. Furthermore, tumour-derived EVs contribute to the formation of metastasis by inducing persistent molecular changes in stromal components, promoting angiogenesis and epithelial–mesenchymal transition (EMT) [8]. On the other hand, EVs derived from MSCs (MSC-EVs) can regulate cell apoptosis, proliferation, invasion of tumour cells and influence several tumour processes by delivering their content in recipient cells (Fig. 1). Although the role of tumour-EVs is rather well-defined, MSC-EVs, as the originating cells, can act on tumour progression with opposite effect and their role has not been clearly elucidated yet. In this review, we report recent findings on the role of MSC-EVs in tumour biology, focusing on their involvement in tumour growth, angiogenesis and drug resistance (Table 1).

2. MSC-derived extracellular vesicles: characterization and composition

Considering the different mechanisms of biogenesis, the composition in surface molecules of exosomes and MVs is quite different. Exosomes derived from MSCs express the well-established exosome markers CD63, CD9, CD81, Alix, LAMP1, HSP70 [9]. The expression of typical MSC markers, such as CD29, CD73, CD90, CD44 and CD105, has been further reported [10]. Membrane lipid composition of MSC-EVs is enriched with diacylglycerol, sphingomyelin, and ceramides; in

* Corresponding author.
E-mail address: mauro.krampera@univr.it (M. Krampera).

https://doi.org/10.1016/j.bbcan.2018.12.001
Received 31 August 2018; Received in revised form 22 November 2018; Accepted 10 December 2018
Available online 30 December 2018

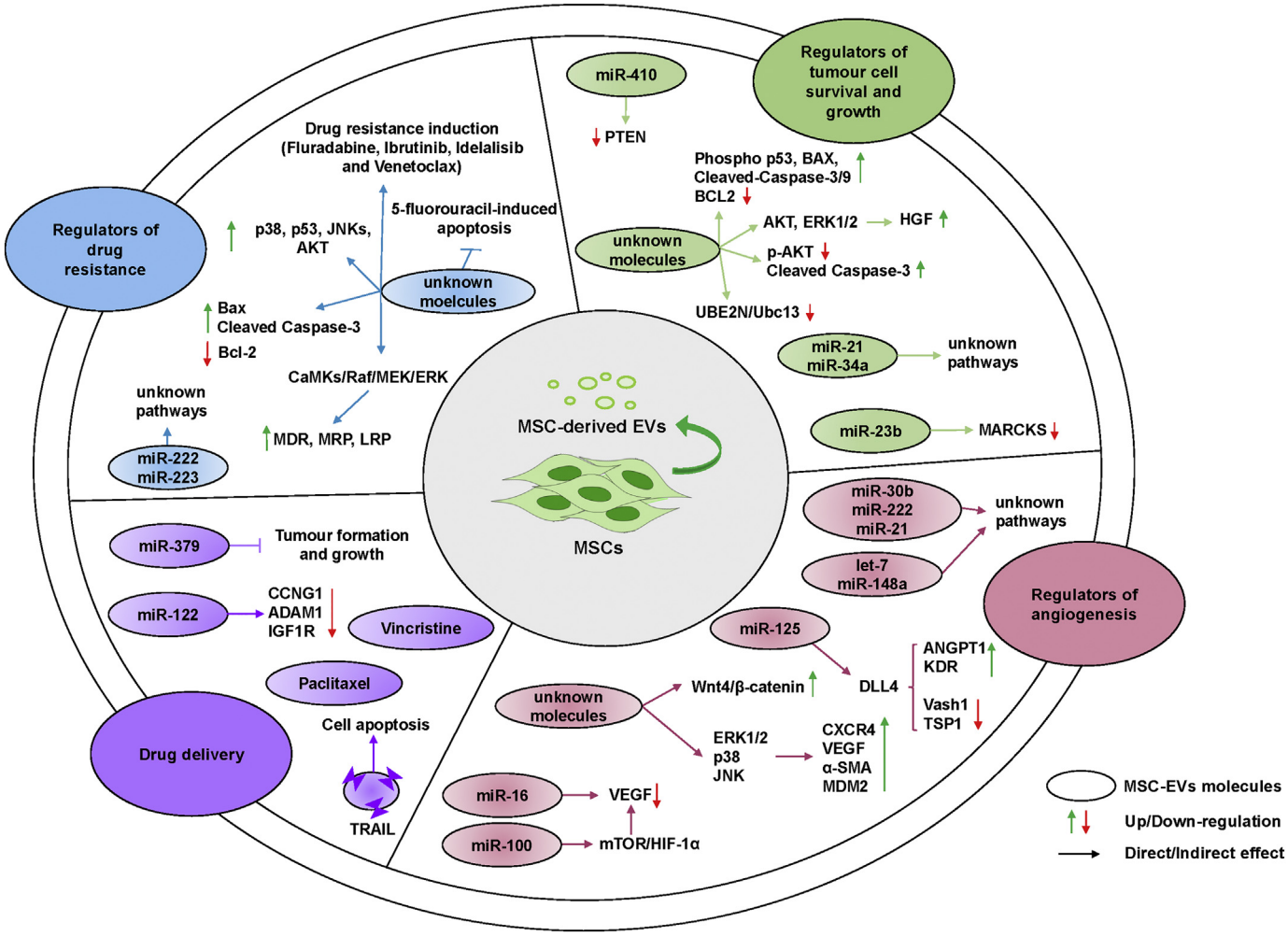

Fig. 1. Bioactive molecules contained in MSC-EVs. Circles represent bioactive molecules contained in MSC-EVs and potentially transferred in tumour cells and/or other cell elements belonging to the tumour microenvironment. These molecules can modulate different genes and pathways in recipient cells, thus influencing tumour cell survival and growth (green), angiogenesis (red) and drug resistance (light blue). Purple circles represent bioactive molecules loaded in EVs to develop EV-based drugs.

addition to their structural role, these lipids have a broad effect on signal transduction and regulation known tumour suppressor, potentiating signalling pathways that drive apoptosis and cell cycle arrest [11,12]. On the other hand, alterations in ceramide turnover favour the opposite effect promoting cell growth [13]. Furthermore, cancer cells are associated to low level of sphingomyelin. The specific activation of sphingomyelin synthases has been shown to lead to cell cycle arrest, cell differentiation and apoptosis in cancer cells [14]. Diacylglycerol kinases, a family of enzymes catalysing the transformation of diacylglycerol into phosphatidic acid, are often up-regulated in many tumour cell types, suggesting that the reduction of diacylglycerol could promote tumour immune-escape [15]. Taken together, these data open an important scenario on the role of lipid transfer mediated by MSC-EVs on tumour processes. Moreover, MSC-EVs contain various bioactive factors that could be involved in several pro or anti-tumour processes, including transcription factors, oncogenes, and nucleic acids, such as mRNAs and small and lncRNAs. MSC-EVs content could be strictly affected by the tissue of origin. Through high-throughput techniques, Vallabhaneni et al. described several known tumour supportive factors in bone marrow-MSC-EVs (BM-MSC-EVs), such as PDGFR-beta, TIMP-1, and TIMP-2 [11]. BM-MSC-EVs showed also the expression of miRNA-21 and miRNA-34a that are involved in cell survival and proliferation [11,16]. MVs released by human umbilical cord-derived MSCs (UC-MSCs) have shown to express many angiogenesis-promoting molecules, including IL-6, bFGF, and UPAR. MSC-MVs also contain angiogenin, VEGF, MCP-1 and the receptor-2 for vascular endothelial growth factor [17]. Furthermore, under hypoxic condition, the expression of some of these cytokines are higher than under normoxic condition [17]. Recently, several works explored the protein expression profile of exosome or MV-enriched fraction derived from MSCs, thus identifying a wide spectrum of proteins associated to inflammation, Wnt signalling pathway components and several putative paracrine effectors of angiogenesis [18,19]. Among the molecules contained in MSC-EVs, many functionally active mRNAs and non-coding RNAs involved in the cross-talk between tumour cells and MSCs have been described [20]. Identified miRNAs can be classified in families or clusters with different functions. Let-7 and miR-15-16 families released by MSC-EVs mainly possess anti-tumour activity, targeting oncogenes such as RAS, MYC, HMAGA2, and LIN28, and the angiogenic factor VEGF-A, respectively [21]. MiR-221 and miR-222 are also involved in angiogenic mechanisms and they have been identified in EVs released by rat MSCs [22]. The expression of lncRNAs has been increasingly studied in human cancer cells and more recently in EVs. In human BM-MSC-EVs the expression of lncRNA involved in cell proliferation, such as lnc-7SK, has been found, thus suggesting their possible role in tumour regulation [11].

Table 1
Effect of MSC-EVs on different tumours.

Human MSC-EVs

Tumour model	*Cell line*	*Source of EVs*	*MVs/ Exosomes*	*Biological function*	*Hypothesized Mechanism*	*Reference*
Lung adenocarcinoma	H1299 PC-9	UC-MSCs	Both	↑ Proliferation ↓ Apoptosis	Transfer of miR-410 in tumour cells directly targeting PTEN.	23
Renal cancer	786-0	WJ-MSCs	MVs	↑ Progression of cell cycle from G0/1 to S ↑ Diffusion	Transfer of RNA in inducing HGF synthesis and AKT and ERK1/2 signalling activation in tumour cells.	24
Gastric cancer	SGC-7901	BM-MSCs	Exosomes	↑ Tumour incidence in vivo	Activation of ERK1/2 signalling in tumour cells.	25
			Exosomes	↑ Angiogenesis	Increase of CXCR4 and VEGF expression mediated by MAPK pathway.	25
	HGC-27 MGC-803 SGC-7901	UC-MSCs	Exosomes	↑ Drug resistance	Increase of multi-drug resistance-associated proteins, including MDR, MRP and LRP.	50
Colon cancer	SW480	BM-MSCs	Exosomes	↑ Tumour incidence in vivo	Activation of ERK1/2 signalling in tumour cells.	25
			Exosomes	↑ Angiogenesis	Increase of CXCR4 and VEGF expression mediated by MAPK pathway.	25
Hepatocellular carcinoma	HepG2	BM-MSCs	Both	↓ Cell cycle progression ↑ Apoptosis	Up-regulation of the anti-proliferative factors DIRAS3, Rbl-1, CDKN2B. Up-regulation of BIRC5, DDX11, and MCM5.	26
Kaposi's sarcoma	KS IMM	BM-MSCs	Exosomes	↓ Cell cycle progression ↑ Apoptosis	Increase of CDKN1A, BIRC5, CCND1, and CDC20 Down-regulation of cell cycle G1/S transition cyclins CCNE1 and CCND2. Down-regulation of CUL3, Rb1, and SKP2.	26
Ovarian cancer	Skov-3	BM-MSCs	Exosomes	↓ Cell cycle progression ↑ Necrosis	Down-regulation of cell cycle progression factors CCND2 and CUL3.	26
	Skov-3 A2780	AT-MSCs	Exosomes	↑ Apoptosis	Upregulation of phosphorylated p53, BAX, activated Caspase9, and activated Caspase3. Down regulation of anti-apoptotic BCL2.	29
Bladder cancer	T24	WJ-MSCs	Both	↑ Cell cycle arrest ↑ Apoptosis ↓ Tumour growth in vivo	Reduction of Akt phosphorylation and up-regulation of p-p53/p21 and cleaved Caspase 3.	28
Multiple myeloma	MM.1S RPMI.8226 U266	Healthy donor-derived BM-MSCs	Exosomes	↓ Tumour growth	miR-15a acts as tumour suppressor that is present in normal BM-MSCs and absent in patient-derived BM-MSCs. The lack of EV-mediated miR-15a transfer to malignant cells promotes the growth of clonogenic cells.	30
		Patient-derived BM-MSCs		↑ Tumour growth		
Chronic lymphocytic leukemia B	Patient-derived CLL cells	BM-MSCs	Exosomes	↑ Drug resistance	Up-regulation of genes involved in the B-cell receptor pathway, including CCL3/4, EGR1/2/3, and MYC.	52
Breast cancer	MDA-MB-231 T-47D	BM-MSCs	Exosomes	↑ Drug resistance	Transfer of miR-222 and miR-223	49
	MDA-MB-231 MCF-7 T47D	BM-MSCs	Exosomes	↓ Angiogenesis	Transfer of miR-100 associated with the modulation of mTORr/HIF1α signalling axis in tumour cells and the consequent reduction of VEGF expression and secretion.	39
	MDA-MB-231 231BrM-2a 231LM-4175 231BM-1833	BM-MSCs	Both	↓ Metastasis formation ↓ Angiogenesis	miR-205 and miR-31 up-regulation in tumour cells targeting UBE2N/Ubc13	31
	MDA-MB-231	BM-MSCs	Exosomes	↓ Proliferation ↓ Invasion ↓ Abundance of stem cell–like surface markers	Transfer of miR-23b inducing a down-regulation of MARCKS.	27

Non-human MSC-EVs

Multiple myeloma	5T33MMvv	Mouse BM-MSCs	Exosomes	↑ Drug resistance	Modulation of p38, p35, c-Jun N-terminal kinase and Akt pathways.	51
Pheochromocytoma	PC12	Rat BM-MSCs	Exosomes	↑ Drug resistance	Inhibition of glutamate-induced cytotoxicity modulating Akt phosphorylation, Bcl-2 expression, Bax expression, and the cleavage of caspase-3.	53
Breast cancer	4T1	Mouse BM-MSCs	Exosomes	↓ Angiogenesis	Transfer of miR-16 targeting VEGF	38

3. MSC-EVs as regulators of tumour cell survival and growth

The role of MSC-EVs during tumour growth has not been clearly elucidated yet. To date, contradictory results have been reported. Some authors have reported a supportive effect toward tumour cells *in vitro* and *in vivo*, while other studies have demonstrated the opposite effect. Recently, Dong et al [23]. showed *in vivo* a pro-tumour activity of EVs derived from UC-MSCs on lung adenocarcinoma (LUAD) growth; in fact, proliferation and apoptosis of LUAD cells were supported by the EV-mediated transfer to cancer cells of miR-410 that directly targets PTEN. MVs released by human Wharton's Jelly MSCs (WJ-MSCs) displayed the same supportive activity on renal cancer cell growth and diffusion both *in vitro* and *in vivo*, promoting the progression of cell cycle from G0/1 to S in tumour cells [24]. In this system, the MSC-MV-mediated transfer of RNA induced HGF synthesis in tumour cells, thus activating AKT and ERK1/2 signalling. The involvement of ERK1/2 signalling was also previously reported by Zhu et al., who showed the activation of such pathway in a human cancer cell line (SGC-7901) treated with MSC-exosomes [25]. Moreover, tumour incidence in mice implanted with SGC-7901 cells or a human colon cancer cell line (SW480) mixed with BM-MSCs or MSC-exosomes was similar and significantly higher than in mice implanted with tumour cells alone [25]. On the other hand, the inhibitory effect of MSC-EVs on tumour growth has been suggested by various reports. MSC-EVs reduced cell cycle progression in HepG2 hepatoma, Kaposi's sarcoma, and Skov-3 ovarian tumour cell lines, while they induced apoptosis in HepG2 and Kaposi's cells and necrosis in Skov-3 [26]. Furthermore, BM-MSC-exosomes promoted dormancy in metastatic breast cancer cells [27]. Such effect has been correlated to the down-regulation of MARCKS expression in cancer cells mediated by the EV-transfer of miR-23b. Human WJ-MSC-EVs induced cell cycle arrest and apoptosis on bladder tumour T24 cells *in vitro* and *in vivo*, through the reduction of phosphorylated AKT protein kinase and the increase of cleaved caspase-3 [28]. Moreover, exosomes secreted by human Adipose Tissue-derived MSCs (AT-MSCs) reduced cell viability, wound-repair capacity, and colony formation ability of A2780 and Skov3 ovarian cancer cells [29]. In particular, A2780 cells responded dose- and time-dependently following AT-MSC-exosomes treatment and they showed a strong upregulation of several pro-apoptotic signalling molecules, including phosphorylated p53, BAX, activated Caspase9, and activated Caspase3, in association with a down regulation of the anti-apoptotic BCL2 protein [29]. The controversial data on the effect of MSC-EVs on tumour growth highlight the complexity of their functions, that could be even opposite depending on several factors, such as the type and the tissue origin of MSCs; in addition, cancer cells may show different responses depending on the tumour model considered. The results can be also influenced by the method of EV isolation: some authors purified exosomes or MV-enriched fraction, while others did not purify the two classes of EVs. Moreover, the method of MSC culture may affect the experimental results. Serum-deprivation, a cell culture system often used for EVs isolation, can modify the expression of important molecules involved in cell survival and apoptosis, such as miR-21 and miR34a, which in turn can affect the release and content of EVs [11]. In the context of multiple myeloma, opposite effects of MSC-exosomes derived from either normal individuals or patients were observed on tumour cell growth. MSC-EVs derived from patients induced tumour growth, whereas MSC-EVs derived from healthy donor showed an anti-tumour effect [30]. Furthermore, the role of MSC-EVs could be different according to the pathological stage of the tumour. Vallabhaneni et al. showed that MSC-EVs supported primary breast tumour progression, but reduced metastasis formation through the UBE2N/Ubc13 pathway [31]. Therefore, tumour model and stage as well as MSC culture conditions are all factors that may influence the release of EVs, their content, and therefore their effects on tumour growth.

3.1. MSC-EVs and angiogenesis

The influence of EVs released by various cell types on vascular development, growth, and maturation has been widely documented and reviewed by Todorova et al. [32]. The role of EVs on angiogenesis has been assessed on both endothelial cells, evaluating their effect in modulating vascular structures, and tumour cells, studying the expression of pro- and anti-angiogenic factors [33].

Angiogenesis plays a crucial role in numerous physiological and pathological responses. Tumour development requires blood vessels for oxygen and nutrients supply, which are both essential factors for tumour growth. Tumour cells frequently overexpress pro-angiogenic factors, such as VEGF, strictly associated with cancer progression [34]. MSCs contribute to this process by controlling blood vessel density and functions [35]. As already underlined for tumour cell survival and growth, the effect of MSCs on angiogenesis have yielded contradictory results, including both anti-angiogenic and pro-angiogenic effects [36,37]. Although the evidence of strong pro-angiogenic properties correlated with the secretion of soluble factors and EVs by tumour components, the effect of MSC-EVs on tumour angiogenesis remains relatively unclear [32]. Murine MSC-EVs may shuttle anti-angiogenic molecules into tumour cells, such as miR-16, a miRNA known to target VEGF, leading to the inhibition of angiogenesis both *in vitro* and *in vivo* models of breast cancer [38]. On the contrary, Zhu et al. clearly demonstrated that BM-MSC-exosomes strongly increase *in vitro* the expression of CXCR4 and VEGF in human gastric carcinoma (SGC-7901) and colon cancer (SW480) cell lines [25]. These authors observed that MAPK pathway, including ERK1/2 and p38, and - in minimal part - JNK pathway, were responsible for the increase of VEGF and CXCR4 after MSC-exosomes treatment. Furthermore, MSC-exosomes strongly induce the expression of α-SMA, CXCR4, VEGF and MDM2 in *in vivo* [25]. In breast cancer cells, BM-MSC-derived exosomes may induce a significant reduction of VEGF expression and secretion by modulating the mTOR/HIF-1α signalling axis and affecting the vascular behaviour of endothelial cells *in vitro* [39]. In addition, VEGF down-regulation has been associated with the horizontal transfer of miR-100 mediated by MSC-exosomes, which has been further confirmed by the ability of anti-miR-100 to rescue the inhibitory effects of MSC-derived exosomes [39]. Liang et al. showed the opposite effect mediated by exosomes secreted by human Adipose Tissue-derived MSCs (AT-MSCs) [33]. In this experimental model, MSC-exosomes promoted angiogenic processes *in vitro* by inducing the upregulation of the pro-angiogenic genes Ang1 (ANGPT1) and Flk1 (KDR) and the downregulation of the anti-angiogenic factors Vash1 and TSP1 in endothelial cells (HUVECs) [33]. MSC-exosomes may also increase the number of vascular structures *in vivo*. These observations correlated with the transfer of miR-125, mediated by MSC-exosomes, targeting the angiogenic inhibitor delta-like 4 (DLL4) [33]. Many other reports show that MSC-EVs contain various miRNAs probably involved in modulating angiogenesis, including miR-30b, miR-222, miR-21 and let-7f, and miR148a [40,41]. Furthermore, McBride et al. showed the reduced ability of BM-MSC conditioned medium to promote angiogenesis after CD63+ exosome depletion [42]. The pro-angiogenic activity was also shown in exosomes isolated from UC-MSCs, with a mechanism dependent on Wnt4/β-catenin [43]. In response to hypoxia, EVs isolated from AT-MSCs stimulated angiogenesis *in vivo* [44]. The ability of MSC-EVs to modulate angiogenic processes was assessed in other contexts different from tumours. Considering their pro-angiogenic properties, MSC-EVs have been proposed as novel therapeutic approaches for cardiovascular diseases as well as brain, renal, and retinal injuries [45].

3.2. MSC-EVs and drug resistance

Drug resistance of cancer cells is a multifactorial process due to dynamic interactions between tumour cells and the surrounding microenvironment. This process includes many mechanisms, such as

defective drug accumulation, alteration of drug targets and cellular pathways and, eventually, lack of induction of tumour cell death [46]. Tumour drug resistance mediated by microenvironmental stimuli has been clearly underlined by several reports and reviewed in the context of different tumours [47]. Tumour-EVs can mediated drug resistance through several mechanisms, including drug sequestration, delivery of specific RNA molecules and proteins, or through the well-known cross-talk between cancer cells and MSCs [48]. MSC-exosomes containing miR-222 and miR-223 may promote quiescence and drug resistance in breast cancer cells [49]. Furthermore, Ono et al. demonstrated that BM-MSC-exosomes decreased the sensitivity of breast cancer cells to docetaxel [27]. UC-MSC-exosomes significantly induced drug resistance of gastric cancer cells both *in vivo* and *ex vivo* [50]. MSC-exosomes inhibited 5-fluorouracil-induced apoptosis and promoted the expression of various multi-drug resistance-associated proteins, such as MDR, MRP and LRP. These findings have been associated to the activation of the CaM-Ks/Raf/MEK/ERK pathway mediated by MSC-exosomes [50]. Furthermore, MSC-EVs may induce drug resistance also in haematological tumours. BM-MSC-exosomes conferred resistance to bortezomib in multiple myeloma cells by influencing several pathways, including p38, p53, c-Jun N-terminal kinase, and Akt pathways [51]. BM-MSC-EVs also increased the chemoresistance to several drugs commonly used for chronic lymphocytic leukemia (CLL) B-cells, including fludarabine, ibrutinib, idelalisib and venetoclax [52]. The treatment with MSC-EVs significantly reduced the number of apoptotic CLL B-cells only after 24 h. Notably, EVs completely suppressed apoptosis induced by cladribine and bortezomib [52]. In addition, rat MSC-EVs may protect rat pheochromocytoma cells from the cytotoxicity induced by glutamate, which induce the reduction of Bcl-2 expression with the concomitant increase of Bax and cleaved caspase-3 expression. This effect was significantly reversed by the pretreatment with MSC-MVs, suggesting their protective effect in pheochromocytoma cells [53].

4. MSC-EVs and drug delivery

Pharmaceutical research has recently focused some strategies to design drug carriers that could be used for delivering drugs specifically to the site of interest [54]. Liposomes are considered an effective drug delivery system based on nanotechnology science and may have numerous therapeutic applications [55,56]. To date, at least 19 therapeutic approaches with liposomal drug delivery systems have been approved by FDA and EMA (www.fda.gov; www.ema.europa.eu/ema/), and many phases I–III open interventional studies are in progress. However, these formulations show several drawbacks related to stability, fatty acyl moiety oxidation, drug loading, liposome targeting, and drug leakage and release [57]. Considering the similarity between liposomes and EVs, the strategy to develop EV-based drugs has been recently proposed. Compared to liposomes, EVs possess some advantages in terms of clearance and immunogenicity. In addition, EV membranes are structurally comparable to cell membranes, thus favouring their biocompatibility and a lower toxicity [57]. Because of their ability to deliver bioactive factors among cells, one of the most intriguing therapeutic potential of EVs is the possibility to load them with the desired drug. EVs can be theoretically loaded with both hydrophilic and lipophilic drugs and, interestingly, they can deliver them to specific tissues, including the brain, considering their capacity to cross the blood-brain barrier, where they have a high stability [58]. Experimentally, there are two different approaches for EV drug loading; *i.e.* exogenously or endogenously. Endogenous loading can be carried out by strong overexpression of the drug molecule in releasing cells, while exogenous loading requires the collection of extracellular vesicles before their loading with the desired drug by using different manipulation, such as electroporation [45]. Pascucci et al. successfully obtained MSC-EVs loaded with Paclitaxel, showing their *in vitro* efficacy against pancreatic cancer cell growth [59]. Additionally, Del Fattore et al. demonstrated that MSC-EV loading with Vincristine promoted cytotoxicity toward glioblastoma cells, with a higher effect as compared to both the free drug and the unloaded EVs [60]. The possibility to directly engineer MSCs to secrete EVs enriched of specific components with anti-tumour activity is emerging. Lou et al. reported encouraging results in the context of hepatocellular carcinoma. Here, AT-MSCs were transfected with a miR-122 expression plasmid, leading to the release of miR-122 enriched exosomes. Such exosomes have shown to increase the chemosensitovity of hepatocellular carcinoma cells *in vitro* by enhancing cell apoptosis and cell cycle arrest, especially when HepG2 cell line was considered [61]. Moreover, intra-tumour injection of miR-122 enriched exosomes sensitized hepatocellular carcinoma cells to sorafenib *in vivo* [61]. Considering that breast cancer cell lines stably expressing miR-379 showed a reduced capability in terms of tumour formation and growth, O'Brien et al. proposed MSC-EVs enriched with miR-397 as an innovative therapy for metastatic breast cancer [62]. They have engineered human BM-MSCs through lentiviral transduction to release EVs enriched with miR-379 for *in vivo* therapeutic approach. The treatment with cell-free EVs loaded with miR-379 had a therapeutic effect with no adverse effects, while the administration of MSC expressing miR-397 did not interfere with tumour growth [57]. The same experimental approach was previously reported by Yuan et al. using the pro-apoptotic TNF-related apoptosis-inducing ligand (TRAIL) [63]. TRAIL and its receptors were used as targets of several anti-cancer drugs, but the clinical application of recombinant TRAIL showed several difficulties in terms of bioavailability and resistance of cancer cells. These problems could be fixed by using MSC-EVs as TRAIL vehicle. Yuan et al. developed TRAIL-enriched MSC-EVs and assessed their cancer cell-killing efficacy [63]. TRAIL enriched MSC-EVs induced apoptosis in 11 cancer cell lines in a dose-dependent fashion. They also demonstrated that caspase activity inhibition or TRAIL neutralisation abolished the cytotoxicity mediated by TRAIL-enriched EVs. Furthermore, the combined treatment of TRAIL-enriched MSC-EVs and CDK9 inhibitor enhanced the induction of apoptosis in TRAIL-resistant cancer cells [63]. Despite these encouraging results, further pre-clinical studies are necessary to clinically harness MSC-EVs as drug vehicle in the context of tumours. Moreover, their therapeutic application is still hampered by the lack of standardized and reproducible isolation and drug loading methods. In addition, understanding which population of EVs is most suitable for delivering drug molecules is also required for future therapeutic approaches.

5. Conclusions

The bidirectional transfer of molecules between tumour cells and tumour microenvironment plays a crucial role in cancer development. In this context, MSC-EVs represent potential vehicles for the delivery of pro- or anti-tumorigenic molecules. By transferring their content into recipient cells, MSC-EVs significantly influence cancer cell survival, growth, angiogenesis and drug resistance, even though some results are still controversial because of the lack of conformity in the assessment of biological functions. We discussed such controversial data specifying relevant experimental information, including the effect mediated by MVs or exosomes and MSC tissue sources. Nevetheless, standardized protocols for isolation, quantification and characterization of EVs are still required to clarify their role in tumour microenvironment and to aim for potential clinical applications. Furthermore, because of the capacity of EVs to encapsulate molecules, including proteins and nucleic acids, therapeutic strategies based on engineered EVs are currently on study and the preliminary results are quite promising, thus supporting encouraging prospects for cancer treatment.

Competing interests

The authors declare no competing financial interests.

References

[1] J.L. Spees, R.H. Lee, C.A. Gregory, Mechanisms of mesenchymal stem/stromal cell function, Stem Cell Res Ther 7 (2016) 125.
[2] S. Kidd, E. Spaeth, J.L. Dembinski, M. Dietrich, K. Watson, L. Battula, M. Weil, M. Andreeff, F.C. Marini, Direct evidence of mesenchymal stem cell tropism for tumor and wounding microenvironments using in vivo bioluminescence imaging, Stem Cells 27 (2009) 2614–2623.
[3] K.M. Bussard, L. Mutkus, K. Stumpf, C. Gomez-Manzano, F.C. Marini, Tumor-associated stromal cells as key contributors to the tumor microenvironment, Breast Cancer Res. 18 (2016) (84–84).
[4] C. Melzer, Y. Yang, R. Hass, Interaction of MSC with tumor cells, Cell Commun. Signal. 14 (2016) (20–20).
[5] H.-Y. Lee, I.-S. Hong, Double-edged sword of mesenchymal stem cells: cancer-promoting versus therapeutic potential, Cancer Sci. 108 (2017) 1939–1946.
[6] S.M. Ridge, F.J. Sullivan, S.A. Glynn, Mesenchymal stem cells: key players in cancer progression, Mol. Cancer 16 (2017) (31–31).
[7] C. Grange, M. Tapparo, F. Collino, L. Vitillo, C. Damasco, M.C. Deregibus, C. Tetta, B. Bussolati, G. Camussi, Microvesicles released from human renal cancer stem cells stimulate angiogenesis and formation of lung premetastatic niche, Cancer Res. 71 (2011) 5346–5356.
[8] A. O'Loghlen, Role for extracellular vesicles in the tumour microenvironment, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 373 (2018) (20160488–20160488).
[9] M. Di Trapani, G. Bassi, M. Midolo, A. Gatti, P.T. Kamga, A. Cassaro, R. Carusone, A. Adamo, M. Krampera, Differential and transferable modulatory effects of mesenchymal stromal cell-derived extracellular vesicles on T, B and NK cell functions, Sci. Rep. 6 (2016) 1–13.
[10] R.S. Lindoso, F. Collino, A. Vieyra, Extracellular vesicles as regulators of tumor fate: crosstalk among cancer stem cells, tumor cells and mesenchymal stem cells, Stem Cell Investigation 4 (2017) (75–75).
[11] K.C. Vallabhaneni, P. Penfornis, S. Dhule, F. Guillonneau, K.V. Adams, Y. Yuan Mo, R. Xu, Y. Liu, K. Watabe, M.C. Vemuri, R. Pochampally, Extracellular vesicles from bone marrow mesenchymal stem/stromal cells transport tumor regulatory microRNA, proteins, and metabolites, Oncotarget (6) (2015) 4953–4967.
[12] S.A.F. Morad, M.C. Cabot, Ceramide-orchestrated signalling in cancer cells, Nat. Rev. Cancer 13 (2013) 51–65.
[13] Y. Baran, A. Salas, C.E. Senkal, U. Gunduz, J. Bielawski, L.M. Obeid, B. Ogretmen, Alterations of ceramide/sphingosine 1-phosphate rheostat involved in the regulation of resistance to imatinib-induced apoptosis in K562 human chronic myeloid leukemia cells, J. Biol. Chem. 282 (2007) 10922–10934.
[14] G. Barcelo-Coblijn, M.L. Martin, R.F.M. de Almeida, M.A. Noguera-Salva, A. Marcilla-Etxenike, F. Guardiola-Serrano, A. Luth, B. Kleuser, J.E. Halver, P.V. Escriba, Sphingomyelin and sphingomyelin synthase (SMS) in the malignant transformation of glioma cells and in 2-hydroxyoleic acid therapy, Proc. Natl. Acad. Sci. 108 (2011) 19569–19574.
[15] I. Mérida, P. Torres-Ayuso, A. Ávila-Flores, J. Arranz-Nicolás, E. Andrada, M. Tello-Lafoz, R. Liébana, R. Arcos, Diacylglycerol kinases in cancer, Adv. Biol. Regul. 63 (2017) 22–31.
[16] F. Meng, R. Henson, H. Wehbe-Jane, K. Ghosal, S.T. Jacobs, T. Patel, MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer, Gastroenterology 133 (2007) 647–658.
[17] J. Chen, Z. Liu, M.M. Hong, H. Zhang, C. Chen, M. Xiao, J. Wang, F. Yao, M. Ba, J. Liu, Z.-K. Guo, J. Zhong, Proangiogenic compositions of microvesicles derived from human umbilical cord mesenchymal stem cells, PLoS ONE 9 (2014) (e115316-e115316).
[18] F. Collino, M. Pomatto, S. Bruno, R.S. Lindoso, M. Tapparo, W. Sicheng, P. Quesenberry, G. Camussi, Exosome and microvesicle-enriched fractions isolated from mesenchymal stem cells by gradient separation showed different molecular signatures and functions on renal tubular epithelial cells, Stem Cell Rev. Rep. 13 (2017) 226–243.
[19] J.D. Anderson, H.J. Johansson, C.S. Graham, M. Vesterlund, M.T. Pham, C.S. Bramlett, E.N. Montgomery, M.S. Mellema, R.L. Bardini, Z. Contreras, M. Hoon, G. Bauer, K.D. Fink, B. Fury, K.J. Hendrix, F. Chedin, S. El-Andaloussi, B. Hwang, M.S. Mulligan, J. Lehtiö, J.A. Nolta, Comprehensive proteomic analysis of mesenchymal stem cell exosomes reveals modulation of angiogenesis via nuclear factor-KappaB signaling, Stem Cells 34 (2016) 601–613.
[20] T. Lopatina, C. Gai, M.C. Deregibus, S. Kholia, G. Camussi, Cross talk between cancer and mesenchymal stem cells through extracellular vesicles carrying nucleic acids, Front. Oncol. 6 (2016) 1–11.
[21] I. Büssing, F.J. Slack, H. Großhans, Let-7 microRNAs in development, stem cells and cancer, Trends Mol. Med. 14 (2008) 400–409.
[22] J. Yang, F. Gao, Y. Zhang, Y. Liu, D. Zhang, Buyang Huanwu Decoction (BYHWD) enhances angiogenic effect of mesenchymal stem cell by upregulating VEGF expression after focal cerebral ischemia, J. Mol. Neurosci. 56 (2015) 898–906.
[23] L. Dong, Y. Pu, L. Zhang, Q. Qi, L. Xu, W. Li, C. Wei, X. Wang, S. Zhou, J. Zhu, X. Wang, F. Liu, X. Chen, C. Su, Human umbilical cord mesenchymal stem cell-derived extracellular vesicles promote lung adenocarcinoma growth by transferring miR-410 article, Cell Death Dis. 9 (2018).
[24] T. Du, G. Ju, S. Wu, Z. Cheng, J. Cheng, X. Zou, G. Zhang, S. Miao, G. Liu, Y. Zhu, Microvesicles derived from human Wharton's jelly mesenchymal stem cells promote human renal cancer cell growth and aggressiveness through induction of hepatocyte growth factor, PLoS ONE 9 (2014) 1–11.
[25] W. Zhu, L. Huang, Y. Li, X. Zhang, J. Gu, Y. Yan, X. Xu, M. Wang, H. Qian, W. Xu, Exosomes derived from human bone marrow mesenchymal stem cells promote tumor growth in vivo, Cancer Lett. 315 (2012) 28–37.
[26] S. Bruno, F. Collino, M.C. Deregibus, C. Grange, C. Tetta, G. Camussi, Microvesicles derived from human bone marrow mesenchymal stem cells inhibit tumor growth, Stem Cells Dev. 22 (2013) 758–771.
[27] M. Ono, N. Kosaka, N. Tominaga, Y. Yoshioka, F. Takeshita, R.u. Takahashi, M. Yoshida, H. Tsuda, K. Tamura, T. Ochiya, Exosomes from bone marrow mesenchymal stem cells contain a microRNA that promotes dormancy in metastatic breast cancer cells, Sci. Signal. 7 (2014) (ra63-ra63).
[28] S. Wu, G.Q. Ju, T. Du, Y.J. Zhu, G.H. Liu, Microvesicles derived from human umbilical cord Wharton's jelly mesenchymal stem cells attenuate bladder tumor cell growth in vitro and in vivo, PLoS ONE 8 (2013) 1–12.
[29] A.M.M.T. Reza, Y.-J. Choi, H. Yasuda, J.-H. Kim, Human adipose mesenchymal stem cell-derived exosomal-miRNAs are critical factors for inducing anti-proliferation signalling to A2780 and SKOV-3 ovarian cancer cells, Sci. Rep. 6 (2016) (38498–38498).
[30] A.M. Roccaro, A. Sacco, P. Maiso, A.K. Azab, Y.T. Tai, M. Reagan, F. Azab, L.M. Flores, F. Campigotto, E. Weller, K.C. Anderson, D.T. Scadden, I.M. Ghobrial, BM mesenchymal stromal cell-derived exosomes facilitate multiple myeloma progression, J. Clin. Investig. 123 (2013) 1542–1555.
[31] K.C. Vallabhaneni, P. Penfornis, F. Xing, Y. Hassler, K.V. Adams, Y.-Y. Mo, K. Watabe, R. Pochampally, K.C. Vallabhaneni, P. Penfornis, F. Xing, Y. Hassler, K.V. Adams, Y.-Y. Mo, K. Watabe, R. Pochampally, Stromal cell extracellular vesicular cargo mediated regulation of breast cancer cell metastasis via ubiquitin conjugating enzyme E2 N pathway, Oncotarget 8 (2017) 109861–109876.
[32] D. Todorova, S. Simoncini, R. Lacroix, F. Sabatier, F. Dignat-George, Extracellular vesicles in angiogenesis, Circ. Res. 120 (2017) 1658–1673.
[33] X. Liang, L. Zhang, S. Wang, Q. Han, R.C. Zhao, Exosomes secreted by mesenchymal stem cells promote endothelial cell angiogenesis by transferring miR-125a, J. Cell Sci. 129 (2016) 2182–2189.
[34] Y. Zhao, A.A. Adjei, Targeting angiogenesis in cancer therapy: moving beyond vascular endothelial growth factor, Oncologist 20 (2015) 660–673.
[35] A. Bronckaers, P. Hilkens, W. Martens, P. Gervois, J. Ratajczak, T. Struys, I. Lambrichts, Mesenchymal stem/stromal cells as a pharmacological and therapeutic approach to accelerate angiogenesis, Pharmacol. Ther. 143 (2014) 181–196.
[36] I.A.W. Ho, H.C. Toh, W.H. Ng, Y.L. Teo, C.M. Guo, K.M. Hui, P.Y.P. Lam, Human bone marrow-derived mesenchymal stem cells suppress human glioma growth through inhibition of angiogenesis, Stem Cells 31 (2013) 146–155.
[37] B.M. Beckermann, G. Kallifatidis, A. Groth, D. Frommhold, A. Apel, J. Mattern, A.V. Salnikov, G. Moldenhauer, W. Wagner, A. Diehlmann, R. Saffrich, M. Schubert, A.D. Ho, N. Giese, M.W. Büchler, H. Friess, P. Büchler, I. Herr, VEGF expression by mesenchymal stem cells contributes to angiogenesis in pancreatic carcinoma, Br. J. Cancer 99 (2008) 622–631.
[38] J.-K. Lee, S.-R. Park, B.-K. Jung, Y.-K. Jeon, Y.-S. Lee, M.-K. Kim, Y.-G. Kim, J.-Y. Jang, C.-W. Kim, Exosomes derived from mesenchymal stem cells suppress angiogenesis by down-regulating VEGF expression in breast cancer cells, PLoS ONE 8 (2013) (e84256-e84256).
[39] K. Pakravan, S. Babashah, M. Sadeghizadeh, S.J. Mowla, M. Mossahebi-Mohammadi, F. Ataei, N. Dana, M. Javan, MicroRNA-100 shuttled by mesenchymal stem cell-derived exosomes suppresses in vitro angiogenesis through modulating the mTOR/HIF-1α/VEGF signaling axis in breast cancer cells, Cell. Oncol. 40 (2017) 457–470.
[40] M. Gong, B. Yu, J. Wang, Y. Wang, M. Liu, C. Paul, R.W. Millard, D.-S. Xiao, M. Ashraf, M. Xu, Mesenchymal stem cells release exosomes that transfer miRNAs to endothelial cells and promote angiogenesis, Oncotarget 8 (2017) 45200–45212.
[41] S.R. Baglio, K. Rooijers, D. Koppers-Lalic, F.J. Verweij, M.P. Lanzón, N. Zini, B. Naaijkens, F. Perut, H.W.M. Niessen, N. Baldini, D.M. Pegtel, Human bone marrow-and adiposemesenchymal stem cells secrete exosomes enriched in distinctive miRNA and tRNA species, Stem Cell Res. Ther. 6 (2015) 1–20.
[42] J.D. McBride, L. Menocal-Rodriguez, A. Candanedo, W. Guzman, M. Garcia-Contreras, E.V. Badiavas, Bone marrow mesenchymal stem cell-derived CD63 + exosomes transport Wnt3a exteriorly and enhance dermal fibroblast proliferation, migration and angiogenesis in vitro, Stem Cells Dev. 26 (2017) (scd.2017.0087-scd.2017.0087).
[43] B. Zhang, X. Wu, X. Zhang, Y. Sun, Y. Yan, H. Shi, Y. Zhu, L. Wu, Z. Pan, W. Zhu, H. Qian, W. Xu, Human umbilical cord mesenchymal stem cell exosomes enhance angiogenesis through the Wnt4/β-catenin pathway, Stem Cells Transl. Med. 4 (2015) 513–522.
[44] C.L. Sicco, D. Reverberi, C. Balbi, V. Ulivi, E. Principi, L. Pascucci, P. Becherini, M.C. Bosco, L. Varesio, C. Franzin, M. Pozzobon, R. Cancedda, R. Tasso, Mesenchymal stem cell-derived extracellular vesicles as mediators of anti-inflammatory effects: endorsement of macrophage polarization, Stem Cells Transl. Med. 6 (2017) 1018–1028.
[45] S. E.L. Andaloussi, I. Mäger, X.O. Breakefield, M.J.A. Wood, Extracellular Vesicles: Biology and Emerging Therapeutic Opportunities, (2013).
[46] C. Holohan, S. Van Schaeybroeck, D.B. Longley, P.G. Johnston, Cancer drug resistance: an evolving paradigm, Nat. Rev. Cancer 13 (2013) 714–726.
[47] J.M. Houthuijzen, L.G.M. Daenen, J.M.L. Roodhart, E.E. Voest, The role of mesenchymal stem cells in anti-cancer drug resistance and tumour progression, Br. J. Cancer 106 (2012) 1901–1906.
[48] P. Samuel, M. Fabbri, D.R.F. Carter, Mechanisms of drug resistance in cancer: the role of extracellular vesicles, Proteomics 17 (2017).
[49] S.A. Bliss, G. Sinha, O.A. Sandiford, L.M. Williams, D.J. Engelberth, K. Guiro, L.L. Isenalumhe, S.J. Greco, S. Ayer, M. Bryan, R. Kumar, N.M. Ponzio, P. Rameshwar, Mesenchymal stem cell-derived exosomes stimulate cycling quiescence and early breast cancer dormancy in bone marrow, Cancer Res. 76 (2016) 5832–5844.

[50] R. Ji, B. Zhang, X. Zhang, J. Xue, X. Yuan, Y. Yan, M. Wang, W. Zhu, H. Qian, W. Xu, Exosomes derived from human mesenchymal stem cells confer drug resistance in gastric cancer, Cell Cycle (Georgetown, Tex.) 14 (2015) 2473–2483.
[51] J. Wang, A. Hendrix, S. Hernot, M. Lemaire, E.D. Bruyne, E.V. Valckenborgh, T. Lahoutte, O.D. Wever, K. Vanderkerken, E. Menu, Bone marrow stromal cell – derived exosomes as communicators in drug resistance in multiple myeloma cells, Blood J. 124 (4) (2014) 555–567.
[52] E. Crompot, M. van Damme, K. Pieters, M. Vermeersch, D. Perez-Morga, P. Mineur, M. Maerevoet, N. Meuleman, D. Bron, L. Lagneaux, B. Stamatopoulos, Extracellular vesicles of bone marrow stromal cells rescue chronic lymphocytic leukemia B cells from apoptosis, enhance their migration and induce gene expression modifications, Haematologica 102 (2017) 1594–1604.
[53] S.S. Lin, B. Zhu, Z.K. Guo, G.Z. Huang, Z. Wang, J. Chen, X.J. Wei, Q. Li, Bone marrow mesenchymal stem cell-derived microvesicles protect rat pheochromocytoma PC12 cells from glutamate-induced injury via a PI3K/Akt dependent pathway, Neurochem. Res. 39 (2014) 922–931.
[54] J.A. Hubbell, A. Chilkoti, Nanomaterials for Drug Delivery Jeffrey, 337 (2012), pp. 303–305.
[55] M.S. Muthu, S.-S. Feng, Theranostic liposomes for cancer diagnosis and treatment: current development and pre-clinical success, Expert Opin. Drug Deliv. 10 (2013) 151–155.
[56] W. Gao, C.-M.J. Hu, R.H. Fang, L. Zhang, Liposome-like nanostructures for drug delivery, J. Mater. Chem. B (2013) 1.
[57] S.-I. Ohno, G.P.C. Drummen, M. Kuroda, Focus on extracellular vesicles: development of extracellular vesicle-based therapeutic systems, Int. J. Mol. Sci. 17 (2016) (172–172).
[58] M.J. Haney, N.L. Klyachko, Y. Zhao, R. Gupta, E.G. Plotnikova, Z. He, T. Patel, A. Piroyan, M. Sokolsky, A.V. Kabanov, E.V. Batrakova, Exosomes as drug delivery vehicles for Parkinson's disease therapy, J. Control. Release 207 (2015) 18–30.
[59] L. Pascucci, V. Coccè, A. Bonomi, D. Ami, P. Ceccarelli, E. Ciusani, L. Viganò, A. Locatelli, F. Sisto, S.M. Doglia, E. Parati, M.E. Bernardo, M. Muraca, G. Alessandri, G. Bondiolotti, A. Pessina, Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit in vitro tumor growth: a new approach for drug delivery, J. Control. Release 192 (2014) 262–270.
[60] A. Del Fattore, R. Luciano, R. Saracino, G. Battafarano, C. Rizzo, L. Pascucci, G. Alessandri, A. Pessina, A. Perrotta, A. Fierabracci, M. Muraca, Differential effects of extracellular vesicles secreted by mesenchymal stem cells from different sources on glioblastoma cells, Expert. Opin. Biol. Ther. 15 (2015) 495–504.
[61] G. Lou, X. Song, F. Yang, S. Wu, J. Wang, Z. Chen, Y. Liu, Exosomes derived from miR-122-modified adipose tissue-derived MSCs increase chemosensitivity of hepatocellular carcinoma, J. Hematol. Oncol. 8 (2015) 122.
[62] K.P. O'Brien, S. Khan, K.E. Gilligan, H. Zafar, P. Lalor, C. Glynn, C. O'Flatharta, H. Ingoldsby, P. Dockery, A. De Bhulbh, J.R. Schweber, K. St John, M. Leahy, J.M. Murphy, W.M. Gallagher, T. O'Brien, M.J. Kerin, R.M. Dwyer, Employing mesenchymal stem cells to support tumor-targeted delivery of extracellular vesicle (EV)-encapsulated microRNA-379, Oncogene 37 (2018) 2137–2149.
[63] Z. Yuan, K.K. Kolluri, K.H.C. Gowers, S.M. Janes, TRAIL delivery by MSC-derived extracellular vesicles is an effective anticancer therapy, J. Extracellular Vesicles 6 (2017) (1265291–1265291).