Stem Cells International

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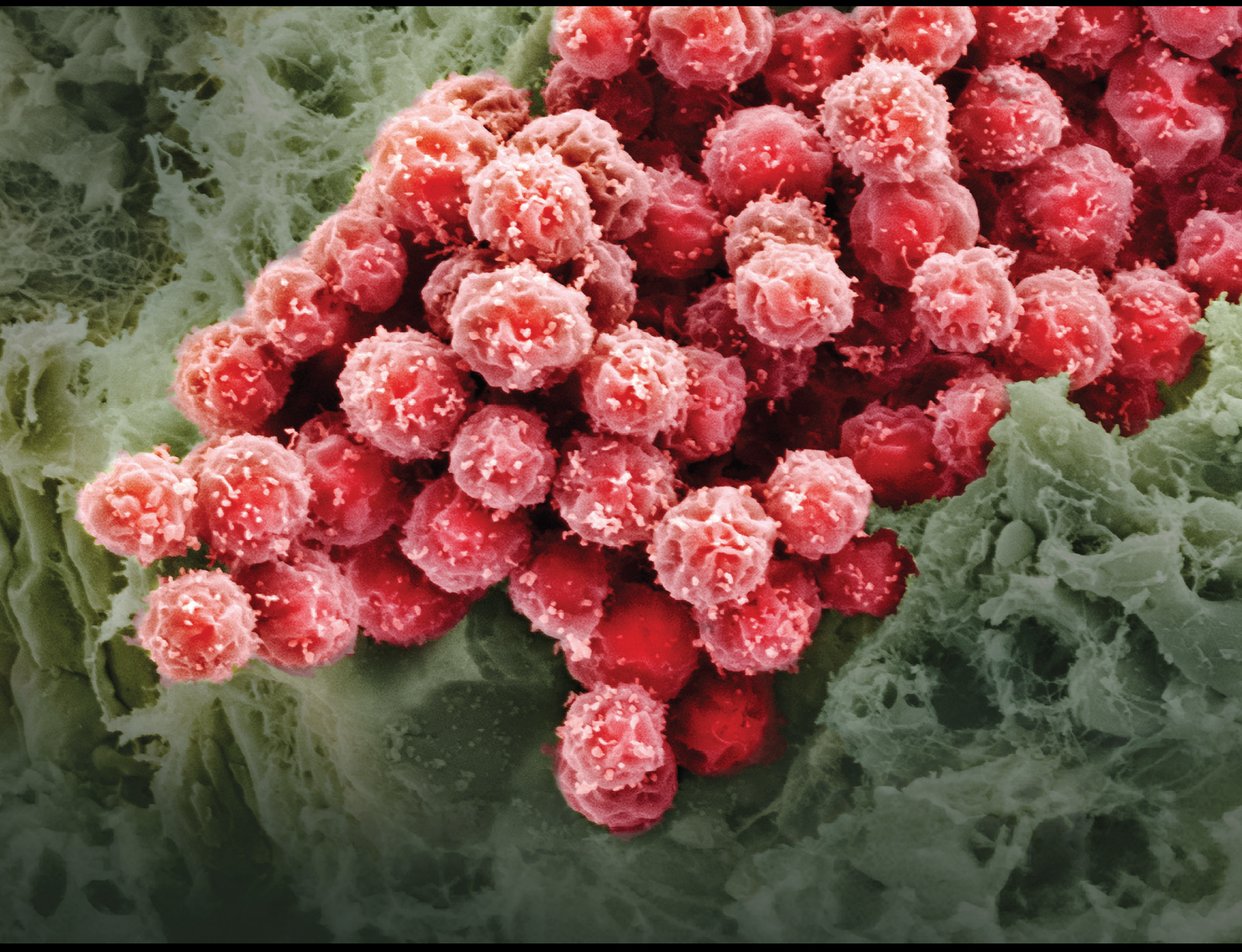

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*Research Article*

# MSC-Derived Extracellular Vesicle-Delivered L-PGDS Inhibit Gastric Cancer Progression by Suppressing Cancer Cell Stemness and STAT3 Phosphorylation

**Benshuai You**,[1] **Can Jin**,[1] **Jiaxin Zhang**,[1] **Min Xu**,[2] **Wenrong Xu**,[1] **Zixuan Sun**,[1] **and Hui Qian**[1,3]

[1]*Zhenjiang Key Laboratory of High Technology Research on Exosomes Foundation and Transformation Application, School of Medicine, Jiangsu University, Zhenjiang, 212013 Jiangsu, China*
[2]*Department of Gastroenterology, Affiliated Hospital of Jiangsu University, Zhenjiang, 212013 Jiangsu, China*
[3]*NHC Key Laboratory of Medical Embryogenesis and Developmental Molecular Biology & Shanghai Key Laboratory of Embryo and Reproduction Engineering, Shanghai 200040, China*

Correspondence should be addressed to Zixuan Sun; zxsun321@126.com and Hui Qian; lstmmmlst@163.com

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Mesenchymal stem cell- (MSC-) derived extracellular vesicles (EVs) serving as delivery system have attracted extensive research interest, especially in cancer therapy. In our previous study, lipocalin-type prostaglandin D2 synthase (L-PGDS) showed inhibitory effects on gastric cancer growth. In this study, we aimed to explore whether MSC-EV-delivered L-PGDS (EVs-L-PGDS) could inhibit gastric cancer progression. EVs-L-PGDS were generated from MSCs transfected with adenovirus encoding L-PGDS. Cell colony-forming, migration, invasion, and flow cytometry assays were used to show the inhibitory effects of EVs on tumor cells in vitro, and the nude mouse subcutaneous tumor model was performed to show the inhibitory effect of EVs on tumor progression in vivo. In vitro, EVs-L-PGDS could be internalized and inhibit the colony-forming, migration, and invasion ability of gastric cancer cell SGC-7901 and promote cell apoptosis. In vivo, EVs-L-PGDS inhibited the tumor growth in nude mouse subcutaneous tumor-bearing model. Compared with the PBS and EVs containing empty vector (EVs-Vector) group, more apoptotic cells and higher L-PGDS expression were detected in tumor tissue of the EVs-L-PGDS treatment group. And these differences are significant. Mechanistically, EVs-L-PGDS reduced the expression of stem cell markers including *Oct4*, *Nanog*, and *Sox2* and inhibited STAT3 phosphorylation in gastric cancer cell SGC-7901. In conclusion, our results imply that MSC-derived EVs could be utilized as an effective nanovehicle to deliver L-PGDS for gastric cancer treatment, which provides a novel idea for the EV-based cancer therapy.

## 1. Introduction

Although the incidence of stomach cancer is generally declining, it has remained a heavy disease burden in developing countries over the past decades [1]. Despite advances in diagnosis and treatment, clinical outcome of advanced gastric cancer remains poor, and new therapeutic methods are urgently needed. Accumulating evidence has shown that mesenchymal stem cells (MSCs) hold promise for a wide range of applications in the treatment of many diseases including cancer [2, 3]. Tang et al. found that human umbilical cord MSCs (huc-MSCs) inhibited the growth of HepG2 cells and promoted their apoptosis [4]. huc-MSC-conditioned medium containing extracellular vesicles (EVs) could effectively induce apoptosis and attenuate the migration of tumor cells, which has attracted increasing attention [5]. These findings point to the positive effects of MSCs in cancer therapy.

EVs, including both microvesicles and exosomes, are small particles secreted by many types of cells and play key roles in intercellular communication [6, 7]. EVs from various origins hold great potential in cell-free anticancer treatment [8]. For

example, tumor-derived EVs contain the similar components to those of the parent cell, indicating that EVs might target cancer sites [9]. Chemotherapy drugs and miRNA-134 and TNF-related apoptosis inducing ligands (TRAIL) could be delivered via tumor-derived EVs, thereby increasing the toxicity and targeting ability of these therapeutic reagents [10–12]. Dendritic cell-derived EVs could exhibit immunostimulatory characteristics, including inducing $CD8^+$ CTL responses in cancer therapy [13, 14]. HEK239T cell-derived EVs are frequently used to achieve targeted delivery to tumor site by genetic modification on ligands [15–17]. MSC-derived EVs are also potential candidates for cancer treatment.

MSC-derived EVs have unique advantages as carriers for anticancer therapy [18]. Many studies have shown that MSCs hold the characteristic of tumor tropism, and EVs are able to carry a variety of biological active molecules from parent cells [19, 20]. Naseri et al. reported that EVs derived from MSCs could migrate to the tumor sites, which is similar to the ability of MSCs [21]. Besides, the immunogenicity of EVs is lower than that of MSCs due to a low amount of membrane proteins such as major histocompatibility complex (MHC) [22]. In addition, MSCs are highly proliferative and produce a large number of EVs under suitable culture conditions, which provides the feasibility for further clinical application [23]. Liu et al. found that MSC-derived extracellular vesicles transmitting miR-34a-5p could suppress tumorigenesis of colorectal cancer [24]. The therapeutic molecules carried by MSC-derived EVs are able to overcome the shortcomings of targeted tumor therapy [25]. These studies highlight the positive role of MSC-derived EVs as therapeutic molecular delivery carriers.

Lipocalin-type prostaglandin D2 synthase (L-PGDS) is the rate-limiting enzyme for the synthesis of prostaglandin D2 (PGD2), which catalyses the isomerization of prostaglandin H2 (PGH2) to PGD2 [26]. Studies have shown that PGD2 plays an important role in regulating physiological sleep and inducing allergic reactions [27, 28], as well as inhibiting tumorigenesis and development [29]. However, the half-life of PGD2 is short because of the presence of prostaglandin F (PGF) synthase and spontaneous dehydration in plasma, which make it limited for direct clinical application [30]. L-PGDS was involved in cyclooxygenase-2- (COX-2-) mediated apoptosis induction after chemotherapeutics [31]. L-PGDS deficiency resulted in decreased apoptosis of tumor cell, and L-PGDS-derived PGD2 was involved in antitumor responses [32]. Furthermore, in our previous study, PGD2/PTGDR2 signaling was found to be involved in regulating self-renewal and tumorigenesis of gastric cancer [29]. Overall, these studies indicate a potential promise of L-PGDS in anticancer therapy.

The promising roles of MSC-derived EVs are highlighted in multiple disease model treatment including cancer therapy. Therefore, the combination of EVs and therapeutic molecules may be a new direction for cancer therapy. In this study, we prepared EVs from huc-MSCs transfected with adenovirus encoding L-PGDS (EVs-L-PGDS) and aimed to evaluate the anticancer effect of the L-PGDS-loaded EVs on gastric cancer. Collectively, we demonstrated that EV-based delivery system is a novel strategy for cell-free cancer therapy.

## 2. Materials and Methods

*2.1. Isolation of Human Umbilical Cord MSCs.* huc-MSCs were isolated and identified as previously described [33]. Briefly, fresh umbilical cords were collected from informed, consenting mothers and rinsed twice with phosphate-buffered saline (PBS) containing penicillin and streptomycin, the cord blood being removed during this process. The washed cords were cut into 1 $mm^2$-sized pieces and floated in Minimum Essential Medium $\alpha$ ($\alpha$-MEM, Invitrogen) containing 10% FBS (Gibco), penicillin, and streptomycin. Tissues were then cultured at 37°C in a humidified 5% $CO_2$ air condition. The medium was replaced every 3 days after the initial plating. When fibroblast-like cells appeared after 10 days, the culture was trypsinised and passaged to a new culture flask for further expansion, and the medium was changed every 2 days. The MSCs were subjected to osteogenic and adipogenic differentiation analysis and flow cytometric analysis of CD105, CD29, CD73, CD11b, CD34, and CD45 to confirm the successful isolation of MSCs. All cells were cultured at 37°C in a humidified 5% $CO_2$ air condition.

*2.2. Generation of EVs-L-PGDS.* Adenovirus was purchased from Fubio Biological Technology Corporation. A schematic representation of the adenovirus vector is presented in Supplementary Figure 1. Adenovirus encoding L-PGDS (Ad-L-PGDS) and vector (Ad-Vector) were added to the MSC medium at $10^7$ PFU/ml when huc-MSCs reached 60–70% confluence. The transfection efficiency was observed by a fluorescence microscope. After 24 hours of transfection, the culture media were replaced with 8 ml of EV-free FBS/$\alpha$-MEM for additional 48 hours. Then, the conditioned medium was collected for EV isolation. Briefly, the conditioned medium was centrifuged for 20 min at 2000*g* to remove dead cells, and subsequently, supernatant was centrifuged for 30 min at 10000*g* to remove cellular debris. Then, the clarified supernatant was concentrated and centrifuged for 30 min at 1500*g* using a 100 kDa MWCO hollow fiber membrane (Millipore, Bedford, MA); after that, the supernatant was fully resuspended with the ExoQuick-TC™ (SBI, EXOTC10A-1) and kept at 4°C overnight. EVs were acquired through centrifugation for 30 min at 1500*g* and stored at -80°C. The protein concentration of EVs was determined by BCA protein assay kit. The particle distribution of EVs was detected by nanoparticle tracking analyzers (Particle Metrix ZetaView®).

*2.3. EV Uptake.* The human gastric cancer cell line SGC-7901 was purchased from the Institute of Biochemistry and Cell Biology at the Chinese Academy of Sciences (Shanghai, China) and preserved in our laboratory and was maintained in RPMI-1640 medium with 10% FBS (Gibco). EVs were incubated with CM-Dil (Invitrogen) dye for 30 min at 37°C and washed twice using ultracentrifugation at $100{,}000 \times g$ for 70 min to remove the excess dye. After that, SGC-7901 cells were incubated with the Dil-labeled EVs for 4 h, 4% PFA and 0.1% Triton-X 100 were used to fix and permeabilize the cells, and subsequently, the cell nuclei were stained using DAPI. Images were acquired using an inverted wide-

field fluorescence microscope (Delta Vision Elite, GE Healthcare Life Sciences).

*2.4. Cell Migration and Invasion Assays.* SGC-7901 cells were seeded on a 6-well plate overnight and treated with PBS, EVs-Vector, and EVs-L-PGDS for 48 h. Then, $10^5$ cells were seeded into the upper chamber of Transwell chamber in serum-free medium and medium containing 10% FBS was added into the lower chamber and, subsequently, culturing in cell incubator for 16 hours. The chambers were fixed in 4% paraformaldehyde for 30 minutes and washed twice with PBS. After crystal violet staining and PBS cleaning, the number of migrated cells on the chamber surface was counted under the microscope, and at least six fields of cells were assayed for each group. For cell invasion assay, 200 $\mu$l of Matrigel at a dilution of 1 : 4 in serum-free medium was precoated into Transwell chambers and cell incubation time was extended to 24 h. The remaining procedures were the same as those in cell migration assay.

*2.5. Colony Formation Assay.* SGC-7901 cells were harvested and seeded into a 6-well plate with the density of 1000 cells per well and incubated in a 5% $CO_2$ humidified incubator at 37°C for 7 days. At the end of the incubation period, cells were fixed with 4% paraformaldehyde and stained with crystal violet. The number of cells was counted under the microscope.

*2.6. Cell Apoptosis Analysis and TUNEL Staining.* After treatment with PBS, EVs-Vector, or EVs-L-PGDS, SGC-7901 cells were harvested and stained with Annexin V and PI (Invitrogen) according to the manufacturer's instruction. The apoptotic cells were detected by flow cytometry. The apoptotic cells in tumor tissue were evaluated by TUNEL Apoptosis Detection Kit (Boster, Wuhan, China) according to the manufacturer's instructions. The positive cells were visualized and photographed with a light microscope.

*2.7. Western Blot.* Cell and tissue lysates were extracted in a lysis buffer (RIPA, Pierce) and proteinase inhibitor. Then, a total of 60 $\mu$g protein was separated in 12% SDS-polyacrylamide gels with 180 kDa prestained protein marker (MP102-02, Vazyme Biotech Co., Ltd.) and transferred to PVDF membranes. The membranes were blocked with 5% BSA and then incubated with primary antibodies against GAPDH (1 : 2000; Bioworld), CD9 (1 : 500; Cell Signal Technology), CD63 (1 : 500; Abcam), CD81 (1 : 500; Abcam), Calnexin (1 : 500; Cell Signal Technology), L-PGDS (1 : 500; Bioworld), Bax (1 : 400; Cell Signal Technology), Bcl2 (1 : 500; Cell Signal Technology), Oct4 (1 : 500; Cell Signal Technology), Nanog (1 : 500; Bioworld), Sox2 (1 : 800; Wanlei bio), p-STAT3 (1 : 500; Cell Signal Technology), and t-STAT3 (1 : 500; Cell Signal Technology) at 4°C overnight. The membranes were washed three times with Tris-buffered saline/Tween and incubated with goat anti-rabbit secondary antibody (1 : 2000, Invitrogen) at 37°C for 1 hour. The signals were visualized using Luminata crescendo western horseradish peroxidase substrate (Millipore) and image software of GE (ImageQuant LAS4000 mini).

*2.8. In Vivo Tumorigenicity.* Xenograft mouse model was established as previously described [34]. Male BALB/c nu/nu mice (Laboratory Animal Center of Shanghai, Academy of Science, Shanghai, China) aged 4–6 weeks (18-20 g weight) were randomly divided into three groups (six mice per group). The mice were allowed free access to food and water and were housed at a controlled temperature (20–25°C) and humidity (50 ± 5%) on a 12 h light–dark cycle. All groups received subcutaneous injections of SGC-7901 cells pretreated with PBS, EVs-L-PGDS, or EVs-Vector at a protein concentration of 320 $\mu$g/ml for 48 h ($1 \times 10^6$ cells in 200 $\mu$l PBS) on side of the upper limbs. Tumor growth was evaluated using tumor weight and tumor volume measurement. Tumor volumes were measured using calipers according to the modified ellipsoidal formula $(\text{length} \times \text{width}^2)/2$. Tumors appeared on the 10th day and mice were sacrificed on the 25th day, and the harvested tumors were subjected to subsequent assays. All experimental protocols were approved by the Medical Ethics Committee of Jiangsu University (2012258).

*2.9. Immunofluorescence and Immunohistochemistry.* Immunofluorescence was used to detect the expression of L-PGDS in tumor tissue (1 : 50; Bioworld, Louis Park, MN). The secondary antibodies were Alexa Fluor 555-labeled donkey anti-rabbit IgG (1 : 300, Invitrogen, Carlsbad, CA). Images were acquired using microscopy (Nikon, Tokyo, Japan). For immunohistochemical analysis, the tumor tissues were fixed in 4% paraformaldehyde (PFA), embedded in paraffin, and cut into 5 $\mu$m thick sections. Then, these sections were incubated with anti-PCNA (1 : 100; Bioworld, Louis Park, MN) overnight at 4°C and, subsequently, incubated with the secondary antibody at 37°C for 30 min. Finally, tissues were counterstained with 3,3′-diaminobenzidine (DAB) and photographed by microscopy.

*2.10. Statistical Analysis.* All data were shown as mean ± standard deviation (SD) and analyzed by GraphPad Prism software (version 7.0). The statistically significant differences between groups were assessed by one-way ANOVA with Tukey's post hoc test. $P < 0.05$ was considered statistically significant.

## 3. Results

*3.1. Identification and Multipotency of huc-MSCs.* huc-MSCs were obtained from fresh human umbilical cords. On the 10th day of culture, the huc-MSCs showed fibrous growth under the microscope (Figure 1(a)). Red fat droplets stained with Oil Red O were observed in huc-MSCs after being induced by adipogenic differentiation medium (Figure 1(b)). Red calcium nodules stained with Alizarin Red were observed in the osteogenic huc-MSCs (Figure 1(c)). Then, the surface markers of the huc-MSCs were detected by flow cytometry. The expression levels of CD105, CD29, and CD73 were positive, and the expression levels of CD11b, CD34, and CD45 were negative (Figure 1(d)). The above results indicate that we have successfully isolated huc-MSCs.

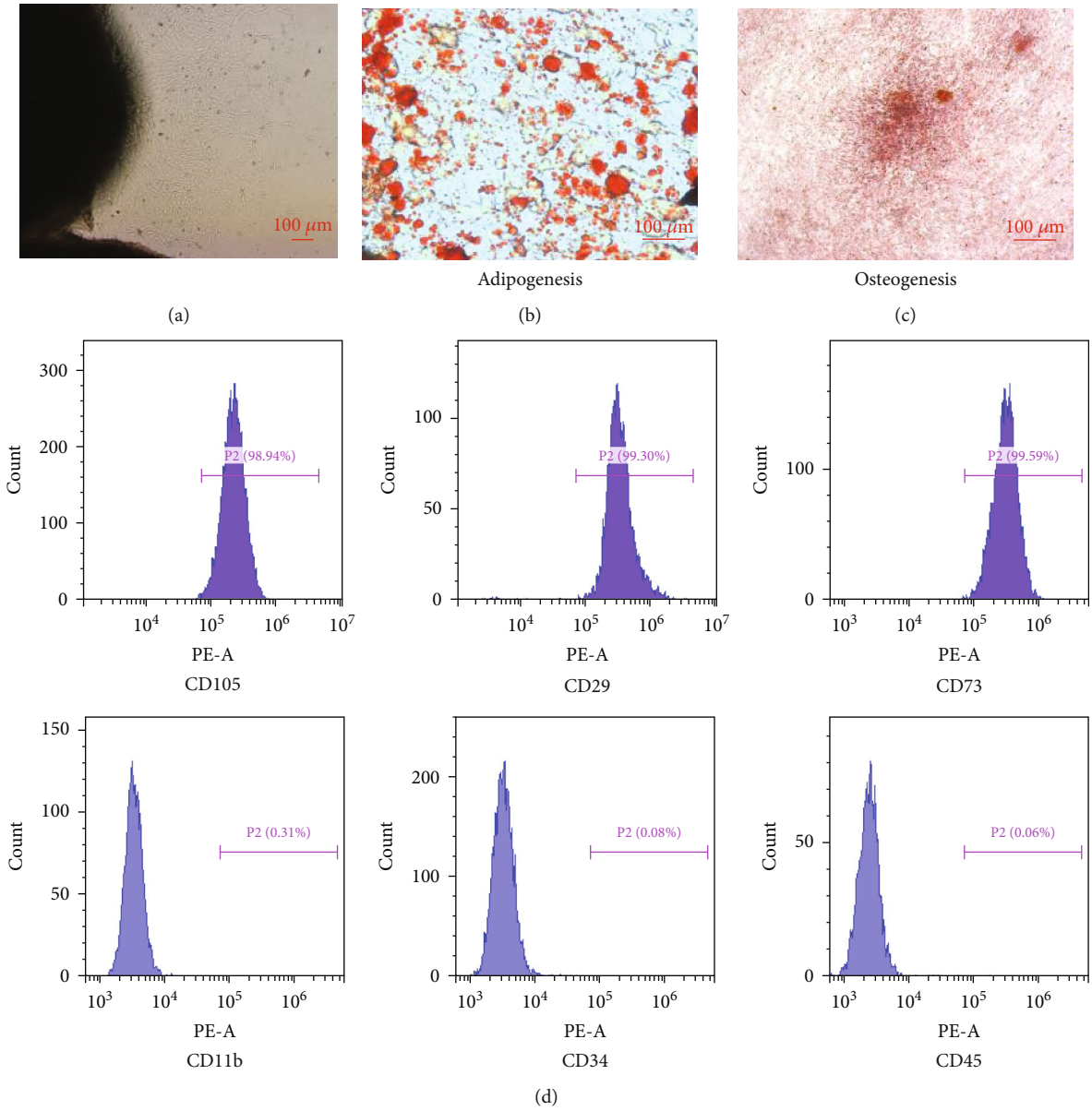


Figure 1: Identification and differentiation potential of huc-MSCs. (a) MSCs with their characteristic fusiform shape were grown on the 10th day of culture (×40). (b) Oil Red O staining for the adipogenic differentiation of huc-MSCs (×100). (c) Alizarin Red staining for the osteogenic differentiation of huc-MSCs (×100). (d) Flow cytometry for the surface antigens of huc-MSCs. The huc-MSCs expressed CD105, CD29, and CD73 but lacked expression of CD11b, CD34, and CD45.

*3.2. Ad-L-PGDS-Modified huc-MSCs Packaged L-PGDS into Secreted EVs.* Adenovirus encoding L-PGDS and vector were used to transfect huc-MSCs, respectively. The GFP expression in huc-MSCs was confirmed by a fluorescence microscope showing that the adenovirus was successfully integrated into the genome (Figure 2(a)). By staining the nucleus, almost all cells were successfully transfected with adenovirus, which showed the coexpression of DAPI and GFP (Supplementary Figure 2). Western blot showed that the expression of L-PGDS was significantly higher in Ad-L-PGDS-transfected MSCs than that in the Ad-Vector treatment group (Figure 2(b)). The supernatant of huc-MSCs transfected by virus was collected for EV isolation. The EVs secreted by huc-MSCs transfected with Ad-L-PGDS and Ad-Vector were marked as EVs-L-PGDS and EVs-Vector, respectively. Western blot showed that the EV markers, CD9, CD63, and CD81, were expressed in both EVs-L-PGDS and EVs-Vector (Figure 2(c)). Calnexin, a negative marker of EVs, was not expressed (Figure 2(c)). Compared with EVs-Vector, the expression of L-PGDS in EVs-L-PGDS was significantly increased (Figure 2(c)). Both kinds of EVs showed typical disc shape under the transmission electron microscope (TEM) (Figure 2(d)). In addition, the NanoSight visible nanoparticle analyzer detected that the particle distribution of EVs was relatively uniform, with a diameter of approximately 100 nm

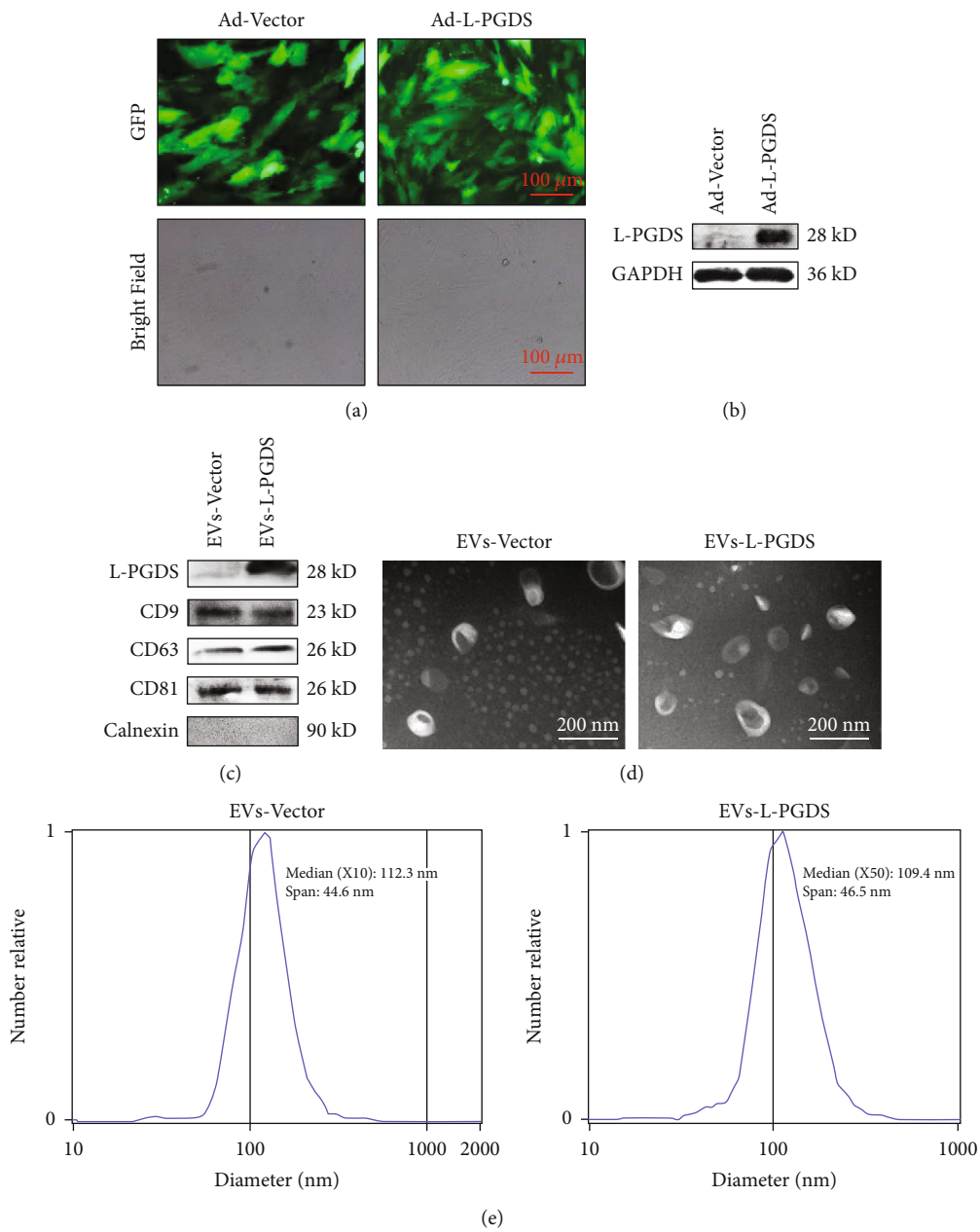


Figure 2: Ad-L-PGDS-modified huc-MSCs packaged L-PGDS into secreted EVs. (a) Representative fluorescence images of GFP in huc-MSCs after treatment with adenovirus for 24 h (×100). (b) Western blot for the expression of L-PGDS in adenovirus-transfected huc-MSCs after 24 h. (c) Western blot for the expression of EV markers CD9, CD63, and CD81 and the expression of L-PGDS in EVs. (d) TEM for the morphology of EVs. (e) NanoSight for the size of EVs. Abbreviations: TEM: transmission electron microscope.

(Figure 2(e)). The results confirmed that L-PGDS-loaded EVs could be successfully generated by adenovirus transfection of MSCs.

*3.3. EVs-L-PGDS Increased L-PGDS and Reduced Stem Cell Marker Expression and Inhibited STAT3 Phosphorylation in the Gastric Cancer Cell SGC-7901.* The EVs were labeled with the lipid membrane dye CM-Dil and incubated with SGC-7901 cells to examine if they could be taken up by cancer cells. Fluorescence images showed the presence of red fluorescent spots in the cytoplasm near the nuclei of cancer cells via confocal microscopy, indicating the successful transfer of EVs into cancer cells (Figure 3(a)). Compared with PBS and EVs-Vector group, western blot showed that the expression of L-PGDS in SGC-7901 cells increased after EVs-L-PGDS treatment (Figures 3(b) and 3(c)). Consistent with our previous research, EVs-L-PGDS also reduced the expression of stem cell markers, including *Oct4*, *Nanog*, and *Sox2* compared with PBS and EVs-Vector group (Figures 3(d) and 3(e)). Besides, western blot analysis showed that the expression of phosphorylated STAT3 significantly decreased after EVs-L-PGDS treatment (Figures 3(f) and 3(g)).

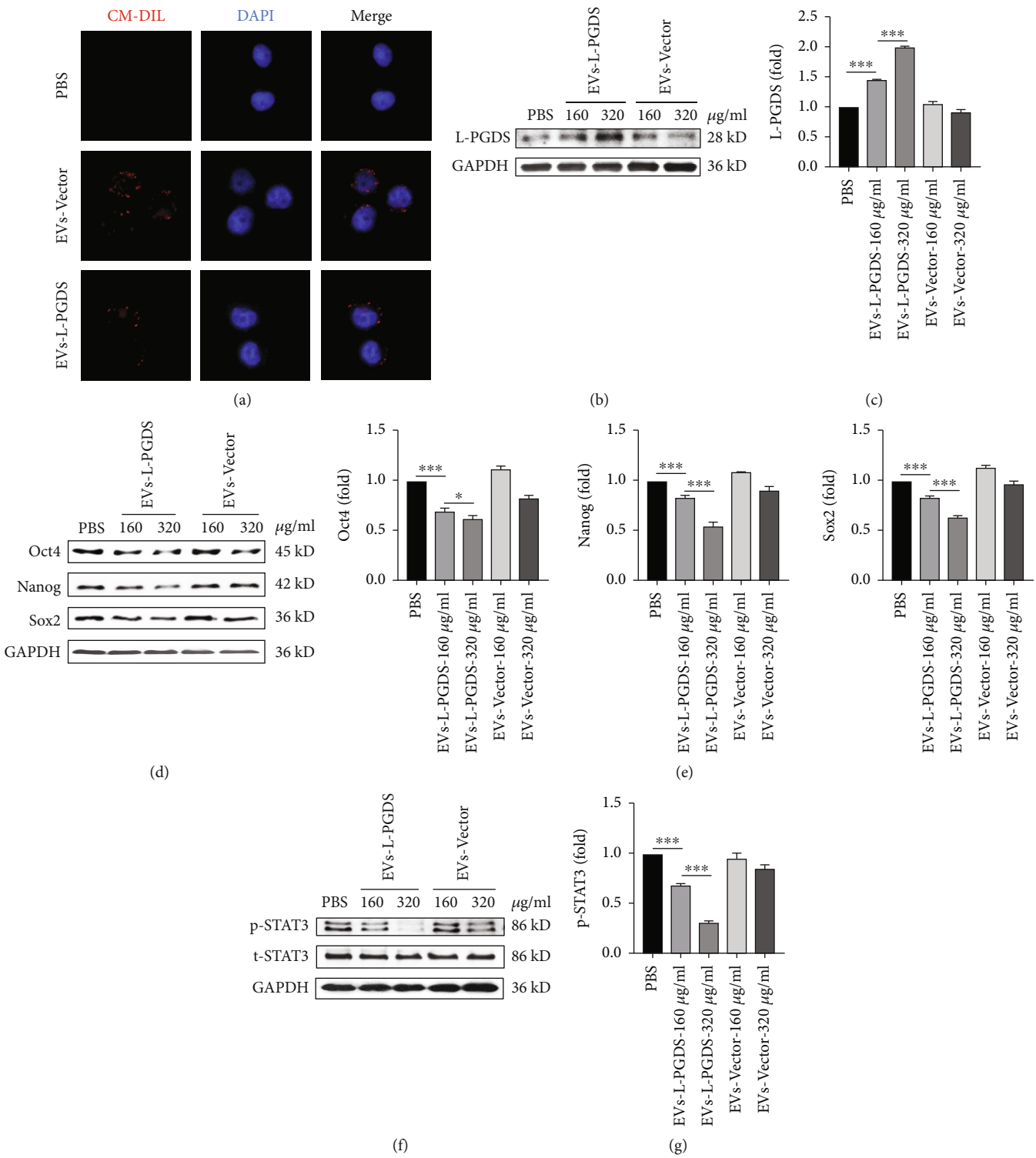


Figure 3: EVs-L-PGDS increased L-PGDS and reduced stem cell marker expression and inhibited STAT3 phosphorylation in the gastric cancer cell SGC-7901. (a) Confocal microscopy for the location of EVs in SGC-7901 cells (×600). (b) Western blot for the expression level of L-PGDS in SGC-7901 cells. (c) Quantitative analyses of protein expression of L-PGDS. (d) Western blot for the expression level of *Oct4*, *Nanog*, and *Sox2* in SGC-7901 cells. (e) Quantitative analyses of protein expression of *Oct4*, *Nanog*, and *Sox2*. (f) Western blot for the expression of p-STAT3 (Thy705), t-STAT3, and GAPDH in SGC-7901 cells. (g) Quantitative analyses of protein expression of p-STAT3 (Thy705). $n = 3$; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

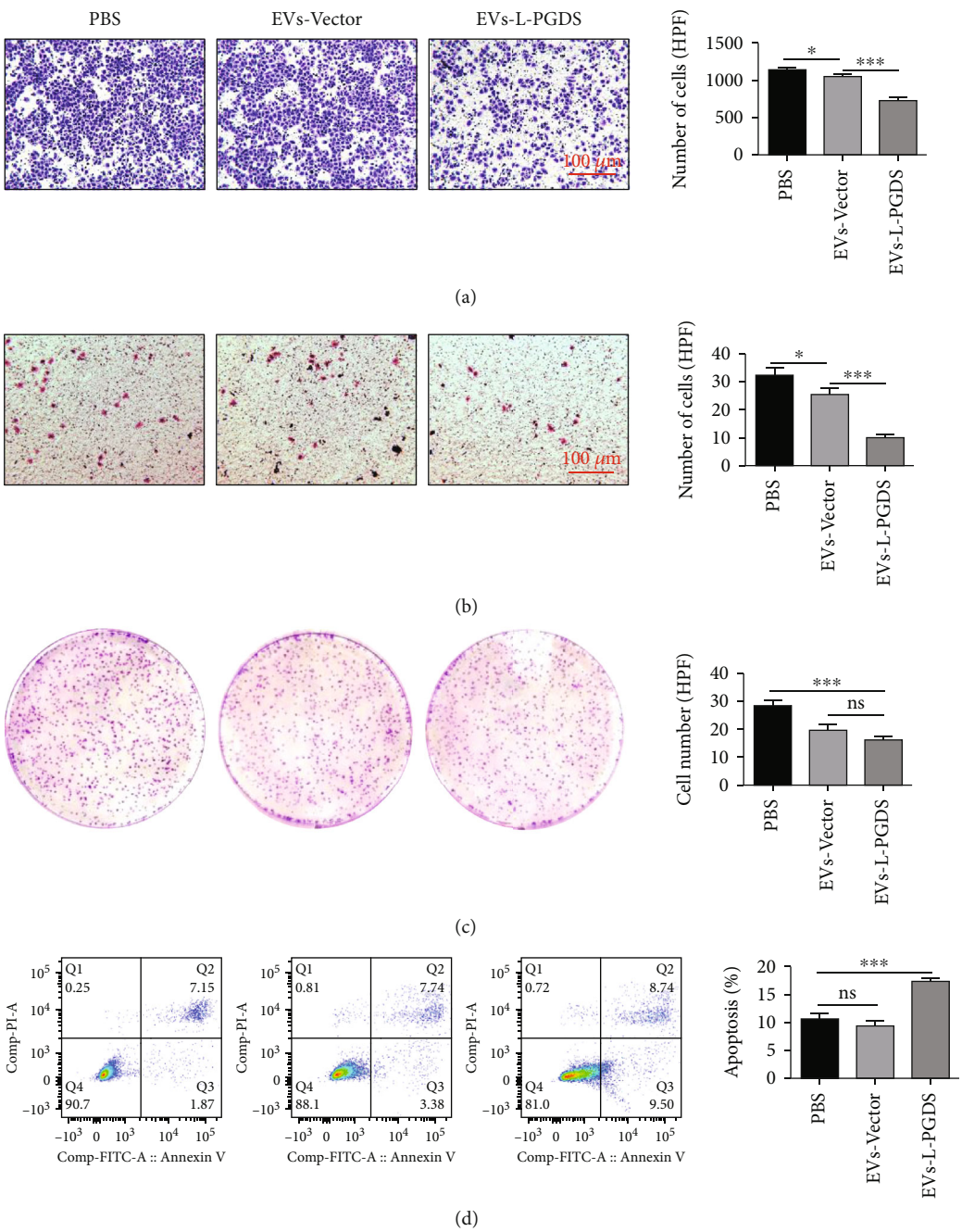


Figure 4: EVs-L-PGDS inhibited migration, invasion, and colony formation and induced apoptosis of gastric cancer cell SGC-7901. (a) Transwell migration experiments for the migration ability of SGC-7901 cells (×100). (b) Transwell invasion experiments for the invasion ability of SGC-7901 cells (×100). (c) Colony-forming assay for the colony formation ability of SGC-7901 cells. (d) Flow cytometry assay for the apoptosis level of SGC-7901 cells. $n = 6$; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$. Abbreviations: HPF: high-power field.

*3.4. EVs-L-PGDS Inhibited Migration, Invasion, and Colony Formation and Induced Apoptosis of Gastric Cancer Cell SGC-7901.* In order to investigate the effects of EVs on biological functions of cancer cells, EVs-L-PGDS and EVs-Vector were used to treat SGC-7901 cells. Compared with PBS, EVs-Vector had a slight inhibitory effect on the migration of tumor cells, while EVs-L-PGDS role in migration restriction was more effective ($P < 0.05$, PBS vs. EVs-Vector; $P < 0.001$, EVs-Vector vs. EVs-L-PGDS) (Figure 4(a)). EVs-L-PGDS also further inhibited the invasion ability of SGC-7901 cells ($P < 0.05$, PBS vs. EVs-Vector; $P < 0.001$, EVs-Vector vs. EVs-L-PGDS) (Figure 4(b)). In colony formation assays, the cancer cells treated with EVs-L-PGDS formed fewer colonies than the PBS group, while compared with the EVs-Vector group, the number of colonies did not change significantly, but the size was smaller ($P < 0.001$, PBS vs. EVs-L-PGDS) (Figure 4(c)). Flow cytometry analysis showed that the apoptosis rates in the PBS, EVs-Vector, and EVs-L-PGDS groups

were 10.81 ± 1.62%, 9.50 ± 1.43%, and 17.62 ± 0.64%, respectively (Figure 4(d)). More apoptotic cells in the EVs-L-PGDS group were observed ($P < 0.001$, PBS vs. EVs-L-PGDS).

*3.5. EVs-L-PGDS Inhibited the Growth of Subcutaneous Tumors in Nude Mice Induced by Gastric Cancer Cell SGC-7901.* A subcutaneous tumor-bearing nude mouse model was performed to estimate the growth ability of SGC-7901 cells upon treatment with the EVs. Representative images of tumor-bearing mice are shown in Figure 5(a). Compared to another two groups, pretreatment with EVs-L-PGDS led to the production of smaller tumor mass (Figure 5(b)). The weight and volume of tumor tissue in the EVs-L-PGDS group were both the smallest ($P < 0.05$, PBS vs. EVs-L-PGDS) (Figures 5(c) and 5(d)). Immunofluorescence and western blot showed that more L-PGDS expression was found in the tumor tissue after EVs-L-PGDS treatment (Figures 5(e)–5(g)). Besides, the expression of Bax, a proapoptotic protein, was also significantly higher in the tumors of mice treated with EVs-L-PGDS compared with those treated with PBS ($P < 0.01$) and EVs-Vector ($P < 0.01$) (Figures 5(e) and 5(f)). And the expression of Bcl2 decreased in the EVs-L-PGDS group ($P < 0.01$, PBS vs. EVs-L-PGDS; $P < 0.05$, EVs-Vector vs. EVs-L-PGDS) (Figures 5(e) and 5(f)). HE staining demonstrated that the tumor tissue in the EVs-L-PGDS group was more porous and had less angiogenesis than that in the PBS and EVs-Vector groups (Figure 5(h)). TUNEL staining showed that there were more apoptotic cells in the tumor tissue in mice following treatment with EVs-L-PGDS compared with those treated with PBS ($P < 0.001$) or EVs-Vector ($P < 0.01$) (Figures 5(i) and 5(j)). Immunohistochemical examination of the expression of PCNA showed that the number of proliferating tumor cells was significantly lower in the tumors from mice in the EVs-L-PGDS group compared with the groups treated with PBS ($P < 0.001$) or EVs-Vector ($P < 0.01$) (Figures 5(k) and 5(l)). In short, our data suggested that L-PGDS-loaded EVs exhibited inhibitory effects on tumor growth in vivo.

## 4. Discussion

Our previous study found that L-PGDS expression was lower in gastric cancer tissues than in adjacent tissues and was associated with poor patient prognosis [29]. We have demonstrated that direct PGD2 stimulation or L-PGDS overexpression is able to inhibit gastric cancer cell growth and migration. Besides, Fukuoka et al. found that exogenous L-PGDS promoted PGD2 secretion of gastric cancer cells, thereby inhibiting the growth of gastric cancer cells by expressing peroxisome proliferator-activated receptor (PPAR $\gamma$) [35]. Furthermore, L-PGDS inhibited tumor growth better than PGD2 injection in tumor-bearing mice which prompted us to optimize the antitumor effects of PGD2 by carrying L-PGDS in EVs. Based on these studies, we hypothesized that MSCs could secrete EVs loaded with L-PGDS, and the modified EVs could restrict the progression of gastric cancer.

Emerging evidence suggests that EVs are useful delivery vehicles in tumor treatment due to their high stability, low immunogenicity, biocompatibility, and natural targeting ability [36, 37]. EVs contain abundant contents, such as mRNAs, proteins, miRNAs, and lipids; EVs could protect these cargos from degradation [38]. Over the past decades, researchers have identified different origin-derived EVs that could be utilized as vehicles to deliver anticancer drugs. Each type of EVs has distinct advantages. In this study, we used huc-MSCs as the origin of EVs. However, the roles of MSCs and MSC-derived EVs on cancer progression have been controversial. Some studies found favorable support of EVs for cancer progression. Roccaro et al. found that bone marrow MSC-derived EVs carry higher levels of oncogenic proteins, cytokines, and adhesion molecules to facilitate multiple myeloma progression [39], while other studies considered that EVs have therapeutic effects on cancer. For example, adipose MSC-derived EVs could inhibit the proliferation and induce apoptosis of ovarian cancer cells [40]. MSC-derived EVs were also shown to suppress hepatocellular carcinoma growth by promoting NK T cell antitumor responses [41]. Furthermore, engineering MSC-derived EVs via genetic or nongenetic methods enhances the antitumor effects [42, 43]. As to huc-MSCs, many studies have shown that huc-MSCs and their conditioned media containing EVs inhibit the growth and migration and induce apoptosis in many kinds of tumors including melanoma, lung cancer, hepatocellular carcinoma, and gliomas [5, 44, 45]. The reason for this discrepancy is unknown. One possible reason may be the timing of EV injection either before or after tumor formation [46, 47]. Another possible explanation for controversial results is due to the source of EVs. EVs may carry different molecules from their parent cells. For example, Zhu et al. showed that bone marrow MSC-derived EVs promoted gastric tumor growth in vivo [48]. Besides, the diverse effects of EVs on tumor progression may also be attributed to different tumor types [2, 49]. In this study, MSC-derived EVs containing empty vector also showed slight antitumor effects, indicating that huc-MSC-derived EVs are suitable candidates as anticancer drug carriers.

The cancer microenvironment contains a small subset of stem-like cells, which play important roles in cancer onset, maintenance, and metastasis [50]. The aggressive cancer cells usually have the enhanced potential in self-renewal ability, resulting in tumor progression. STAT3 is considered to have an important regulatory effect on the behavior of cancer stem-like cells [51]. By binding to the promoters of *Oct4*, *Nanog*, and *Sox2*, STAT3 could regulate the gene expression of some cancer stem-like cell markers, which may contribute to carcinogenesis and progression [52, 53]. In our previous study, we showed L-PGDS overexpression could restrict cancer cell stemness and suppress the activation of STAT3 [29]. In this study, our data indicated that the inhibition of STAT3 phosphorylation induced by EVs-L-PGDS is crucial for the expression of stem cell markers. Therefore, we speculate that EVs-L-PGDS may inhibit the gastric cancer progression by inhibiting STAT3 phosphorylation.

Various methods are employed to achieve EV modification. Incubation is often used to load chemotherapeutic

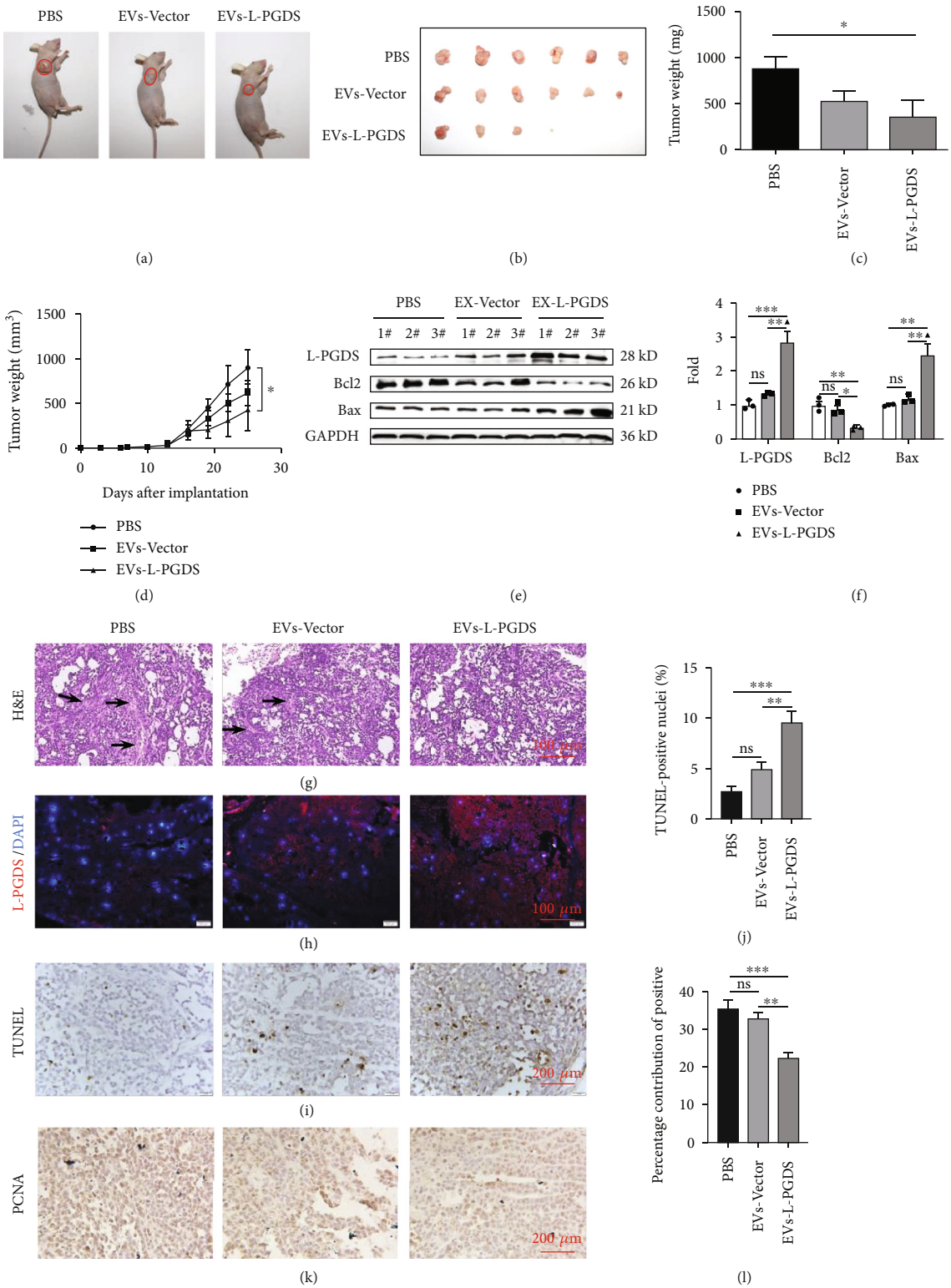


Figure 5: EVs-L-PGDS inhibited the growth of subcutaneous tumors in nude mice induced by gastric cancer cell SGC-7901. (a) Representative images of tumor-bearing mice. (b) Representative view of subcutaneous tumors. (c, d) Weight statistics and volume changes of tumor in mice treated with different EVs and PBS. (e) Western blot for the expression of L-PGDS, Bcl2, and Bax in tumor tissues. (f) Quantitative analyses of protein expression of tumor tissue. (g) HE staining of tumor tissues (×100). Arrowheads point to blood vessels. (h) Immunofluorescence staining for the expression of L-PGDS in tumor tissues (×100). (i) TUNEL assay for the apoptosis level in tumor tissues (×400). (j) Quantitative analyses of TUNEL-positive cells in tumor tissue. (k) Immunohistochemical staining for the expression of PCNA in tumor tissues (×400). (l) Quantitative analyses of PCNA expression in tumor tissue. $n = 6$; $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

drugs into EVs [54]. For loading miRNAs or siRNAs into isolated EVs, electroporation is the most commonly employed [55]. Most researchers use virus or plasmid transfection to genetically modify donor cells [56]. Herein, L-PGDS-loaded EVs were obtained via adenovirus-transfected huc-MSCs and characterized by TEM and western blot. We found that EVs-L-PGDS were internalized by cells, thereby inhibiting the colony formation, migration, and invasion and promoting apoptosis of SGC-7901 cells. Tumor growth was inhibited in SGC-7901 tumor-bearing mice after pretreatment with EVs-L-PGDS. These data indicate that adenovirus-transfected huc-MSCs are capable of secreting EVs enriched with therapeutic cargos and these cargos could be functionally delivered to tumor cells.

Before employing EVs, there are still many limitations. At present, there is no standard method for the isolation and extraction of EVs. Different methods including Exo-Quick EV extraction kit, ultracentrifugation, microfluidic separation, and immunomagnetic bead sorting are developed for EV separation, which makes EVs present different qualities [57, 58]. Moreover, potential side effects and oncogenicity after long-term use should be mentioned and monitored in safety evaluations [59]. In addition, large-scale production of clinical-grade EVs is still difficult to achieve so far [60]. Additional efforts to these problems will improve the feasibility of clinical application of EVs.

In conclusion, our study demonstrates that EVs overexpressing L-PGDS inhibit gastric cancer progress by regulating cancer cell stemness and suppressing STAT3 phosphorylation. Our findings provide novel insights into the EV-based cancer therapy by genetic modification.

## Data Availability

The data used to support the findings of this study are included within the article.

## Conflicts of Interest

The authors declare that they have no conflict of interest.

## Acknowledgments

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## Supplementary Materials

Figure S1: schematic representation of the adenovirus vector. Figure S2: representative fluorescence images of GFP and DAPI in huc-MSCs after treatment with adenovirus. Supplementary Figure 3: uncropped western blot images for all figures. *(Supplementary Materials)*

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*Review Article*

# Roles of Mesenchymal Stem Cell-Derived Exosomes in Cancer Development and Targeted Therapy

**Zixuan Sun, Jiaxin Zhang, Jiali Li, Mi Li, Jing Ge, Peipei Wu, Benshuai You, and Hui Qian**

*Zhenjiang Key Laboratory of High Technology Research on Exosomes Foundation and Transformation Application, School of Medicine, Jiangsu University, Zhenjiang 212013, China*

Correspondence should be addressed to Hui Qian; lstmmmlst@163.com

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Exosomes have emerged as a new drug delivery system. In particular, exosomes derived from mesenchymal stem cells (MSCs) have been extensively studied because of their tumor-homing ability and yield advantages. Considering that MSC-derived exosomes are a double-edged sword in the development, metastasis, and invasion of tumors, engineered exosomes have broad potential use. In this review, we focused on the latest development in the treatment of tumors using engineered and nonengineered MSC-derived exosomes (MSC-EXs). Nonengineered MSC-EXs exert an antitumor effect on several well-studied tumors by affecting tumor growth, angiogenesis, metastasis, and invasion. Furthermore, engineered exosomes have promising research prospects as drug-carrying tools for the transport of miRNAs, small-molecule drugs, and proteins. Although exosomes lack uniform standards in terms of definition, separation, and purification, they still have great research value because of their unique advantages, such as high biocompatibility and low toxicity. Future studies on MSC-EXs should elucidate the mechanisms underlying their anticancer effect and the safety of their application.

## 1. Introduction

The diagnosis and treatment of tumors, which are a key global health concern, have long been a focus of research. Although surgery is still an effective strategy for early cancer treatment, owing to the hidden onset of cancer and the limitations of diagnostic techniques, most patients with cancer have already reached intermediate and advanced tumor stages by the time their cancers are detected, minimizing the therapeutic effect of surgery [1]. Although emerging immunotherapy has been successfully applied, the heterogeneity of tumors hinders the wide application of immunotherapy for all tumors [2, 3]. Hence, radiotherapy and chemotherapy are still the preferred methods of treating tumors. However, drug delivery issues and drug resistance have become major challenges related to chemotherapy and radiotherapy.

MSC-EXs, which are natural nanovesicles with low immunogenicity, good biocompatibility, and low cytotoxicity, have a unique antitumor effect. As MSC-EXs are high-quality drug delivery carriers, constructing engineered MSC-EXs for drug loading has long been a key research topic in the field of exosome-based therapy for cancer. Moreover, natural exosomes that function through their own inclusions or surface markers also exert antitumor effects; some of them may be used as biomarkers for cancer diagnosis or prognosis [4–7]. Given that the role of natural exosomes in tumors is still controversial, in this study, we reviewed both the positive and negative effects of natural MSC-EXs on tumor occurrence and development.

## 2. Biogenesis and Uptake of Exosomes

Extracellular vesicles (EVs) are classified as apoptotic bodies (500 nm to 2 $\mu$m), microvesicles (MVs; 100 to 1000 nm), and exosomes (30 to 150 nm) mainly based on their diameter and origin [8]. MVs, also known as exfoliated vesicles or extracellular bodies [9, 10], germinate from the plasma membrane,

and apoptotic bodies are formed by bubbles in the apoptotic cell membrane [11].

Exosomes are formed from the double invagination of the plasma membrane [12]. The plasma membrane that invades inward for the first time invaginates to form early endosomes which germinate inward to form intraluminal vesicles (ILVs) [9, 12, 13]. Two mechanisms are behind the formation of ILVs: the pathway mediated by the endosomal sorting complex required for transport (ESCRT) and the ESCRT-independent pathway [14, 15]. The ESCRT is the most studied mechanism [16]. The formed ILVs can selectively capture certain molecules in the cell [17]. The composition of exosomes is highly heterogeneous. This feature is related to the cell shape, stimulation state, stress state, metamorphosis, and differentiation function of the original cell type. This shows that content such as miRNA and protein recruitment into exosomes may be a regulated process [11, 18]. Late endosomes containing ILVs are termed multivesicular bodies (MVBs) [9]. Some MVBs are transported to the trans-Golgi network for endosomal circulation and finally reach the lysosome to degrade all the carried substances [11]. Some MVBs are transported under the influence of the Rab GTPase family, cytoskeleton (microtubules and microfilaments), molecular motors (dynein and kinesin), and membrane fusion devices (SNARE complex) [19]. They fuse with the plasma membrane and eventually release exosomes.

Target cells uptake exosomes mainly through fusion, receptor-mediated endocytosis, macrophage phagocytosis, or phagocytosis [16]. In addition, a study reported that cells preferentially absorb smaller exosomes, especially those with diameters of 40–50 nm [9]. The uptake of exosomes depends not only on the nature of exosomes but also on the type and physiological state of the recipient cells [9] (Figure 1).

## 3. Characterization of Exosomes

The surfaces of exosomes contain various markers. At present, 9769 proteins have been identified in exosomes from different sources, such as CD9, CD63, CD81, Alix, EP-CAM, Hsp70, Tsg101, CD55, and CD59 [20]. Studies have reported that all MSC-EXs express markers CD9, CD63, and CD81 [21, 22]. CD55 and CD59 help avoid the activation of opsonin and coagulation factors, thus stabilizing the distribution of exosomes in biological fluids [23]. In addition, CD9, CD63, Alix, and EP-CAM can be used for the isolation of exosomes. Magnetic beads, which play a central role in capture-based techniques, are a new type of tool that can be modified to bind to target proteins on the membrane surface. CD9, CD63, Alix, and EP-CAM can be enriched by antibody-coated magnetic beads. According to the binding of antibody-coated magnetic beads to the target protein on the membrane surface, the process of collecting immobilized specific exosomes can be achieved by washing in the stationary phase [24].

## 4. Direct and Indirect Effects of MSC-EXs on Tumors

Extensive evidence has confirmed that MSC-EXs play a key role in angiogenesis, tumor growth, and metastasis (invasion). Among the various sources of exosomes, human umbilical cord MSCs (hUC-MSCs) and human umbilical cord Wharton's jelly MSCs (hWJ-MSCs) are the most commonly used. Whether natural MSC-EXs exert positive or negative effects on tumors is still controversial. A previous study reported that the dual effect seems to be related to the source of MSC-EXs, dose, and time of MSC injection, cancer type, and other factors [25].

*4.1. Dual Effects of MSC-EXs on Tumors.* A group of studies has reported that natural MSC-EXs promote tumor progression [26]. However, some reliable evidence still indicates that MSC-EXs can inhibit the occurrence and development of tumors.

*4.1.1. Angiogenesis.* Abnormal and excessive angiogenesis during cancer development could exacerbate the disease [27]. In the tumor microenvironment, MSC-EXs can enhance intracellular communication and promote angiogenesis [28]. The mechanism for promoting angiogenesis could be enhancing the tube-forming ability of endothelial cells (ECs) via exosomes. Evidence has suggested that microRNAs could play a key role in it. For example, Gong et al. [29] reported that the vascular precursor receptors (e.g., miR-30b) in MSCs derived from mouse embryos were transported to human umbilical vein endothelial cells (hUVECs) by MSC-EXs, which could directly promote the formation of the tube-like structure of hUVECs *in vitro*.

*4.1.2. Tumor Growth.* MSC-EXs are a double-edged sword in tumor growth. Kalimuthu et al. [30] used flow cytometric analysis of FITC-Annexin V staining to assess the apoptotic effects of EV treatment and confirmed that MSC-derived extracellular vesicle (MSC-EV) treatment induced apoptosis in Lewis lung carcinoma cells. By contrast, Huang et al. [31] reported that exosomes derived *in vitro* from bone marrow MSCs (BM-MSCs) could enhance tumorigenesis by promoting oncogenic autophagy in osteosarcoma.

*4.1.3. Metastasis and Invasion.* Tumor metastasis and invasion are regulated not only by the cancer cells themselves but also by the entire tumor microenvironment. As a part of the tumor microenvironment, the role of exosomes in information transmission cannot be ignored. Gu et al. [32] observed that exogenous hUC-MSC-derived exosomes (hUC-MSC-EXs) could facilitate the growth and migration of gastric cancer cells by activating the Akt pathway. In addition, endogenous MSC-EVs derived from murine and human bone marrow can induce breast cancer cells to enter the bone marrow and survive as cancer stem cells (CSCs) in a dormant state for decades [33].

*4.2. Influence of MSC-EXs on the Growth and Development of Different Types of Tumors.* Considering the heterogeneity of tumors, different tumor types may be among the reasons for the seemingly contradictory effects of MSC-EXs.

*4.2.1. Effects on Breast Cancer.* The incidence of breast cancer has been increasing in recent years [34]. According to statistics by Miller et al., breast cancer was still the most common

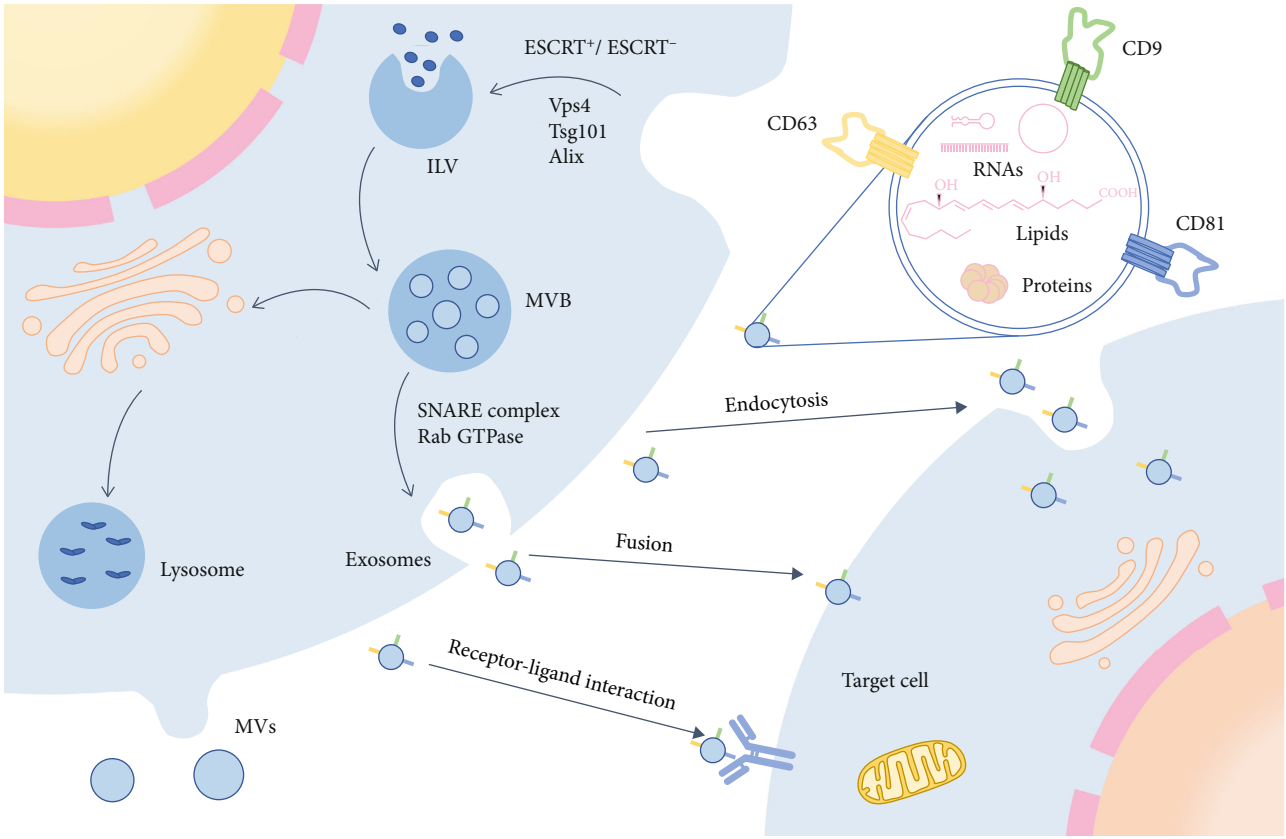


Figure 1: Biogenesis and cellular uptake of exosomes. The plasma membrane invaginates through the ESCRT-dependent and ESCRT-independent pathways to form ILVs. Late endosomes containing ILVs are called multivesicular bodies (MVBs). Some MVBs are transported to the Golgi complex circulation and finally transported to the lysosome for degradation, and some MVBs are fused with the plasma membrane under the influence of the Rab family, SNARE complex, and tubulin before being released from the cell. Exosomes released from cells can enter target cells through fusion, receptor-mediated endocytosis, macrophage phagocytosis, or phagocytosis. The surfaces of exosomes contain many molecules. This figure shows three common molecules (CD9, CD63, and CD81) on the surface of MSC-EXs.

cancer among women in the United States as of 2019, and it may affect at least 1,000,000 women by 2030 [35]. The promotional effect of MSC-EXs on tumors has been largely confirmed. Furthermore, MSC-EXs also have an inhibitory effect on tumors. Research has shown that exogenous MSC-EXs activate the extracellular signal-regulated kinase pathway to promote the proliferation and migration of breast cancer cells *in vitro* [36]. In addition, as exosomes derived from adipose-derived MSCs (ADSCs) *in vivo* contain various miRNAs that regulate epithelial-mesenchymal transition (EMT), they can promote breast cancer cells to enter the dormant phase, which is related to higher chemoresistance [37]. Pakravan et al. [38] found that BM-MSC-derived exosomes (BM-MSC-EXs) *in vitro* are rich in miR-100, which modulates the mTOR/HIF-1$\alpha$/VEGF signaling axis and inhibits the angiogenesis of breast cancer (Table 1).

*4.2.2. Effects on Multiple Myeloma.* Multiple myeloma (MM) accounts for about 10% of all hematological malignancies, with an overall survival in affected patients of only 3 years [43]. Extensive research has been conducted in this regard. Roccaro et al. [44] found that homogeneous MSC-EXs from different sources exerted completely different effects on MM. Interestingly, Umezu et al. [45] found that young donor-derived BM-MSC-EXs *in vitro* could be more competent in inhibiting MM-induced angiogenesis than BM-MSC-EXs from old donors and could thus improve the overall survival of patients.

*4.2.3. Effects on Gastric Cancer.* Gastric cancer (GC) is one of the most common malignancies worldwide [46]. Chemotherapy is still the main treatment for GC. Further, the approach that inhibits human epidermal growth factor receptor 2 is clinically proven to significantly improve survival rates [47]. Nevertheless, the treatment and prognosis of these patients are still poor. The research of exosomes brings new possibilities, which can be further applied to the discovery, treatment, and prognosis of cancers (Table 2).

*4.2.4. Effects on Liver Cancer.* Extensive research has shown that MSCs and MSC-EXs have considerable potential for the treatment of liver cancer. MVs derived from BM-MSCs inhibit cell cycle progression and induce apoptosis in HepG2 cells *in vitro*. Moreover, intratumor administration of BM-MSC-derived MVs *in vivo* remarkably inhibited tumor growth [50]. hUC-MSC-EXs containing miR-451a can limit

Table 1: Effects and mechanisms of MSC-EXs on breast cancer.

| Source | Effect | Mechanism | Model | Ref |
|---|---|---|---|---|
| ADSC | Stimulating metastasis of BCC | Type 2 diabetes mellitus altered the functions of MSC-EVs | *In vivo* | [39] |
| ADSC | Reduced tumor cell proliferation and migration and enhanced tumor cell apoptosis | CD90 expression in different concentrations ($CD90^{high}$ ADSCs and $CD90^{low}$ ADSCs) on ADSC-EVs affected the antitumor activity | *In vitro* | [40] |
| BM-MSC | Suppressed the growth of triple-negative breast cancer | By secreting miR-106a-5p | *In vivo* | [41] |
| hUC-MSC | Promoted the invasion and migration potential of breast cancer cells | By activating the Akt pathway to promote epithelial-mesenchymal transition | *In vitro* | [36] |
| hMSC or mMSC | Promoted the progression of breast cancer | By inducing monocytic myeloid-derived suppressor cells to differentiate into highly immunosuppressed M2 polarized macrophages | *In vivo* | [42] |

MSC: mesenchymal stem cell; EV: extracellular vesicle; ADSC: adipose-derived MSC; BCC: breast cancer cell; MSC-EV: MSC-derived EV; ADSC-EV: ADSC-derived EV; BM-MSC: bone marrow MSC; hUC-MSC: human umbilical cord MSC; hMSC: human MSC; mMSC: mouse MSC.

Table 2: Effects and mechanisms of MSC-EXs on GC.

| Source | Effect | Mechanism | Model | Ref |
|---|---|---|---|---|
| $p53^{-/-}$ mBM-MSC | Promotion of the growth and metastasis of gastric cancer | Delivery of UBR2 to $p53^{+/+}$ mBM-MSC and MFC cells by modulating the Wnt/$\beta$-catenin pathway | *In vitro* | [48] |
| BM-MSC | Promotion of the growth of osteosarcoma (MG63) and GC (SGC7901) cells | Activation of the Hedgehog signaling pathway | *In vitro* | [49] |

MSC: mesenchymal stem cell; $p53^{-/-}$ mBM-MSC: p53 deficient mouse bone marrow MSC; $p53^{+/+}$ mBM-MSC: p53 wild-type mouse bone marrow MSC; MFC: murine foregastric carcinoma; BM-MSC: bone marrow MSC; GC: gastric cancer; hUC-MSC: human umbilical cord MSC.

the EMT of hepatocellular carcinoma (HCC) cells by targeting ADAM10; this may provide a new target for HCC therapy [51].

*4.2.5. Effects on Bladder Cancer.* In recent years, the morbidity and mortality of bladder cancer have had an evident upward trend, making bladder cancer among the most common urinary system malignancies. Exosomes and the engineered ones are of great importance to bladder cancer patients for their future research and treatment. The inhibitory effect of MSC-EXs on bladder cancer is greater than that on other major tumors. Cai et al. [52] investigated the effect of BM-MSC-EXs on bladder cancer cells through loss- and gain-of-function experiments. The experimental results showed that exosomal miR-9-3p upregulation could inhibit the expression of endothelial cell-specific molecule 1 (EMS1), thereby inhibiting the progression of bladder cancer. Some researchers have also studied the effect of MSC-derived exosomal miRNA on bladder cancer cells. Fu et al. [53] extracted BM-MSC-EXs for an miR-19b-1-5p inhibition and elevation test and found that BM-MSC-derived exosomal miR-19b-1-5p could inhibit the growth of bladder cancer by downregulating nonreceptor protein tyrosine kinase Arg (ABL2). Similarly, Jia et al. [54] found that hUC-MSC-derived exosomal miR-139-5p could inhibit the progression of bladder cancer through targeting and downregulating PRC1.

*4.2.6. Effects on Prostate Cancer.* In cancer targeted therapy research, up- or downregulation of the expression of a certain molecule often arouses the interest of researchers, who then, through interference measures, attempt to reverse this phenomenon and observe whether it has clinical value. Che et al. [55] chose MSC-EXs to implement this intervention for prostate cancer, which is the second most common cause of cancer-related deaths among men in developed countries [56]. miR-143 expression is downregulated in prostate cancer cells, and TFF3 expression is upregulated. Through their own exosomes, MSCs can deliver overexpressed miR-143 to prostate cancer cells, and this downregulates TFF3 expression, thereby inhibiting the proliferation and invasion of prostate cancer cells and promoting their apoptosis. Similarly, Jiang et al. [57] initially found that miR-205 expression was downregulated and rhophilin Rho GTPase binding protein 2 (RHPN2) expression was upregulated in prostate cancer cells. The upregulated miR-205 inhibited the proliferation, invasion, and migration of prostate cancer cells and promoted apoptosis by targeting RHPN2, and this was effected through miR-205-expressing exosomes derived from human bone marrow mesenchymal stem cells.

*4.2.7. Effects on Ovarian Cancer.* Ovarian cancer has the highest mortality rate among gynecological malignancies; its detection in early stages is challenging [58]. Even with advancements in medical technology, the standard treatments for ovarian cancer are still cytoreductive surgery and platinum-based adjuvant chemotherapy [58]. Research on MSC-EXs revealed their potential applicability in the treatment of ovarian cancer. Reza et al. [59] demonstrated that miRNA contained in exogenous exosomes derived from human ADSCs can effectively reduce the viability of A2780 and SKOV-3 ovarian cancer cells and inhibit their

proliferation. Qiu et al. [60] stated that hUC-MSC-derived exosomal miR-146a can target LAMC2 to regulate the PI3K/Akt signaling pathway, thereby inhibiting the growth of ovarian cancer cells and their chemoresistance. Li et al. [61] observed that MSC-EVs overexpressing miR-424 can suppress hUVEC proliferation, migration, and tube formation by inhibiting MYB, thereby further inhibiting the proliferation, migration, and invasion of ovarian cancer cells. Together, these studies provide new insights into the prevention, treatment, and prognosis of ovarian cancer.

## 5. Engineered MSC-EXs for Cancer Treatment

Exosomes were originally considered cell cleaners for the disposal of unnecessary components [62–64]. However, their low immunogenicity and toxicity, long half-life, high biocompatibility, tumor-homing ability, and other advantages make them a high-quality drug delivery tool for cancer treatment [65]. In addition to traditional methods for constructing engineered exosomes such as cocultivation, electroporation, freezing and thawing, and mechanical extrusion, genetic engineering has become a more attractive option.

*5.1. miRNAs: A Tumor Treatment Tool.* Currently, targeted drug delivery for tumors has been investigated to target specific subcellular compartments, and receptor-mediated endocytosis is the most promising approach [66]. As promising tumor treatment tools [67], miRNAs are difficult to pass through cell membranes owing to their negative charge and hydrophilic nature. Moreover, they are easily degraded after entering the body. As high-quality carriers, exosomes can address this concern [68]. In related research, the exosomes of hUC-MSCs expressing miRNAs have been highlighted as important carriers for gene or drug therapy [69].

Gene modification is the most commonly used strategy for miRNA transfection of MSC-EXs [70]. For example, Wu et al. [71] used bone marrow mesenchymal exosomes overexpressing miR-126-3p in a coculture with pancreatic cancer cells and found that miR-126-3p inhibited the development of pancreatic cancer by targeting ADAM9. Similarly, Yuan et al. [69] cocultured MSC-EXs overexpressing miR-148b-3p with breast cancer cell line MDA-MB-231 and found that miR-148b-3p inhibited proliferation, invasion, and migration but promoted apoptosis in breast cancer cells by downregulating TRIM59. Another study reported that the exosomal miR-205 derived from hBM-MSCs delayed the progression of prostate cancer by inhibiting RHPN2 [57]. MSC-EXs enriched with miR-185 were expected to serve as a new treatment option for oral leukoplakia because they can reduce inflammation, inhibit cell proliferation and angiogenesis, and induce cell apoptosis [72]. Notably, $\beta$-catenin, a key molecule of the Wnt/$\beta$-catenin signaling pathway, plays an important role in tumor EMT. Wan et al. [73] first conducted a study on the inhibitory effect of miR-34c on $\beta$-catenin in nasopharyngeal carcinoma (NPC). They obtained exosomes overexpressing miR-34c by transfecting MSCs with lentivirus, and they found that exosomes overexpressing miR-34c considerably increased radiation-induced apoptosis in NPC cells. miR-34c reduced the expression of $\beta$-catenin by directly targeting the $3'$-UTR region of $\beta$-catenin mRNA, which contributes to a reduction in EMT and radioresistance. In addition, Jeong et al. [68] used 2D and 3D microfluidic devices cultured with hUVECs and A549 cells to simulate the tumor-like microenvironment of non-small-cell lung cancer, and they demonstrated that the miR-497 exosomes could act synergistically on endothelial cells and tumor cells to inhibit tumor growth, migration, and angiogenesis. This indicates that the combination of exosome-mediated miRNA therapeutic technology and microfluidic technology could become a predictive tool for the development of tumor targeted therapy. Liang et al. [74] constructed tumor-derived exosomes carrying both 5-FU and miR-21 inhibitor oligonucleotide (miR-21i) through electroporation and lentiviral transfection. Compared with miR-21i or 5-FU alone, the combinational delivery of miR-21i and 5-FU effectively reversed drug resistance and remarkably enhanced the cytotoxicity of 5-FU-resistant colon cancer cells.

*5.2. High-Quality Transportation System for Small-Molecule Drugs.* Chemotherapeutic drugs can be loaded onto MSC-EXs for administration, which can help address concerns related to their low aqueous solubility and specificity; this loading can improve the effects of related cancer therapy [75].

Taxol is a widely used chemotherapy drug. Melzer et al. [76] treated MSC544 cells with Taxol, and then exosomes were isolated using a serum-free MSC544 medium under continuous centrifugation conditions after 24 h. Human MDA-hyb1 triple-negative breast cancer cells were injected subcutaneously to induce subcutaneous tumors in 15 NOD/SCID mice. Subsequently, Taxol-loaded MSC544 exosomes were injected intravenously into tumor-bearing mice. The results showed that Taxol-loaded MSC544 exosomes showed superior tumor-reducing abilities. Some researchers have investigated the possibility of encapsulating paclitaxel into exosomes derived from other cells. For example, Agrawal et al. [77] successfully loaded Taxol on milk-derived exosomes and effectively overcame the barriers of the low oral bioavailability and cytotoxicity of Taxol. Similarly, Han et al. [78] discovered that natural killer cell-derived exosome-encapsulated paclitaxel exerted antitumor effects by inducing the upregulation of Bax and caspase-3 in tumor cell apoptosis signaling pathways.

Some researchers have focused on doxorubicin (DOX), which is also a common chemotherapy drug. Wei et al. [79] mixed exosomes with DOX-HCl, desalted the mixture with triethylamine, and then dialyzed it with PBS overnight to prepare EXs (EX-DOX) containing adriamycin. They found that EX-DOX had a favorable therapeutic effect against osteosarcoma. Similar results were also observed in the mouse breast cancer models used by other researchers. They used electroporation to load DOX into exosomes derived from MSCs to treat breast cancer and observed that EX-DOX significantly reduced the growth rate of tumors in a mouse breast cancer model [80].

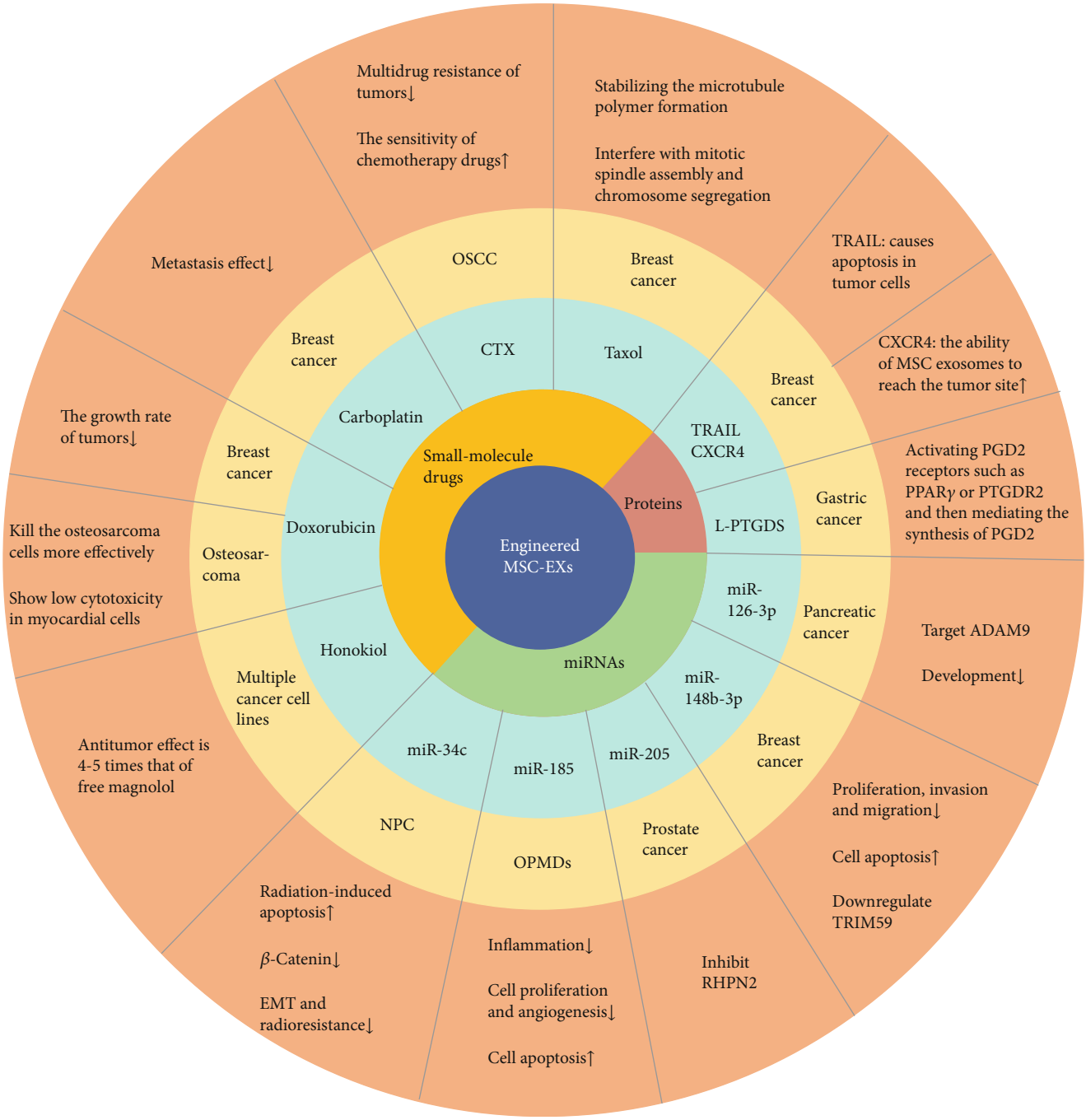


Figure 2: Engineered MSC-EXs for cancer treatment. The substances used for tumor treatment carried by the engineered MSC-EXs mainly include miRNAs, small-molecule drugs, and proteins. Four recent studies provide detailed information about miRNA loading in engineered MSC exosomes. Small-molecule drugs mainly include paclitaxel, CTX, carboplatin, doxorubicin, and magnolol. Compared with miRNAs and small-molecule drugs, few studies have reported the successful loading of proteins into exosomes.

Honokiol is a newly discovered chemotherapy drug for tumor treatment. Kanchanapally et al. [81] loaded honokiol into MSC-EXs by the sonication method, and the results indicated that its antitumor effect was 4–5 times greater than that of free honokiol.

In addition to the application of single drugs, the combined use of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and other drugs is also a popular research direction for tumor treatment. Qiu et al. [82] obtained MSCT-EXs/CTX by combining CTX (a taxane drug) with TRAIL for the treatment of oral squamous cell carcinoma. The delivery system showed good antitumor activity *in vitro*; it effectively reversed the multidrug resistance of tumors and improved the sensitivity of chemotherapy drugs. In another study, researchers obtained CXCR4/TRAIL-rich exosomes from MSCs overexpressing

both CXCR4 and TRAIL, and these exosomes acted synergistically with carboplatin (a first-line drug for the treatment of metastatic breast cancer) to exert antibreast metastasis effects *in vivo* [83].

Drug resistance has reduced the efficacy of chemotherapy and radiotherapy, which are first-line cancer treatments [75]. Therefore, a new generation of tumor treatment methods, including EVs, immunotherapy, and nanotechnology, is being gradually developed [75, 84]. Currently, MSCs are the only known cells capable of producing exosomes on a large scale [75, 83, 85]. Thus, MSC-EXs seem to be the most promising carriers for delivering specific drugs to tumor cells.

*5.3. Research Difficulties of Engineered Exosomes: Proteins.* The cargos carried by engineered exosomes are mainly short RNA sequences, small-molecule drugs, and proteins [86]. Few studies have reported the successful loading of proteins into exosomes [87, 88]. This may be attributable to the higher molecular weight of proteins, unclear mechanism of protein sorting by exosomes, and lack of related loading methods.

Mizrak et al. [89] first reported protein loading into EVs for antitumor therapy. Sterzenbach et al. [87], who were inspired by the release of enveloped viruses, developed a new method for loading proteins into exosomes based on the evolutionarily conserved late-domain (L-domain) pathway. Using this new method, they demonstrated that Cre recombinase labeled with a WW tag could be ubiquitinated and loaded into exosomes after being recognized by the L-domain-containing protein Ndfip1.

As previously mentioned, Liu et al. [83] were the first to use TRAIL in combination with CXCR4. CXCR4 is the most common chemokine receptor in human cancer cells. TRAIL can induce apoptosis in various cancer cells. Liu et al. transfected MSCs with CXCR4 and TRAIL through lentiviral transfection to obtain $Exo^{CXCR4+TRAIL}$, and they observed that $Exo^{CXCR4+TRAIL}$ exerted a significant synergistic effect with carboplatin in the mouse model.

These results indirectly indicate that extensive research with regard to proteins is warranted. For instance, a clinical case showed that the expression of lipocalin-type prostaglandin D synthase (L-PTGDS) in GC tissue was significantly reduced, and low expression of L-PTGDS was associated with shorter patient survival time [90]. Overexpression of L-PTGDS in GC cells inhibited their growth, clone formation, and migration ability. This suggested that L-PTGDS could inhibit the progression of GC and could be a potential therapeutic molecule for GC treatment. L-PTGDS likely exerts its antitumor effect by mediating the synthesis of PGD2 and activating PGD2 receptors such as PPAR$\gamma$ or PTGDR2 (prostaglandin D2 receptor 2) on the surface of tumor cells, thereby inhibiting the malignant progression of tumors. By combining the carrier advantages of MSC-EXs and the antitumor effect of L-PTGDS, we applied genetic engineering technology to construct MSC-EXs carrying L-PTGDS to inhibit the malignant progression of GC in order to provide new ideas and methods for the biological treatment of GC (Figure 2).

## 6. Conclusions and Prospects

Since Rothman, Schekman, and Sudhof won the Nobel Prize in 2013 for revealing the transport regulation mechanism of intracellular vesicles such as exosomes, perspectives on exosomes have finally transformed; they were once considered to be "cell cleaners" but are now taken seriously in the field of scientific research.

Although the role of natural MSC-EXs in cancer is still controversial and a group of studies has proved that MSC-EXs play a role in promoting cancer progression, their tumor suppressor effects have also been reported. However, some scholars attribute these antitumor effects to differences in the materials and methods used in these studies, indicating that their effects are not universal.

In addition to the functions of surface markers and the content of MSC-EXs, MSC-EXs themselves are also high-quality drug delivery carriers. Further research is required to explore the role of MSC-EXs in cancer development and treatment and to identify solutions to the following challenges. First, the identification of exosomes lacks uniform international standards. Second, the low yield and high cost of this process limit its application. Third, traditional centrifugation methods require a long time to extract exosomes, and existing kits are expensive; moreover, the purity of the isolated exosomes is not ideal. Finally, because the quality of exosomes is greatly affected by temperature and time, the storage of exosomes is also challenging [91]. In short, MSC-EXs have high potential for use in cancer treatment, but they need to solve the problems of using themselves as tools first.

## Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

## Authors' Contributions

Zixuan Sun, Jiaxin Zhang, and Jiali Li contributed equally to this article.

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