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REVIEW

EVs vs. EVs: MSCs and Tregs as a source of invisible possibilities

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Abstract

Extracellular vesicles (EVs) are produced by various cells and exist in most biological fluids. They play an important role in cell–cell signaling, immune response, and tumor metastasis, and also have theranostic potential. They deliver many functional biomolecules, including DNA, microRNAs (miRNA), messenger RNA (mRNA), long non-coding RNA (lncRNA), lipids, and proteins, thus affecting different physiological processes in target cells. Decreased immunogenicity compared to liposomes or viral vectors and the ability to cross through physiological barriers such as the blood–brain barrier make them an attractive and innovative option as diagnostic biomarkers and therapeutic carriers. Here, we highlighted two types of cells that can produce functional EVs, namely, mesenchymal stem/stromal cells (MSCs) and regulatory T cells (Tregs), discussing MSC/Treg-derived EV-based therapies for some specific diseases including acute respiratory distress syndrome (ARDS), autoimmune diseases, and cancer.

Keywords Extracellular vesicles · Mesenchymal stem · Stromal cells · Regulatory T cells

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Introduction

Extracellular vesicles (EVs) play an important role in cell–cell communication and extracellular matrix remodeling [1, 2]. They are secreted by most cell types including mesenchymal stromal cells (MSCs), immune cells, endothelial cells, epithelial cells, neuronal cells, embryonic stem cells, and cancer cells [3–5]. Cell-free therapies, being the safe, available, and cost-effective alternative to cell-based therapies [6], have boosted the EV research over the past years.

Although several classes of EVs have been discovered, the International Society for Extracellular Vesicles emphasizes that potential heterogeneity and undefined biogenesis of the obtained samples should imply the use of the term EVs rather than referring to the specific EV subgroup [7]. To avoid any bias or misunderstanding and to support this important point raised in the MISEV2018 guidelines, in this article, we will stick to this term.

MSC-derived EVs (MSC-EVs) seem to be the most researched ones, which is hardly surprising given the wide use of MSCs in cell therapy. Not only MSCs' potential for self-renewal and multi-lineage differentiation, but also their paracrine activity is currently believed to contribute to their unique therapeutic properties. Moreover, there is evidence that conditioned media of MSCs demonstrate therapeutic effects similar to those of transplanted cells [8–10], which means that cytokines, chemokines, and most importantly EVs come to the fore as the probable key factors determining MSCs' therapeutic potential [11, 12]. Hence, MSC-EVs are being extensively investigated and employed to treat a wide range of diseases, including cardiovascular, neurological, immunological, and kidney pathologies [13].

Immune regulation could become an unequaled tool in treating plenty of pathologic conditions from inflammatory and autoimmune ones to cancer. While MSCs and their EVs exhibit some immunomodulatory properties, immune cells, and most importantly regulatory T cells (Tregs), might be a possible source of EVs with even more potent and specific effects.

Tregs are a subpopulation of CD4 + T lymphocytes that play a crucial role in the creation of immunological self-tolerance unlike CD8 + cytotoxic T lymphocytes [23, 24]. Although the mechanism of their regulatory effects is not fully elucidated, Tregs are reported to produce anti-inflammatory factors including interleukin-10 (IL-10), transforming growth factor beta 1 (TGF-β), and IL-13, thus suppressing the inflammatory response and reducing the inflammatory damage [25, 26]. Accordingly, numerous studies have indicated that type 1 diabetes, multiple sclerosis, myasthenia gravis, rheumatoid arthritis, and other autoimmune diseases are caused by Treg deficiencies [27]. At the same time, Tregs were reported to promote tumor formation by reducing the anticancer activity of immune cells [28]. Hence, a reduction in Treg's activity may facilitate anticancer immune responses in vivo [29], and targeting Tregs may improve tumor treatment efficacy [30].

In contrast to MSC-EVs, which have been extensively studied, Treg-EV-based studies are still in their infancy. However, Treg-EVs are starting to gain more interest due to their immunosuppressive effects, such as extending survival in animal allograft models [31].

Up to now, there have been 257 clinical trials (www.clinicaltial.gov) evaluating the Treg's effects in treating different conditions and no clinical trials evaluating Treg-EV effects. However, both Tregs and their EVs hold great promise in increasing the efficacy of autoimmune disease therapies or improving the transplant patients' condition.

This review aims to discuss the research of understudied Treg-EVs by contrast with the widely studied MSC-EVs (Table 1; Fig. 1) and encourage further studies in this field. We aimed to focus on the immunomodulatory properties of the EVs and therefore chose to discuss the pathological conditions that would illustrate the duality of their effect. Thus, we chose those characterized by immune system overreactivity (ARDS accompanied by cytokine storm, autoimmune diseases, allograft rejection) and cancer, characterized by immune system suppression.

MSC-EVs and Treg-EVs: contents and immunomodulatory functions

Multiple studies reported MSC-EVs' specific cargo and associated biological effects, including immunomodulatory ones. In addition to numerous miRNAs, which will be further discussed in this review, there is evidence that MSC-EVs contain growth factors and cytokines, including hepatocyte growth factor (HGF), TGFβ, interleukin-6 (IL-6), and IL-10, which contribute to immunoregulation mechanisms [54].

In contrast, the contents of Treg-EVs are not so thoroughly explored. Like their parent cells, Treg-EVs were reported to express CD73, CD39, and CD25 [22] (Table 2). Both CD73 and CD 39 are involved in the adenosinergic pathway, which plays a key role in modulating immune responses [55]. At the same time, CD25 is speculated to promote T-effector apoptosis; however, this assumption requires further validation [22].

ARDS/ALI

ARDS, as well as its milder form, acute lung injury (ALI), is a complex inflammatory lung disease which leads to substantial reductions in lung diffusing capacity and is characterized by

Table 1 MSC-EV and Treg-EV effect on over-reactive immune conditions and cancer development: in vivo studies

Cell source	EV source	Animal model	Modeled pathological condition	miRNA studied	Native/loaded	Outcomes	Ref
Mouse	AMSCs	Mouse	T1D	-	Native	Stabilizing blood glucose levels; pancreatic islets regeneration	[32]
Human	AMSCs	Mouse	HCC	miR-199a	Loaded	↓ Tumor growth	[33]
Human	BMMSCs	Mouse	ALI	miR-145 (blocked)	Loaded	↓ Antimicrobial effect	[34]
Rat	BMMSCs	Rat	EAE	-	Native	↓ Inflammatory CNS infiltration ↓ Demyelination ↑ M2-related cytokines ↓ M1-related cytokines	[35]
Mouse	BMMSCs	Mouse	EAE	-	Native	↓ Inflammatory response; ↑ Fibers within lesion; ↑ Thickness of the myelin sheaths	[36]
Human	BMMSCs	Mouse	T1D + islet transplantation	miR-375 (blocked)	Loaded	↑ Allograft survival	[37]
Rat	BMMSCs	Mouse	T1D	-	Native	↓ Learning and memory impairment; ↓ Oxidative stress; ↑ Synaptic density	[38]
Mouse	BMMSCs	Mouse	CIA	-	Native	↓ Swelling; ↓ Global clinical score	[39]
Mouse	BMMSCs	Mouse	CIA	miR-150-5p	Loaded	↓ VEGF and MMP14; ↓ Hind paw thickness; ↓ Clinical arthritis score	[40]
Human	BMMSCs	Mouse	CIA	miR-320a	Loaded	↓ Arthritic index; ↓ Inflammatory cell infiltration	[41]
Human	BMMSCs	Mouse	CML	-	Native	↑ Tumor incidence and growth	[42]
Mouse	BMMSCs	Mouse	Breast cancer	miR-16	Native	↓ Tumor weight; ↓ Angiogenesis	[43]
Human	BMMSCs	Mouse	Glioma	miR-29a-3p	Loaded	↓ Tumor growth; prolonged survival time	[44]
Human	BMMSCs	Mouse	Glioma	miR-199a	Loaded	↓ Tumor weight	[45]
Human	BMMSCs	Mouse	GBM	miR-512-5p	Loaded	↓ Tumor growth; ↑ Survival time	[46]
Human	ESC-MSCs	Mouse	Psoriasis	-	Native	↓ IL-17 and C5b-9 in the skin	[47]
Human	MBMSCs	Rat	T1D	-	Native	Stabilizing blood glucose levels; pancreatic islets regeneration	[48]
Human	UCMSCs	Mouse	Psoriasis	-	Native	↓ Epidermis proliferation ↓ PASI scores	[49]
Mouse	Tregs	Mouse	AMI	-	Native	↓ iNOS, IL-1β, and TNF-α in the myocardial tissues; ↓ Infarct size; ↓ Myocardial cells apoptosis; ↑ M2 macrophage polarization	[50]
Mouse	Tregs	Mouse	CIA	miR-449a-5p	Loaded	Maintaining Th17/Treg cells balance	[51]
Rat	Tregs	Rat	kidney allo-transplantation	-	Native	↑ Allograft survival	[52]
Mouse	Tregs	Mouse	IBD	miR-195a-3p	Native	↑ Colon structure integrity; ↓ Mucosal damage	[53]

ALI acute lung injury, *AMI* acute myocardial infarction, *AMSCs* adipose tissue–derived MSCs, *BMMSCs* bone marrow–derived MSCs, *CIA* collagen-induced arthritis, *CML* chronic myeloid leukemia, *CNS* central nervous system, *EAE* experimental autoimmune encephalomyelitis, *ESC-MSCs* embryonic stem cell–derived MSCs, *EVs* extracellular vesicles, *GBM* glioblastoma, *HCC* hepatocellular carcinoma, *IBD* inflammatory bowel disease, *IL* interleukin, *iNOS* inducible nitric oxide synthase, *MBMSCs* menstrual blood–derived MSCs, *MMP* matrix metalloproteinase, *PASI* Psoriasis Area and Severity Index, *T1D* type 1diabetes, *Th* T helper cell, *TNF-α* tumor necrosis factor α, *Treg* regulatory T cell, *UCMSCs* umbilical cord–derived MSCs, *VEGF* vascular endothelial growth factor

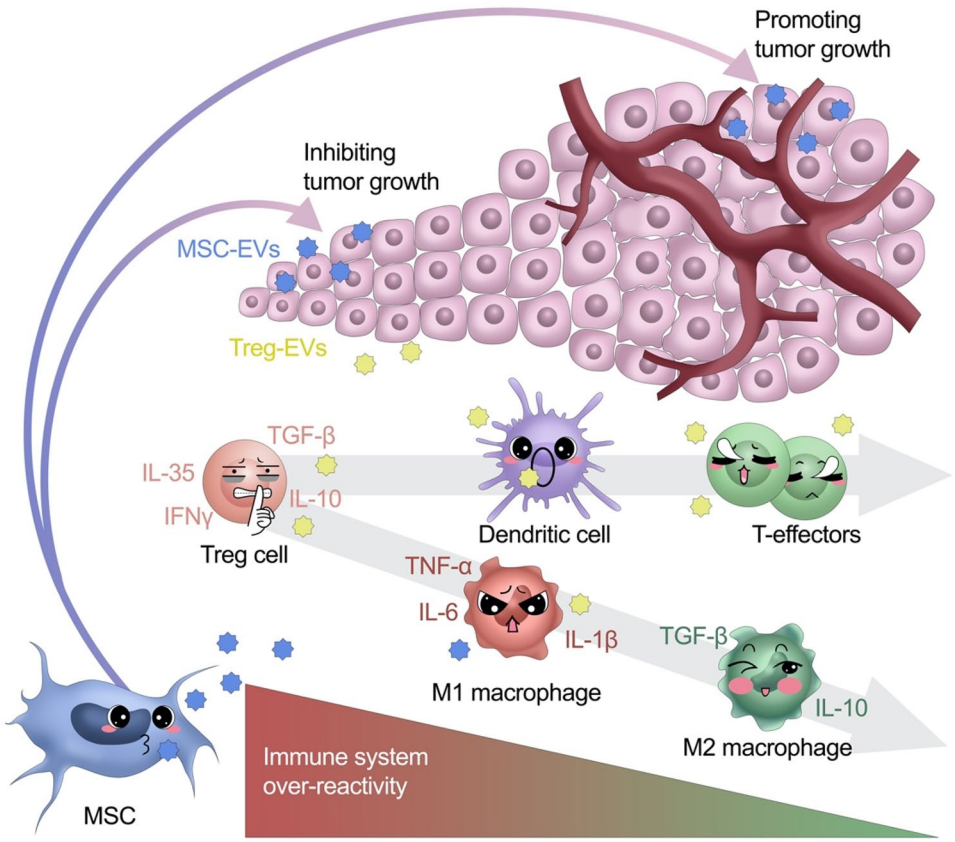

Fig. 1 MSC-EVs andTreg-EVs effect on over-reactive immune conditions and cancer development

high morbidity and mortality in intensive care unit–admitted patients. It results from a variety of pathological conditions, including pulmonary (e.g., aspiration, pneumonia) and non-pulmonary ones (e.g., trauma, sepsis) [56]. The pathogenesis of ARDS involves alveolar-capillary membrane damage, pulmonary edema due to increased inflammation, inactivation of surfactant, reduced alveolar fluid clearance, impairment of oxygenation, and pulmonary fibrosis [57, 58]. It is thought that uncontrolled inflammation underlies all these pathophysiological mechanisms [59], often leading to life-threatening complications [60]. Despite the therapeutic advances [61], new effective ARDS treatments are urgently needed because of the high mortality rate (34.9–46.1%) [62]. The increase in deaths due to COVID-19-related ARDS during the pandemic in 2019–2022 has once again emphasized the importance of developing new therapeutic options [63, 64].

Table 2 Immunomodulatory cargo of MSC-EVs and Treg-EVs

Bioactive molecule	Functions	Ref
MSC-EVs		
HGF	IL-10 production ↑ T cell proliferation ↓	[14, 15]
TGFβ	T cell proliferation ↓	[16–18]
IL-6	Dendritic cell differentiation ↓	[19]
IL-10	T cell proliferation ↓	[20]
Treg-EVs		
CD73	Adenosine production ↑ T-effector functions ↓	[21]
CD39	Adenosine production ↑ T-effector functions ↓	[22]
CD25	IL-2 production ↓ T-effector apoptosis ↑	[22]

MSC-EVs and ARDS/ALI

There is evidence that MSC-EVs can target epithelial cell damage in ARDS patients, preventing the endothelial and epithelial lung barrier deficiency, increased alveolus permeability, and decrease pulmonary edema. Thus, Bari et al. reported adipose-derived MSC-EVs to express alpha-1-anti trypsin, the main elastase inhibitor in the lung, which could repress proteolytic enzymes derived from neutrophils and demonstrated anti-inflammatory and immune-regulating properties which could protect lung epithelial cells [65]. Other studies revealed that bone marrow–derived MSC-EVs could reduce pulmonary edema [66] and restore alveolar fluid clearance [67].

Furthermore, there is evidence that bone marrow–derived MSC-EVs can shift the balance between pro-inflammatory

M1 and anti-inflammatory M2 macrophages [68]. It could be the turning point in inflammation modulation and enhancing tissue repair for ARDS patients experiencing cytokine storm, which practically uncontrollably leads to cytotoxic tissue damage, exacerbating the disease prognosis. Moreover, the presence of miR-145 in bone marrow–derived MSC-EVs was reported to improve the lung tissue maintenance and regeneration [34].

Treg-EVs and ARDS/ALI

Given that Tregs play a crucial role in inhibiting excessive inflammation, Treg-EVs seem a valid option to manage the uncontrolled inflammation occurring in ARDS.

For instance, Treg-EVs can transport specific miRNAs such as miR-150, miR-142-3p, miR-146a-5p, and let-7d from Tregs to effector T cells and dendritic cells, performing targeted mRNA suppression in these cells [69–71]. There is evidence that two of these miRNAs, namely, miR-142-3p and miR-150, alleviate lipopolysaccharide-induced ALI [72, 73].

Although there is no study yet on the use of Treg-EVs in the treatment of ALI/ARDS, their application in other immune system–related conditions may be instructive. As we emphasized earlier, promoted M2 macrophage polarization controls excessive inflammation in ALI/ARDS [74], and Treg-EVs were reported to increase M2 polarization in macrophages in a murine model [50].

It is well known that the maintenance of T helper 17 (Th17)/Treg balance is essential to prevent immunological diseases, but this balance is also crucial for the control of lung inflammation in ALI/ARDS [75–77]. In a recent study, Chen et al. showed that Treg-EVs from TGF-β-induced Tregs could maintain the balance of Th17/Tregs and regulate Notch1 signaling via miR-449a-5p in a murine model [51]. Finally, Sullivan et al. reported Treg-EVs to suppress CD8 + cytotoxic T lymphocyte proliferation [78], which could possibly alleviate the cytokine storm-mediated tissue damage.

Autoimmune conditions

Autoimmune conditions are characterized by abnormal immune response resulting in healthy cells' destruction. They include, but are not limited to, psoriasis, multiple sclerosis (MS), type 1 diabetes (T1D), rheumatoid arthritis (RA), and inflammatory bowel disease (IBD). Allograft rejection also involves an autoimmune component.

Whether they affect the skin, central nervous system (CNS), pancreas, joints, or other tissues and organs, autoimmune diseases are commonly treated with immune system suppressants, which often have limited efficacy [79] while causing a number of adverse effects including nephrotoxicity, hepatotoxicity, hypertension, hyperkalemia, lymphadenopathy, lymphopenia, lipoatrophy, and dyspnea [80–84]. Therefore, the search for alternative treatment strategies in this field is of great need.

MSC-EVs and autoimmune conditions

Psoriasis Human umbilical cord–derived MSC-EVs were reported to significantly suppress the proliferation of epidermis and to reduce Psoriasis Area and Severity Index (PASI) scores in the imiquimod (IMQ)–induced murine model. These EVs also reduced the expression of inflammatory IL-17, IL-23, and chemokine C–C-motif ligand 20 as well as suppressed phosphorylation of signal transducer and activator of transcription 3 both in the skin of IMQ-induced murine model and in human keratinocytes [49]. A similar study reported embryonic stem cell–derived MSC-EVs to reduce psoriasis-associated inflammation in a murine model of IMQ psoriasis via inhibition of complement stimulation in the stratum corneum and reduction in C5b-9 complex and IL-17 [47].

Multiple sclerosis MSC-EVs were confirmed to be involved in microglial polarization and improvement of motor function in the experimental autoimmune encephalomyelitis (EAE) in a rat model. Comparing the treated and untreated EAE groups, Li et al. showed that bone marrow–derived MSC-EVs significantly reduced neural behavioral scores, decreased the infiltration of inflammatory cells in CNS, and reduced demyelination in the treated group compared to the untreated one. Moreover, they showed that treatment with EVs significantly increased the M2-related cytokines such as TGF-β and IL-10. However, at the same time, significant increases in the production of M1-related tumor necrosis factor alpha (TNF-α) and IL-12 were observed [35]. Hosseini Shamili et al. bio-conjugated the bone marrow–derived MSC-EVs to an aptamer targeting the oligodendrocyte markers. The armed EVs increased the proliferation rate of oligodendroglia cell line (OLN93) in vitro and alleviated demyelinated CNS lesions in vivo in a murine EAE model [36].

Type 1 diabetes mellitus Menstrual blood–derived MSC-EVs were reported to induce the islet regeneration via pancreatic and duodenal homeobox 1 pathway [48]. Moreover, there is evidence that bone marrow–derived MSC-EV injection combined with islet transplantation could suppress peripheral blood mononuclear cell proliferation, induce regulatory T cells, and increase survival in the T1D

murine model [37]. Similarly, Nojehdehi et al. demonstrated adipose-derived MSC-EVs to alleviate clinical symptoms of the streptozotocin-induced T1D model including blood glucose stabilization [32]. The authors also reported the increase of Tregs population, upregulation of IL-10, IL-4, and TGF-β, and downregulation of IL-17 and IFNγ. It is noteworthy that MSC-EVs could improve not only metabolic-related disorders, but also T1D-associated complications, such as cognitive impairment, promoting neurons and astrocytes repair [38]. Due to these promising results, umbilical cord–derived MSC-EVs have entered the II/III clinical trial phase for T1D treatment (NCT02138331) [85].

Rheumatoid arthritis Bone marrow–derived MSC-EVs were observed to have an anti-inflammatory effect on T and B lymphocytes in a collagen-induced arthritis (CIA) murine model [39], and this effect was enhanced when MSC-EVs were additionally loaded with specific miRNAs. For example, miR-150-5p-enriched MSC-EVs blocked migration and invasion of rheumatoid arthritis–related fibroblast-like synoviocytes (RA-FLS), decreased hind foot thickness, and inhibited angiogenesis in a murine CIA model [40]. Likewise, bone marrow–derived MSC-EVs miR-124a could block proliferation and promote apoptosis of FLS cell line in vitro [86]. There is also evidence of correlation between the expression of miR-320a and chemokine C-X-C motif ligand 9 in the RA synovial tissue. Being a target of miR-320a, CXCL9 was reported to reestablish the function of RA-FLSs when upregulated and suppress their activation, migration, and invasion when knocked down [87]. Accordingly, bone marrow–derived MSC-EVs containing miR-320a demonstrated the same effect in vitro and decreased arthritis and bone injury in CIA mice. [41].

Treg-EVs and autoimmune conditions

Allograft rejection While Treg's role in suppressing alloreactivity has been shown in various studies [52, 88, 89], Treg-EVs in this light have only been studied by Aiello et al. [52]. This study investigated Treg-EVs from dnIKK transgenic mice, and it was demonstrated that modified DnIKK2-Treg-EVs contained a unique molecular cargo of specific miRNAs and iNOS which could convert T cells into regulatory cells. Moreover, inhibiting either miRNAs or NOS alone only partially reduced the suppressive capacity of DnIKK2-Treg-EVs suggesting both are critical. The authors have also found modest but significant improvement with DnIKK2-Treg-EVs in kidney allograft survival in rats arguing that Treg-EVs may provide clinical benefit to transplant patients.

Inflammatory bowel disease models The effectiveness of Treg-EVs in IBD treatment has been demonstrated by two studies in murine models. Okoye et al.'s studies in mice showed that Tregs utilize miRNAs and deliver them via EVs to T cells to suppress T helper 1 (Th1) response. The authors have demonstrated this by using Dicer-/- as well as Rab27a/Rab27b double knockout mice which are deficient in miRNA or EV biogenesis, respectively [69]. The authors have tested Treg-EVs' therapeutic potential in an adoptive T cell transfer model of colitis, induced by $CD4^{+}CD45RB^{hi}$ T cells in Rag1-deficient mice. The suppression of inflammatory bowel disease development by Tregs was impaired when Tregs from Dicer-/-, as well as Rab27a/Rab27b double knockout mice-derived Tregs, were used, suggesting that Treg-EVs are indeed contributing to the suppression of inflammation mediated by T cells in vivo. Let-7d has been proposed to be a critical mediator of such suppression [69]. More recently, Liao et al. showed that murine splenic Treg-EVs also ameliorated dextran sodium salt-induced colitis, an acute model of colitis induced by chemical destruction of the intestinal epithelia. This study suggested a role for miR-195a-3p, a miRNA shown to be present in Treg-EVs, in mediating the suppression [53].

Cancer

The crosstalk between the tumor and its microenvironment was proven to have a direct impact on tumor growth and metastasis [90], immune cells including macrophages [91] and Tregs [92] being both the key players and targets in this process. Such orchestrating of the tumor microenvironment is partially provided by the tumor cells' EVs [93–97], which means that the use of EVs derived from non-tumor cells, either native or loaded with specific miRNAs, could possibly modulate the tumor microenvironment in the way favorable for antitumor therapies.

MSC-EVs and cancer

Native MSC-EVs There is evidence of both antitumor and tumorigenic action of MSC-EVs on angiogenesis, the tumor cell proliferation, invasion, metastasis, and response to medicines and to radiotherapy [98]. For example, human bone marrow–derived MSC-EVs were reported to inhibit the proliferation of human chronic myeloid leukemia cells in vitro via miR-15a, whereas in vivo, they were shown to increase tumor incidence and speed up tumor growth [42]. This is not an isolated case, and it suggests that the use of native MSCs-EVs, just like the use of MSCs, should require cautiousness in terms of tumorigenicity. However, some pathways determining the possible tumorigenic effect of MSCs-EVs are already determined, which is encouraging, since deepening our knowledge about these pathways will probably give us the chance of managing the unfavorable

ones. For example, human bone marrow–derived MSC-EVs were reported to promote gastric cancer cell growth through the activation of the hedgehog signaling pathway [99], while another study reported native umbilical cord–derived MSC-EVs to increase gastric cancer cell proliferative and metastatic potential predominantly via the activation of the protein kinase B signaling pathway [100]. At the same time, adipose-derived MSC-EVs promoted migration and proliferation of breast cancer cell line MCF7 through the activation of the Wnt signaling pathway [101]. Paradoxically, MSC-EV immunomodulatory properties can be another concern regarding their use in oncology. While their ability to switch macrophages' phenotype from pro-inflammatory M1 to anti-inflammatory M2 is beneficial in treating such inflammatory conditions as ARDS/ALI [68], it can have undesirable effect in tumor treatment.

On the other hand, many studies report the beneficial effect of native MSC-EVs. For example, native bone marrow–derived MSC-EVs were shown to inhibit tumor development and progression in an experimental rat model of diethylnitrosamine-induced hepatocellular carcinoma, which manifested as apoptosis activation, as well as inhibition of angiogenesis and epithelial–mesenchymal transition [102].

Just like reporting the signaling pathways mediating the tumorigenic effect of MSC-EVs, many researchers report specific MSC-EV miRNAs to have antitumor properties. For instance, miR-16 of native bone marrow–derived MSC-EVs was reported to inhibit angiogenesis via downregulation of the expression of vascular endothelial growth factor (VEGF) [43]. Time-dependent downregulation of VEGF in breast cancer cells was also reported to be caused by bone marrow–derived MSC-EVs-miR-100 targeting the mTOR/HIF-1α signaling axis [103]. Another study reported miR-4461 to be a potential target for the diagnosis and treatment of colorectal cancer, since bone marrow–derived MSC-EVs-miR-4461 inhibited the proliferation, migration, and invasion of colorectal cancer cells by downregulating coatomer protein complex subunit beta 2 expression [104]. Furthermore, human bone marrow–derived MSC-EVs carrying miR-205 were reported to suppress rhophilin Rho GTPase binding protein 2, thus enhancing prostate cancer cell apoptosis [105]. miR-143 of human bone marrow–derived MSC-EVs demonstrated the similar effect by downregulating trefoil factor 3 [106].

Functionalized MSC-EVs Modified or engineered MSC-EVs, however, are reported to have enhanced specificity, reduced immunogenicity, and better targeting capabilities than the native ones [107]. Thus, once the important pathways and essential miRNAs are determined, MSC-EVs can be modified in a way maximizing their beneficial properties and minimizing the unfavorable ones. One way of doing so is loading the EVs with a specific miRNA, since there is evidence that EV-mediated miRNA transport protects them, providing an improved therapeutic effect compared to direct miRNA treatment [108].

Thus, it was determined that miR-1228-loaded human bone marrow–derived MSC-EVs inhibit proliferation, invasion, and migration and accelerate apoptosis of gastric cancer cells, by downregulating the expression of matrix metalloproteinase-14 (MMP-14) [109]. Similar effect was observed in PANC-1 cells treated with human umbilical cord–derived MSC-EVs transfected with hsa-miRNA-128-3p, which targeted Galectin-3 [110]. At the same time, human glioma cell line (U87MG and A172) migration and vasculogenic mimicry formation were suppressed by miR-29a-3p-loaded bone marrow–derived MSC-EVs targeting roundabout guidance receptor 1 [44], while another study reported miRNA-512-5p-loaded bone marrow–derived MSC-EVs to target Jagged Canonical Notch Ligand 1 and inhibit glioblastoma cell proliferation and G1-S phase cell cycle by inhibiting the expression of CDK4, CDK6, and Cyclin D1 [46].

Another approach suggests loading EVs with agents blocking specific miRNAs; for example, LNA-antimiR-142-3p-loaded bone marrow–derived MSC-EVs were demonstrated to decrease expression of the miR-142-3p and miR-150, thus reducing clone-formation and tumor-initiating abilities of the MCF7-derived cancer stem-like cells [111]. Or else, EVs can be engineered in a way allowing to target specific oncogenes. For instance, engineered bone marrow–derived MSC-EVs with the ability to target oncogenic KRAS could induce apoptosis of PANC-1 cells, and their use in a murine model of pancreas ductal adenocarcinoma (PDAC) improved the histopathology of pancreas, reduced tumor burden, and exhibited a trending decrease in tumor weight [112]. Moreover, there is an ongoing clinical trial evaluating the efficacy and safety of MSC-EVs with KrasG12D siRNA in treating patients suffering from metastatic pancreatic cancer with KrasG12D mutation (NCT03608631).

Some miRNAs can enhance the chemosensitivity of tumor cells. For example, human adipose MSC-derived miR-199a-loaded EVs targeting and inhibiting mammalian target of rapamycin increased the chemosensitivity of hepatocellular carcinoma cells to doxorubicin both in vivo and in vitro [33]. At the same time, miR-199a-loaded human bone marrow–derived MSC-EVs enhanced the chemosensitivity of glioma cells to temozolomide by downregulating ankyrin repeat and PH domain 2 [45]. Finally, MSC-EVs can act as a vehicle for targeted therapy and can be directly loaded with therapeutic agents. For instance, taxol-loaded umbilical cord–derived MSC-EVs were demonstrated to significantly decrease SK-OV-3 ovarian cancer cell viability [113].

Treg-EVs and cancer

As it was mentioned earlier, Tregs' immunosuppressive properties beneficial for treating conditions characterized by excessive inflammation may at the same time impede the establishment of effective antitumor immunity in patients with advanced malignancies and even accelerate tumor progression. Despite the fact that cancer immunotherapy is very promising, new anticancer drugs and vaccines have failed to show promising benefits against cancer, which is at least partly due to Treg infiltration into the tumor region and suppression of anticancer drug and vaccine activities [114].

Therefore, studies involving the use of Treg-EVs in cancer models are lacking since targeting them rather than activating seems the right strategy to improve tumor treatment efficacy [30]. On the other hand, there is evidence that Treg-EVs contain proapoptotic or antiproliferative miRNAs (miR-466 family [115], miR-195 [116], and miR-16 [117]), which suggests that they still have some antitumor potential which can be realized when sufficiently studied.

Furthermore, a detailed characterization of Tregs in tumor areas would help us better understand how Treg transcription changes in tumor-specific contexts [118]. For example, Treg cell death pathways [119], Treg-produced IL-35 [120–125], and epigenetic pathways [121, 126] could all be potential therapeutic targets and warrant further study.

Challenges and prospects

Based on the above analysis, it is clear that EVs, and particularly MSC-EVs and Treg-EVs, are a relevant research topic. At the same time, their translation to the clinical practice may be hampered by some considerable challenges. First of all, standardized protocols for different stages of the EV-based biomedical product development are lacking. Although the approaches to the EV isolation, classification, purification, characterization, and storage have been developed in the past years, they need to be standardized for large-scale production and clinical applications.

One of the main problems remains the heterogeneity of the obtained samples [7]. In order to address rigor and reproducibility issues in EV research, many aspects should be considered. Those should include not only their size distribution, but also more specific features, such as characteristics of their lipid membranes, the ratio of membrane lipids to proteins or RNA, and the enzyme activity of the surface proteins [127]. Thorough identification of these attributes might help to overcome another challenge, which is predicting therapeutic potency of the obtained samples. Despite some progress in this field, robust potency assays for EV preparations reflecting their mechanism of action are yet to be developed [128].

It is noteworthy that some researchers suggest protein-mediated mechanism of action to be more likely for EVs than miRNA-mediated one [129]. There is evidence that miRNAs may not be the key players determining the EV biological activity [130] since they may not be present in sufficient quantities in EV preparations [131] and therefore may not elicit a biologically relevant response. However, speaking of MSC-EVs, while numerous studies evaluated their molecular composition and associated functions [132–134], the contribution of particular miRNAs is still discussed much more often than that of particular proteins. Therefore, more studies assessing the contribution of certain proteins to EV biological activity would be of great scientific value.

Moreover, speaking of Treg-EVs, in contrast to MSC-EVs, their research is still in the investigative stage. In this regard, more systematic studies and experimental models could probably bridge the knowledge gap that still exists in the field of Treg-EV research.

The critical role of Treg-EVs in Treg-mediated suppression of immune cells demonstrated in recent in vitro [135] and in vivo [22, 50, 51, 136] studies offers the prospect of using Treg-EV-based therapies for conditions characterized by immune system over-reactivity. However, Treg-EV successful use was reported not only in immune-related disorders and transplantation tolerance models, but also in models of myocardial infarction [50], and scar formation in wound healing [137]. This is a very important insight suggesting that the range of Treg-EV possible applications is wider than treating excessive immune reactions.

EVs are known to recapitulate a number of the parent cell's properties; therefore, possible targets of Treg-EV application can be inspired by the Tregs' reported effects, such as facilitating blood flow recovery after ischemia [138], controlling adipose tissue inflammation, promoting muscle repair [139], and maintaining tissue/organ homeostasis [140].

Another particular field requiring further investigation is the use of Treg-EVs in oncology, since the immune component in cancer development is well documented. Moreover, Tregs are reported to play a crucial role in tumor formation [28, 29, 141, 142] and are known as a target of tumor cell EVs [93–97]. Hopefully, having disclosed these complex interactions between the pool of cells involved in the tumor development and their EVs, we might get answers to the questions still existing in cancer research.

Conclusions

The variety of available sources and the unique role of cell–cell communication mediators make EVs an accessible and promising tool in modulating many vital processes

such as immune signaling, inflammation, and angiogenesis. Furthermore, EVs can be used both as diagnostic biomarkers for different pathologies due to their specific cargo of lipids, proteins, and RNAs and as a drug delivery system due to their ability to protect internal biomolecules from degradation and suitable candidates to pass through physiological barriers such as the blood–brain barrier. MSC-EVs, being widely studied, are known for their immunomodulatory properties, but due to the duality of their effect on the tumor growth, their use in oncology would remain a double-edged sword until the sufficient body of knowledge is accumulated and the tools for their undesired effects management are developed. At the same time, Treg-EVs remain largely unstudied but still hold great promise for treating a wide range of diseases.

EVs exert their effects both on cell-mediated and humoral (including the complement system) immunity. Treg-EVs' immunosuppressive effect is beneficial for conditions characterized by immune system over-reactivity (ARDS accompanied by cytokine storm, autoimmune diseases, allograft rejection), but at the same time fatal in tumor development, being favorable for the tumor growth. On the other hand, MSC-EVs, while alleviating immune system over-reactivity, exhibit a dual effect on the tumor development, being able to both promote and inhibit tumor growth.

Abbreviations ALI: Acute lung injury; ARDS: Acute respiratory distress syndrome; CIA: Collagen-induced arthritis; CNS: Central nervous system; EAE: Experimental autoimmune encephalomyelitis; EVs: Extracellular vesicles; IBD: Inflammatory bowel disease; IL: Interleukin; IMQ: Imiquimod; MMP: Matrix metalloproteinase; MS: Multiple sclerosis; MSCs: Mesenchymal stromal cells; MSC-EVs: MSC-derivedextracellular vesicles; PASI: Psoriasis Area and Severity Index; RA-FLS: Rheumatoid arthritis-related fibroblast-likesynoviocytes; RA: rheumatoid arthritis; T1D: type 1 diabetes; Th: T-helper; Tregs: regulatory T cells; Treg-EVs: regulatory T cells-derived extracellular vesicles; VEGF: vascularendothelial growth factor

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