Journal of Pharmaceutical Sciences 106 (2017) 2265-2269

Contents lists available at ScienceDirect

Journal of Pharmaceutical Sciences

journal homepage: www.jpharmsci.org

Mini Review

Pharmacokinetics of Exosomes—An Important Factor for Elucidating the Biological Roles of Exosomes and for the Development of Exosome-Based Therapeutics

Masaki Morishita, Yuki Takahashi*, Makiya Nishikawa, Yoshinobu Takakura

Department of Biopharmaceutics and Drug Metabolism, Graduate School of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan

ARTICLE INFO

Article history:
Received 8 February 2017
Accepted 28 February 2017
Available online 7 March 2017

Keywords:
exosomes
pharmacokinetics
biological functions
therapeutics

ABSTRACT

Exosomes are small membrane vesicles containing lipids, proteins, and nucleic acids. Recently, researchers have uncovered that exosomes are involved in various biological events, such as tumor growth, metastasis, and the immune response, by delivering their cargos to exosome-receiving cells. Moreover, exosomes are expected to be used in therapeutic treatments, such as tissue regeneration therapy and antitumor immunotherapy, because exosomes are effective delivery vehicles for proteins, nucleic acids, and other bioactive compounds. To elucidate the biological functions of exosomes, and for the development of exosome-based therapeutics, the pharmacokinetics of exosomes is important. In this review, we aim to summarize current knowledge about the pharmacokinetics and biodistribution of exosomes. The pharmacokinetics of exogenously administered exosomes is discussed based on the tissue distribution, types of cells taking up exosomes, and key molecules in the pharmacokinetics of exosomes. In addition, recent progress in the methods to control the pharmacokinetics of exosomes is reviewed.

Introduction

Exosomes are cell-derived secretory membrane vesicles that contain lipids, proteins, and nucleic acids.[1] Since the discovery that the microRNAs contained in exosomes were involved in the regulation of expression of the microRNA-target genes in the cells that received the exosomes, the involvement of intercellular delivery of bioactive molecules through exosomes have been widely investigated in various biological events, including tumor growth, metastasis, and the immune response.[2-5]

The therapeutic application of exosomes is also anticipated because the exosome-mediated intercellular delivery of bioactive molecules affects cellular function. It have been reported that exosomes collected from mesenchymal stem cells (MSCs) showed a protective effect against ischemia/reperfusion injury because of their immunosuppressive and anti-inflammatory effects.[6-9] Based on these results, the treatment of type I diabetes using umbilical cord blood–derived MSC exosomes is under clinical trial.[10] The development of cancer immunotherapies using exosomes is also foreseen. Tumor cell–derived exosomes contain endogenous tumor antigens and can induce an antitumor immune response, by transferring tumor antigens to antigen-presenting cells, such as dendritic cells (DCs).[11-13] Furthermore, exosomes collected from DCs pulsed with tumor antigens can also induce antitumor immunity because DCs pulsed with tumor antigens secrete exosomes that contain tumor antigens and MHC molecules displaying tumor antigen epitopes.[14,15] Moreover, clinical trials has demonstrated the effectiveness of exosomes as a cancer vaccine.[16]

For the elucidation of the biological functions of exosomes, and the practical application of exosome-based therapeutics, a systematic understanding of the pharmacokinetics of exosomes, that is, the *in vivo* behavior of exosomes is important. In this review, current findings regarding the pharmacokinetics and biodistribution of exosomes are described. Moreover, recent progress in controlling the pharmacokinetics of exosomes is reviewed.

Pharmacokinetics of Exosomes

The distribution of exosomes to organs and their subsequent cellular uptake are the main steps in the pharmacokinetics of exosomes. In addition, it is considered that the cellular uptake of exosomes occurs through cellular recognition of the surface molecules on the exosomes. In this section, current findings about the pharmacokinetics of exosomes are described; they are based on the

* *Correspondence to:* Yuki Takahashi (Telephone: +81-75-7534616; Fax: +81-75-7534614).
E-mail address: ytakahashi@pharm.kyoto-u.ac.jp (Y. Takahashi).

http://dx.doi.org/10.1016/j.xphs.2017.02.030
0022-3549/

Table 1
Summary of Methods Used for Exosome Labeling and Biodistribution

Exosome Source	Exosome Labeling	Administration	Biodistribution	Reference
B16F10	PKH 67	i.v.	Lung, bone marrow, liver, and spleen	17
MSCs	DiD, DiR	i.v. (normal mice)	Spleen and liver	18
		i.v. (AKI model mice)	Kidney, spleen, and liver	
B16F10, C2C12, BMDCs, HEK293T	DiR	i.v.	Liver, spleen, lung, and gastrointestinal tract	19
HEK293T		i.p.	Liver, pancreas, and gastrointestinal tract	
		s.c.	Liver, pancreas, and gastrointestinal tract	
4T1	DiR	i.v. (60 μg)	Liver and spleen	20
		i.v. (400 μg)	Lung	
Bovine milk	DiR	i.v.	Liver	21
		p.o.	Liver, lung, kidney, pancreas, spleen, ovaries, colon, and brain	
B16BL6	gLuc-LA	i.v.	Lung, spleen, and liver	22
B16BL6, C2C12, NIH3T3, MAEC, RAW264.7	gLuc-LA	i.v.	Liver	23
HEK293T	gLuc fused to the transmembrane domain of platelet-derived growth factor receptor	i.v.	Spleen, liver, lungs, and kidneys	24
B16BL6	SAV-LA and ^{125}I-IBB	i.v.	At 4 h, 28%, 1.6%, and 7%/ID/organ was accumulated in the liver, spleen, and lungs, respectively.	25
PC3	^{111}In	i.v.	12% ID/g in the liver at 24 h	20
B16F10	SPION5	Foot pad injection	Popliteal lymph nodes	26

BMDC, bone marrow-derived dendritic cell; ID, injection dose; i.p., intraperitoneal; i.v., intravenous; MAEC, murine aortic endothelial cells; p.o., oral; SAV, streptavidin; s.c., subcutaneous.

tissue distribution, types of cells taking up exosomes, and the key molecules in the pharmacokinetics of exosomes.

Biodistribution of Exosomes

To elucidate the pharmacokinetics of exosomes, the first step comprises the evaluation of tissue distribution, that is, biodistribution of exosomes. As several labeling methods have been used to evaluate the biodistribution of exogenously administered exosomes, the following part of this section describes current findings in this field, based on the exosome labeling method (Table 1).

So far, small lipophilic fluorescent dyes have been widely used to label exosomes for *in vivo* tracking. Although the reliability of the *in vivo* analysis would be impaired by the free dye released from exosomes, this strategy is a useful approach to evaluate the localization of exosomes delivered to tissues.[18,27] Peinado et al.[17] examined the effect of tumor cell–derived exosomes on tumor metastasis by investigating the biodistribution of exosomes. PKH67, a lipophilic fluorescent dye, was used to label highly metastatic B16F10 murine melanoma cell–derived exosomes. Intravenously administered B16F10 exosomes accumulated in the lung, bone marrow, spleen, and liver. Furthermore, B16F10 exosomes enhanced endothelial permeability in the lung and facilitated tumor metastasis to the lung.

In addition to fluorescent dyes, such as PKH, lipophilic near-infrared dyes, such as 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine perchlorate (DiD) and 1,1′-dioctadecyltetramethyl indotricarbocyanine iodide (DiR), have been widely used for the imaging of exogenously administered exosomes. It has been demonstrated that intravenously injected DiD-labeled MSC exosomes were distributed to the spleen and liver in normal mice.[18] On the other hand, in acute kidney injury model mice, exosomes accumulated in the kidney, in addition to the spleen and liver, after intravenous injection. This finding may be helpful for understanding the mechanism through which the administration of MSC-derived exosomes facilitates the recovery from acute kidney injury.[28,29] Wiklander et al.[19] analyzed the *in vivo* behavior of DiR-labeled exosomes from 4 different types of cells: B16F10 murine melanoma cells, C2C12 murine myoblast cells, bone marrow–derived DCs, and HEK293T human embryonic kidney cells. All exosomes were mainly distributed to the liver, spleen, lung, and gastrointestinal tract after intravenous injection. Among these exosomes, B16F10 exosomes largely accumulated in the lung compared with the exosomes collected from the other 2 types of murine cells. The highest accumulation in the spleen and liver was observed in DC exosomes and C2C12 exosomes, respectively. Moreover, the influence of the injection route on the biodistribution of HEK293T exosomes was investigated. Although intravenously injected, HEK293T exosomes mainly accumulated in the liver, HEK293T exosomes administered by intraperitoneal or subcutaneous injection accumulated in the liver, pancreas, or gastrointestinal tract, respectively. Smyth et al.[20] investigated the dose–effect on the biodistribution of exosomes. Sixty micrograms of DiR-labeled 4T1 murine mammary carcinoma cell–derived exosomes mainly distributed to the liver and spleen after intravenous injection. However, when 400 μg of 4T1 exosomes was intravenously injected into mice, a massive accumulation of exosomes was observed in the lung, which resulted in asphyxiation of the mice. The elucidation of the mechanism of the remarkable accumulation of exosomes in the lung is required for the practical application of exosome-based therapeutics.

The biodistribution of exosomes purified from body fluid samples rather than cultured cells has also been reported.[21] Bovine milk–derived exosomes were labeled with DiR and administered orally or intravenously into mice. The exosomes distributed to the liver, lung, kidney, pancreas, spleen, ovaries, colon, and the brain at 4 days after oral administration. On the other hand, intravenously injected bovine milk–derived exosomes predominantly accumulated in the liver, which was in good agreement with previous findings regarding the tissue distribution of exosomes collected from cultured cells.

Although the earlier studies investigating the biodistribution of exosomes based on fluorescence labeling methods have offered useful information about the localization of exosomes in target tissues, an analysis of their pharmacokinetic profiles, such as an elimination profile from the blood circulation and an accumulation profile in the organs, could not be performed due to a lack in sensitivity and quantitative capacity. Bioluminescence emitted from luciferase can be used with high sensitivity and has been used to analyze the time-dependent behavior of exogenously administered cells.[30] To analyze the pharmacokinetics of exogenously administered exosomes, we designed a fusion protein named gLuc-LA, consisting of *Gaussia* luciferase (gLuc) and lactadherin (LA).[22]

Table 2
Summary of Key Molecules in Pharmacokinetics of Exosomes

Exosome Source	Injection Route	Distribution of Exosomes (Exosome-Receiving Cells)	Key Molecules	References
B16BL6	i.v.	Liver (macrophages)	Negative charge of PS on exosomes	36
HEK293	i.v.	Liver	Negative charge on exosomes (recognition by SR-A on macrophages)	37
B lymphocytes	i.v.	Spleen (CD169 + macrophages)	α2,3-Linked sialic acid on exosomes	39
4175	i.v.	Lungs	Integrin $\alpha_6\beta_4$ on exosomes	40
BxPC-3		Liver	Integrin $\alpha_v\beta_5$ on exosomes	

i.v., intravenous.

The pharmacokinetic analysis demonstrated that gLuc-LA–labeled B16BL6 exosomes quickly disappeared from the blood circulation, with a half-life of approximately 2 min, after intravenous injection into mice. Moreover, *in vivo* imaging revealed that intravenously injected B16BL6 exosomes mainly distributed to the lung, spleen, and liver. Based on this finding, we further evaluated the pharmacokinetics of exosomes derived from 5 different types of mouse cell lines: the B16BL6 murine melanoma cells, C2C12 murine myoblast cells, NIH3T3 murine fibroblasts cells, murine aortic endothelial cells (MAEC), and RAW264.7 murine macrophage-like cells.[23] We observed that all exosomes quickly disappeared from the blood circulation, with a half-life of approximately 2-4 min, and mainly distributed to the liver after intravenous injection into mice. This exosome labeling method based on bioluminescence has allowed the evaluation of pharmacokinetic properties and tissue distribution of exogenously administered exosomes, which is essential for the systematic understanding of the *in vivo* behavior of exosomes. Lai et al.[24] also performed *in vivo* imaging of exosomes, using a fusion protein of gLuc and a transmembrane domain of the platelet-derived growth factor receptor. gLuc-labeled HEK293T exosomes mainly accumulated in the spleen, followed by the liver, lungs, and kidneys, after intravenous administration.

The labeling of exosomes with a radiotracer is more suitable for quantitative evaluation of the pharmacokinetics and biodistribution of exosomes than labeling with fluorescence dyes or chemiluminescent proteins due to its high sensitivity and stability. We modified B16BL6 exosomes with a streptavidin–LA fusion protein and added biotin derivatives labeled with ^{125}I to obtain ^{125}I-labeled B16BL6 exosomes.[25] This technique enabled us to analyze the accumulation profile of exosomes in the organs in a quantitative manner. At 4 h after intravenous injection of ^{125}I-labeled exosomes, 28%, 1.6%, and 7% of the injected radioactivity/organ was detected in the liver, spleen, and lung, respectively. Smyth et al.[20] have also evaluated the pharmacokinetics of human prostate adenocarcinoma PC3 exosomes labeled with ^{111}In. ^{111}In-labeled PC3 exosomes rapidly disappeared from blood circulation and primarily distributed to the liver (12% injection dose [ID]/g at 24 h) after intravenous injection.

Magnetic resonance imaging of exosomes was performed by loading 5-nm superparamagnetic iron oxide nanoparticles into B16F10 exosomes.[26] An accumulation of exosomes at the popliteal lymph nodes was detected by magnetic resonance imaging, after foot pad injection of SPION5-loaded B16F10 exosomes.

These previous studies demonstrate that several labeling methods can be used to evaluate the pharmacokinetics and biodistribution of exosomes and that various types of tissues are targets for exosomes. However, irrespective of the exosome-labeling methods, it is apparent that the liver is the main organ that takes up intravenously administered exosomes.

Cellular Uptake of Exosomes

Exosomes are recognized and taken up by cells to transport their cargos. Therefore, identifying the types of cells that take up exogenously administered exosomes is important for further investigation of the biological functions of exosomes and for the development of exosome-based therapeutics.

We investigated the types of cells responsible for the uptake of intravenously injected B16BL6 exosomes.[31] It has been demonstrated that macrophages are responsible for the hepatic and splenic uptake of B16BL6 exosomes. In contrast, the B16BL6 exosomes detected in the lung were taken up mainly by endothelial cells. Moreover, in macrophage-depleted mice, the clearance of intravenously injected B16BL6 exosomes from the blood circulation was extremely delayed compared with that in untreated mice, which suggests the importance of macrophages in the pharmacokinetics of intravenously injected exosomes. We also observed that exosomes collected from C2C12 cells, NIH3T3 cells, MAEC cells, and RAW264.7 cells were mainly taken up by macrophages in the liver after intravenous administration.[23] Another study also reported that mouse DC-derived exosomes were taken up by macrophages in the spleen and liver after intravenous administration.[32] Moreover, CD11c + DCs also contributed to the uptake of exosomes in the spleen and liver. It has been reported that exosomes collected from MDA-MB-231 breast cancer cells were taken up by macrophages in the lung and brain, after intravenous administration.[33] These findings indicate that macrophages are the primary cells that actively take up exogenously administered exosomes.

Key Molecules in the Pharmacokinetics of Exosomes

It is predicted that exosomes are taken up by cells through the recognition of surface molecules on the exosomes. There are several studies investigating the molecules that contribute to the pharmacokinetics of exosomes (Table 2). It has been reported that macrophages take up apoptotic cells through the recognition of the phosphatidylserine (PS) exposed on the outer leaflet of the plasma membrane.[34] Because exosomes expose PS on their surface,[35] we investigated the role of PS in the recognition and uptake of intravenously administered exosomes by macrophages.[36] Pre-injection of negatively charged, PS- or phosphatidylglycerol-containing liposomes delayed the clearance of B16BL6 exosomes from the blood circulation. Moreover, accumulation of intravenously administered B16BL6 exosomes in the liver was reduced by the pre-injection of PS-containing liposomes, suggesting that the negative charge of PS exposed on the exosomes is involved in the recognition and uptake of exosomes by macrophages. Watson et al.[37] evaluated the contribution of the scavenger receptor class A family (SR-A), a receptor that is expressed on macrophages and recognizes negatively charged molecules, to the pharmacokinetics of exosomes. They found that the blocking of SR-A by pre-treatment with dextran sulfate reduced the accumulation of intravenously administered HEK293 cell-derived exosomes in the liver. An *in vitro* cellular uptake experiment demonstrated that carbohydrate moieties on the exosomes contributed to the uptake of exosomes by cells.[38] Saunderson et al.[39] investigated the involvement of sialic acid on exosomes, in the recognition by CD169 on macrophages *in vivo*. CD169 is a sialic acid–binding immunoglobulin-like lectin

Table 3
Summary of Controlled Pharmacokinetics of Exosomes

Exosome Source	Injection Route	Functional Molecules	Control of Pharmacokinetics	References
DCs	i.v.	RVG-lamp2b	Selective distribution to the brain	41
DCs	i.v.	RVG-lamp2b	Selective distribution to the brain	42
HEK293T	i.v.	RVG-lamp2b	Selective distribution to the brain	43
Immature DCs	i.v.	iRGD-lamp2b	Selective distribution to the αv integrin-positive tumor tissues	44
HEK293	i.v.	GE11 peptide	Selective distribution to the EGFR-expressing tumor tissues	45
Neuro2A	i.v.	PEG	Prolonged circulation time	46

i.v., intravenous.

(Siglec-1) and functions as a sialic acid receptor. After intravenous injection, B cell–derived exosomes expressing α2,3-linked sialic acid distributed to the spleen and were predominantly taken up by CD169+ macrophages in the marginal zone, followed by SIGN-R1+ macrophages in the outer marginal zone rim, and by F4/80+ macrophages in the red pulp. When B cell–derived exosomes were intravenously injected into CD169−/− mice, SIGN-R1+ and F4/80+ macrophages largely took up the exosomes, as opposed to what was observed in wild-type mice. These findings indicate that the CD169 on macrophages played an important role in the recognition and uptake of exosomes. Hoshino et al.[40] investigated the influence of the integrins on the surface of exosomes on their pharmacokinetics. Exosomes derived from tumor cells that metastasize to the lung (MDA-MB-231 and 4175) or to the liver (BxPC-3 and HPAF-II), primarily accumulated in the lung and liver, respectively. A proteomic analysis of exosomes revealed that 4175 exosomes and BxPC-3 exosomes highly expressed integrins α6β4 and αVβ5, respectively. Exosomes collected from integrin β4–knocked down 4175 cells showed a reduced accumulation in the lung. Moreover, liver accumulation of BxPC-3 cell–derived exosomes was reduced by the knockdown of integrin β5 in exosome-producing BxPC-3 cells. These results indicate that integrins play a key role in the pharmacokinetics of exosomes.

These previous studies demonstrate that the exosomal surface molecules, including phospholipids and proteins, are important factors in determining the *in vivo* behavior of exosomes. Further studies, aimed at discovering the molecules on the surface of exosomes that are recognized by cells, are needed for a proper understanding of their pharmacokinetics.

Control of the Pharmacokinetics of Exosomes

For the development of exosome-based therapeutics, it is important to control the pharmacokinetics of exosomes, that is, the selective delivery of exosomes to target organs and cells. To control the pharmacokinetics of exosomes, the modification of the surface of exosomes with targeting proteins or peptides has been widely used (Table 3). Wood and colleagues genetically engineered DC-derived exosomes with Lamp2b, an exosomal membrane protein, and the neuron-specific RVG peptide. Small interfering RNA was loaded into the exosomes by electroporation and intravenously injected into mice. RVG-modified exosomes selectively delivered small interfering RNA to the brain and exerted gene-silencing effects against target mRNA.[41] Subsequent studies also demonstrated the usefulness of RVG-modified exosomes for the targeted delivery of exosomes to the brain.[42,43] Tian et al.[44] genetically engineered the immature DCs to express Lamp2b fused to the αv integrin-specific iRGD peptide. Exosomes purified from the immature DCs expressing the iRGD peptide showed a selective distribution to αv integrin–positive tumor tissues. Ohno et al.[45] modified HEK293 cell–derived exosomes using the GE11 peptide, which binds to the epidermal growth factor receptor, fused with the transmembrane domain of the platelet-derived growth factor receptor. After the loading of the tumor suppressor gene *let-7a* into GE11-modified exosomes, the exosomes were administered into epidermal growth factor receptor–expressing breast cancer mice. GE11-modified exosomes selectively distributed to tumor tissues and exhibited an antitumor effect mediated by *let-7a*.

In addition to their targeting capacity, the escape of exosomes from a nonspecific interaction with nontarget cells is also important for the development of exosome-based therapeutics. Kooijmans et al.[46] modified Neuro2A cell–derived exosomes with polyethylene glycol, which could shield nanoparticles from interactions with plasma proteins, and enhance their circulation time. Whereas unmodified exosomes were rapidly cleared from the blood circulation after intravenous injection in mice, the modification of exosomes with polyethylene glycol prolonged their circulation time.

These findings demonstrated that modifying the surface of exosomes is useful to control their pharmacokinetics. However, there are no clinical trials for exosome-based therapeutics reporting controlled pharmacokinetics of exosomes. Besides the studies investigating the pharmacokinetics of exosomes, the development of further useful strategies to control their pharmacokinetics will be needed in the future.

Conclusions

Data on the pharmacokinetics of exosomes are important for the elucidation of the biological roles of exosomes and the development of exosome-based therapeutics. In this review, we summarized recent findings regarding the pharmacokinetics and biodistribution of exosomes. By virtue of the development of exosome labeling methods, including our studies, we have obtained a systematic understanding of the *in vivo* behavior of exosomes. We believe that these pieces of information will be efficiently used for the development of exosome research in the future.

References

1. Théry C, Ostrowski M, Segura E. Membrane vesicles as conveyors of immune responses. *Nat Rev Immunol*. 2009;9:581-593.
2. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat Cell Biol*. 2007;9:654-659.
3. Tominaga N, Kosaka N, Ono M, et al. Brain metastatic cancer cells release microRNA-181c-containing extracellular vesicles capable of destructing blood–brain barrier. *Nat Commun*. 2015;6:6716.
4. Skog J, Würdinger T, van Rijn S, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. *Nat Cell Biol*. 2008;10:1470-1476.
5. Miller IV, Grunewald TG. Tumour-derived exosomes: tiny envelopes for big stories. *Biol Cell*. 2015;107:287-305.
6. Bruno S, Grange C, Deregibus MC, et al. Mesenchymal stem cell-derived microvesicles protect against acute tubular injury. *J Am Soc Nephrol*. 2009;20:1053-1067.
7. Lai RC, Arslan F, Lee MM, et al. Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Res*. 2010;4:214-222.
8. Xin H, Li Y, Buller B, et al. Exosome-mediated transfer of miR-133b from multipotent mesenchymal stromal cells to neural cells contributes to neurite outgrowth. *Stem Cells*. 2012;30:1556-1564.

9. Lee C, Mitsialis SA, Aslam M, et al. Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension. *Circulation*. 2012;126:2601-2611.
10. Lener T, Gimona M, Aigner L, et al. Applying extracellular vesicles based therapeutics in clinical trials—an ISEV position paper. *J Extracell Vesicles*. 2015;4:30087.
11. Wolfers J, Lozier A, Raposo G, et al. Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. *Nat Med*. 2001;7:297-303.
12. Morishita M, Takahashi Y, Matsumoto A, Nishikawa M, Takakura Y. Exosome-based tumor antigens—adjuvant co-delivery utilizing genetically engineered tumor cell-derived exosomes with immunostimulatory CpG DNA. *Biomaterials*. 2016;111:55-65.
13. Dai S, Wei D, Wu Z, et al. Phase I clinical trial of autologous ascites-derived exosomes combined with GM-CSF for colorectal cancer. *Mol Ther*. 2008;16:782-790.
14. Zitvogel L, Regnault A, Lozier A, et al. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell derived exosomes. *Nat Med*. 1998;4:594-600.
15. Pitt JM, Charrier M, Viaud S, et al. Dendritic cell-derived exosomes as immunotherapies in the fight against cancer. *J Immunol*. 2014;193:1006-1011.
16. Viaud S, Thery C, Ploix S, et al. Dendritic cell-derived exosomes for cancer immunotherapy: what's next? *Cancer Res*. 2010;70:1281-1285.
17. Peinado H, Alečković M, Lavotshkin S, et al. Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Nat Med*. 2012;18:883-891.
18. Grange C, Tapparo M, Bruno S, et al. Biodistribution of mesenchymal stem cell-derived extracellular vesicles in a model of acute kidney injury monitored by optical imaging. *Int J Mol Med*. 2014;33:1055-1063.
19. Wiklander OP, Nordin JZ, O'Loughlin A, et al. Extracellular vesicle in vivo biodistribution is determined by cell source, route of administration and targeting. *J Extracell Vesicles*. 2015;4:26316.
20. Smyth T, Kullberg M, Malik N, Smith-Jones P, Graner MW, Anchordoquy TJ. Biodistribution and delivery efficiency of unmodified tumor-derived exosomes. *J Control Release*. 2015;199:145-155.
21. Munagala R, Aqil F, Jeyabalan J, Gupta RC. Bovine milk-derived exosomes for drug delivery. *Cancer Lett*. 2016;371:48-61.
22. Takahashi Y, Nishikawa M, Shinotsuka H, et al. Visualization and in vivo tracking of the exosomes of murine melanoma B16-BL6 cells in mice after intravenous injection. *J Biotechnol*. 2013;165:77-84.
23. Charoenviriyakul C, Takahashi Y, Morishita M, Matsumoto A, Nishikawa M, Takakura Y. Cell type-specific and common characteristics of exosomes derived from mouse cell lines: yield, physicochemical properties, and pharmacokinetics. *Eur J Pharm Sci*. 2017;96:316-322.
24. Lai CP, Mardini O, Ericsson M, et al. Dynamic biodistribution of extracellular vesicles in vivo using a multimodal imaging reporter. *ACS Nano*. 2014;8:483-494.
25. Morishita M, Takahashi Y, Nishikawa M, et al. Quantitative analysis of tissue distribution of the B16BL6-derived exosomes using a streptavidin−lactadherin fusion protein and Iodine-125-labeled biotin derivative after intravenous injection in mice. *J Pharm Sci*. 2015;104:705-713.
26. Hu L, Wickline SA, Hood JL. Magnetic resonance imaging of melanoma exosomes in lymph nodes. *Magn Reson Med*. 2015;74:266-271.
27. Kotmakçı M, Çetintaş V. Extracellular vesicles as natural nanosized delivery systems for small-molecule drugs and genetic material: steps towards the future nanomedicines. *J Pharm Pharm Sci*. 2015;18:396-413.
28. Bruno S, Grange C, Collino F, et al. Microvesicles derived from mesenchymal stem cells enhance survival in a lethal model of acute kidney injury. *PLoS One*. 2012;7:e33115.
29. Gatti S, Bruno S, Deregibus MC, et al. Microvesicles derived from human adult mesenchymal stem cells protect against ischaemia-reperfusion-induced acute and chronic kidney injury. *Nephrol Dial Transpl*. 2011;26:1474-1483.
30. Badr CE, Tannous BA. Bioluminescence imaging: progress and applications. *Trends Biotechnol*. 2011;29:624-633.
31. Imai T, Takahashi Y, Nishikawa M, et al. Macrophage-dependent clearance of systemically administered B16BL6-derived exosomes from the blood circulation in mice. *J Extracell Vesicles*. 2015;4:26238.
32. Kim SH, Bianco NR, Shufesky WJ, Morelli AE, Robbins PD. Effective treatment of inflammatory disease models with exosomes derived from dendritic cells genetically modified to express IL-4. *J Immunol*. 2007;179:2242-2249.
33. Chow A, Zhou W, Liu L, et al. Macrophage immunomodulation by breast cancer-derived exosomes requires toll-like receptor 2-mediated activation of NF-κB. *Sci Rep*. 2014;4:5750.
34. Fadok VA, Bratton DL, Rose DM, Pearson A, Ezekewitz RAB, Henson PM. A receptor for phosphatidylserine-specific clearance of apoptotic cells. *Nature*. 2000;405:85-90.
35. Subra C, Laulagnier K, Perret B, Record M. Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. *Biochimie*. 2007;89:205-212.
36. Matsumoto A, Takahashi Y, Nishikawa M, et al. Role of phosphatidylserine-derived negative surface charges in the recognition and uptake of intravenously injected B16BL6-derived exosomes by macrophages. *J Pharm Sci*. 2017;106:168-175.
37. Watson DC, Bayik D, Srivatsan A, et al. Efficient production and enhanced tumor delivery of engineered extracellular vesicles. *Biomaterials*. 2016;105:195-205.
38. Hao S, Bai O, Li F, Yuan J, Laferte S, Xiang J. Mature dendritic cells pulsed with exosomes stimulate efficient cytotoxic T-lymphocyte responses and antitumour immunity. *Immunology*. 2007;120:90-102.
39. Saunderson SC, Dunn AC, Crocker PR, McLellan AD. CD169 mediates the capture of exosomes in spleen and lymph node. *Blood*. 2014;123:208-216.
40. Hoshino A, Costa-Silva B, Shen T, et al. Tumour exosome integrins determine organotropic metastasis. *Nature*. 2015;527:329-335.
41. Alvarez-Erviti L, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol*. 2011;29:341-345.
42. Cooper JM, Wiklander P, Nordin JZ, et al. Systemic exosomal siRNA delivery reduced alpha-synuclein aggregates in brains of transgenic mice. *Mov Disord*. 2014;29:1476-1485.
43. Liu Y, Li D, Liu Z, et al. Targeted exosome-mediated delivery of opioid receptor mu siRNA for the treatment of morphine relapse. *Sci Rep*. 2015;5:17543.
44. Tian Y, Li S, Song J, et al. A doxorubicin delivery platform using engineered natural membrane vesicle exosomes for targeted tumor therapy. *Biomaterials*. 2014;35:2383-2390.
45. Ohno S, Takanashi M, Sudo K, et al. Systemically injected exosomes targeted to EGFR deliver antitumor microRNA to breast cancer cells. *Mol Ther*. 2013;21:185-191.
46. Kooijmans S, Fliervoet L, Van Der Meel R, et al. PEGylated and targeted extracellular vesicles display enhanced cell specificity and circulation time. *J Control Release*. 2016;224:77-85.