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Research paper

Cancer cell-type tropism is one of crucial determinants for the efficient systemic delivery of cancer cell-derived exosomes to tumor tissues

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ABSTRACT

Exosomes are gaining increasing attention as drug delivery vehicles due to their low toxicity and ability to functionally transfer biological cargos between cells. However, the therapeutic applicability of exosomes is partially hampered by a lack of cell-type specificity. In this study, therefore, we investigated the impact of cell-type tropism on the *in vivo* systemic delivery of exosomes to tumor tissues. Exosomes derived from murine colorectal cancer cells (C26) (C26-Exos) and murine melanoma cells (B16BL6) (B16BL6-Exos) were collected. *In vitro* cellular uptake of either autologous (C26) or allogeneic (B16BL6) exosomes by C26 tumor cells was determined. *In vivo* tumor accumulation of each type of exosomes in mice bearing C26 tumors was monitored with an *in vivo* imaging system (IVIS). In *in vitro* studies, autologous C26-Exos were more efficiently taken up by C26 cancer cells, compared to allogeneic B16BL6-Exos. For *in vivo* studies, exosomes were modified with surface polyethylene glycol (PEG) to improve their circulation lifetimes. Although both types of PEGylated exosomes accumulated in C26-tumor tissue, autologous exosomes were preferentially accumulated within C26-tumor tissue compared to allogeneic exosomes. The increased tumor accumulation of autologous PEGylated exosomes was accompanied by the preferential uptake of exosomes by not only C26-tumor cells but also tumor-associated immune cells. This study implies that cancer cell-type tropism is an important factor in the achievement of tumor cell targeting with cancer cell-derived exosomes.

1. Introduction

Extracellular vesicles (EVs) have emerged in the field of nanotechnology as potential delivery systems for various diagnostics and/or therapeutics [1–8]. EVs are secreted by many types of cells including cancer cells, and they exist in various biological fluids. They are classified into different subtypes according to their size and intercellular origin [5,9,10]. Exosomes are a nano-sized subtype of EVs that are derived from the endosomal pathway. Exosomes, especially tumor-derived exosomes, have the ability to carry molecular cargos which work as signatures reflecting the real-time status of disease such as cancer [6,11,12]. They also play roles in cancer chemoresistance and progression [13]. In addition, they possess several promising characteristics that make them potentially exploitable as delivery vehicles [8,11,12,14] such as the presence of uptake-related surface proteins, and favorable *in vivo* biological tolerability and stability. They are also amenable to modification for targeting specific cell types [15,16]. Therefore, tumor-derived exosomes have been picked out as potential cancer biomarkers, therapeutic targets and drug delivery platforms for cancer therapy.

Nevertheless, despite some useful properties of exosomes for use as delivery vehicles, major challenges still hinder the clinical utilization of naked exosomes compared to conventional drug delivery vehicles such as liposomes. The highly complex and variable composition of exosomes hinders the prediction of their cell specificity and biological effects and makes establishing suitable characterization protocols a difficult and time-consuming mission[10,17,18]. Their low yield and low uptake by specific target cells are among the critical challenges facing exosome field [9,18]. Furthermore, in contrast to the general perception that exosomes are non-immunogenic because of their endogenous origin, the ability of exosomes to elicit immune responses may contribute to toxicities and/or rapid exosome clearance following systemic administration [19–21]. These hurdles have to be overcome before exosomes can be exploited as drug carriers.

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In spite of numerous publications claiming the utility of exosomes as delivery vehicles, especially for cancer therapy [2,8,12,14,16,22–25], the question how, or even if, exosomes are specifically taken up by recipient cells is still subject to debate. It has been suggested that exosomes are trafficked (and act) in both autocrine and paracrine manners. Some *in vitro* studies, including our previous study, indicate that exosomes are preferentially taken up by the same cell type that produces them [26,27]. In addition, some combinations of exosomes and cells result in higher uptake than others [28–30]. These results suggest that the cellular uptake of exosomes may have some cell specificity. It has been reported that exosomes from some immune cells such as NK cells or T cells [31], and from stem cells such as mesenchymal stromal stem cells (MSCs) express cell receptors that recapitulate the tumor tropism of their parent cells [32,33]. Nevertheless, for drug delivery purposes, *in vivo* cell-type tropism of exosome uptake should be confirmed so it can be exploited to improve the cell-specific uptake of exosomes.

We recently showed that *in vitro* incubation of B16BL6 and C26 cancer cells with liposomes can increase exosome secretion, increasing the yield of harvested exosomes [34]. We further showed that donor cells (B16BL6) have increased uptake of exosomes compared to allogeneic cells (C26) [26]. In the current study, we investigated the impact of cell-type tropism on the *in vivo* systemic delivery of exosomes to tumor tissues and found that cancer cell-type tropism represents a crucial determinant that dictates target cell specificity of cancer cell-derived exosomes.

2. Materials and methods

2.1. Materials and antibodies

1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*n*-[methoxy(polyethylene-glycol)-2000] (mPEG$_{2000}$-DSPE) was purchased from NOF (Tokyo, Japan). PKH67 Green Fluorescent Cell Linker kit was purchased from Sigma Aldrich (MO, USA). 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR; DiIC18(7)), 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate (DiI; DiIC18(3)), anti-F4/80 monoclonal antibody (BM8, eFluor 660, eBioscience™) and anti-CD11c monoclonal antibody (N418, FITC, eBioscience™) were purchased from Thermo Fisher Science (MA, USA). Anti-CD3 antibody (17A2, APC) was purchased from BioLegend (CA, USA). Anti-IgM antibody (A135FS, FITC) was purchased from American Qualex (CA, USA). Exosome-depleted fetal bovine serum was purchased from System Biosciences (CA, USA). All other reagents were of analytical grade.

2.2. Animals and cancer cells

BALB/c mice (male, 5 weeks old) were purchased from Japan SLC (Shizuoka, Japan). The experimental animals were allowed free access to water and mouse chow, and were housed under controlled environmental conditions (constant temperature, humidity, and a 12-h dark–light cycle). All animal experiments were evaluated and approved by the Animal and Ethics Review Committee of Tokushima University.

Two cancer cell lines, the Colon 26 (C26) murine colorectal cancer cell line and the B16BL6 murine melanoma cell line, were purchased from the Cell Resource Center for Biomedical Research (RIKEN RBC CELL BANK, Saitama, Japan). These cells were cultured at 5% CO_2 at 37 °C in RPMI-1640 culture medium (Wako Pure Chemical, Osaka, Japan) supplemented with 10% exosome-depleted FBS, 100 IU/ml penicillin, and 100 μg/ml streptomycin (MP Biomedicals, CA, USA).

2.3. Exosome isolation and PEGylation

Both cell lines were adapted for collecting C26-Exos and B16BL6-Exos from their respective cell lines, as previously described [26,34]. Briefly, at 80–90% confluence, the cells were incubated for a further 48 h following the replacement of culture medium with a fresh exosome-depleted medium. Then, the culture medium was collected and centrifuged at 4 °C (300*g* for 10 min, 2000*g* for 20 min and 12,500*g* for 30 min) to exclude cell debris, apoptotic bodies and microvesicles. After that, exosomes were enriched from the final supernatant by ultracentrifugation at 100,000*g* for 70 min at 4 °C [35–37]. The resultant pellet was washed twice with phosphate-buffered saline (PBS), and then suspended in 125 μl of PBS. The diameters of the collected exosomes were determined in PBS at 25 °C using a Zetasizer Nano ZS (Malvern Instruments Ltd., WR, UK). Bio-Rad DC® protein assay (Bio-Rad Laboratories Inc., CA, USA) was conducted to measure the protein concentration of the harvested exosomes according to the manufacturer's recommended protocol using a linear standard curve of bovine serum albumin (BSA) for calculating the protein concentration.

PEGylation of exosomes was conducted after the exosome labeling step via co-incubation of labeled exosomes with PEGylated lipid-based micelles. Briefly, PEGylated lipid-based micelles were prepared by dissolving mPEG$_{2000}$-DSPE in PBS above the critical micelle concentration of PEG$_{2000}$-DSPE [38]. Then, exosomes were incubated at 37 °C for 1 h in the presence of different ratios of PEGylated lipid-based micelles (1:10, 1:50 and 1:100 (μg exosome protein:μg PEGylated lipid)).

2.4. Evaluation of the in vitro uptake of exosomes by cancer cells

In vitro cellular uptake of either C26 or B16BL6-derived exosomes was evaluated using the two cancer cell lines, C26 and B16BL6, as previously described [34]. To track the uptake of exosomes, exosomes were labeled with the fluorescence dye, PKH67, following the manufacturer's protocol with minor modifications [39–41]. Then, exosomes were washed twice by centrifugation using PBS. To evaluate the uptake of exosomes by cancer cells, the cells were pre-cultured at a density of 1.5×10^5 cell/well in a 6-well plate for 24 h. Labeled exosomes at a final concentration of 5 μg/ml were added to the cancer cells and then incubated for 4 h at 37 °C because the amount of exosome uptake increased up to 4 h and then remained unchanged (data not shown). The cells were harvested and the cellular uptake of exosomes was analyzed using Gallios flow cytometer (Beckman Coulter, CA, USA). The data were analyzed using Kaluza software (Beckman Coulter).

2.5. Evaluation of the tumor accumulation and cellular uptake of exosomes in tumor-bearing mice

The tumor accumulation of both exosome types was evaluated using C26 tumor-bearing mice. To track the tumor accumulation of exosomes, as well as the *in vivo* exosome uptake by tumor tissue, either DiR or DiI was employed for exosome labeling according to the manufacturer's protocol [20]. Each dye was incorporated into its respective exosome type at a final concentration 0.5% (μg dye/μg exosome protein) via incubation at 37 °C for 1 h and then the exosomes were washed twice with PBS by centrifugation.

To evaluate tumor accumulation of exosomes in C26 tumor-bearing mice, C26 cells (2×10^6 cells/200 μl PBS) were subcutaneously inoculated into the back region of 5-week-old male BALB/c mice. Tumor-bearing mice were divided into five cohorts; one received PBS (control), two cohorts were treated with non-PEGylated C26- or B16BL6-Exos, and other two cohorts were treated with PEGylated C26- or B16BL6-Exos. DiR-labeled exosomes (60 μg/200 μl PBS/mouse), having approximately the same of number of exosomes/mouse, were intravenously injected on day 9 post tumor inoculation (average tumor volume of 150–200 mm^3) via the tail vein of C26 tumor-bearing mice. At indicated time points post-injection (4, 8, 24, 48, 72 and 96 h), the mice were anesthetized and then the tumor accumulation of DiR-labeled exosomes was visualized using IVIS (Xenogen, CA, USA). To verify the tumor accumulation of exosomes, mice were euthanized at 24, 48 and 96 h post-injection and tumor tissues were collected. The tumor accumulation of DiR-labeled exosomes was monitored via *ex vivo* imaging using IVIS.

To evaluate the *in vivo* exosome uptake by tumor and tumor-associated cells, DiI-labeled PEGylated exosomes (60 μg/200 μl PBS/mouse)

were intravenously injected into the same C26 tumor-bearing BALB/c mouse model. At 24 h post-injection, mice were euthanized and tumor tissues were collected and further treated to harvest tumor cells and tumor-associated cells. The uptake of DiI-labeled exosomes by these cells was then evaluated by flow cytometry analysis by gating on the tumor cells. Tumor-associated cells were sorted using fluorescently labeled specific antibodies against F4/80, CD3, CD11c and IgM for tumor-associated macrophages (TAMs), T cells, dendritic cells (DCs) and B cells, respectively. The uptake of DiI-labeled exosomes by each cell population was determined using a flow cytometer.

2.6. Statistical analysis

Statistical analysis was performed via an unpaired *t* test and one way ANOVA (Tukey's test and Dunnett's test) using Graphpad Prism 6.01 software (GraphPad Software Inc., CA, US). The level of significance was set at $p < 0.05$.

3. Results

3.1. In vitro uptake of exosomes

To investigate effect of cancer cell-type tropism on *in vitro* uptake of cancer cell-derived exosomes, the uptake of two different types of cancer cell-derived exosomes (C26-Exos and B16BL6-Exos) was studied in two different types of cancer cells (C26 and B16BL6) (Fig. 1). Both types of exosomes were taken up by both donor and recipient cells. The uptake of C26-Exos by C26 donor cells was higher than that by B16BL6 recipient cells (31.1% vs. 23.6%, respectively). Similarly, the uptake of B16BL6-Exos by B16BL6 donor cells was higher than that by C26 recipient cells (59.6% vs. 42.6%, respectively). These results indicate that cancer cell-derived exosomes were taken up preferentially to a small degree by donor cancer cells rather than recipient cancer cells. We confirmed with this *in vitro* study that cancer cell-type tropism contributes to the uptake of exosomes by cancer cells.

3.2. Biodistribution and tumor accumulation of exosomes in tumor-bearing mice

Many groups have studied *in vivo* behavior of exosomes after intravenous injection and have shown that exosomes are rapidly cleared from blood circulation into liver and spleen [19,42]. To study the effect

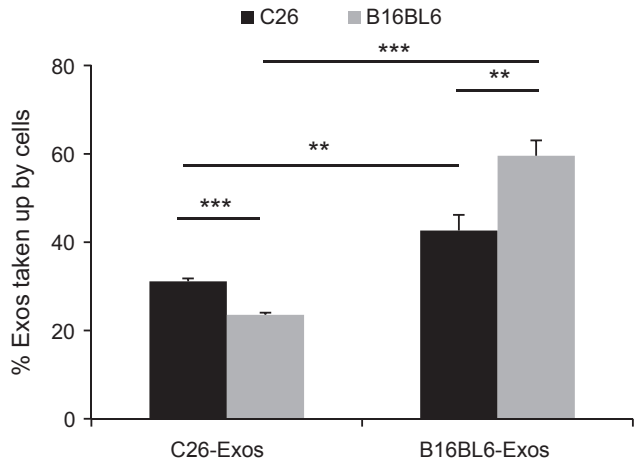

Fig. 1. *In vitro* uptake of exosomes by donor cancer cells and recipient cancer cells. PKH67-labeled exosomes (C26-Exos and B16BL6-Exos) were individually incubated with two different cancer cell lines, C26 and B16BL6. After 4 h-incubation, cells were harvested and analyzed using a flow cytometer. All data represent the mean ± SD. One way ANOVA test (Tukey's test) was applied. ** $p < 0.01$ and *** $p < 0.001$.

Table 1
Mean diameter of naked exosomes and PEGylated exosomes.

Type of exosomes		Size (nm)	PDI*
C26-Exos	Naked	187 ± 9.15	0.36
	PEGylated	192 ± 14.40	0.62
B16BL6-Exos	Naked	190 ± 7.11	0.36
	PEGylated	185 ± 21.02	0.48

* PDI: polydispersity index.

of cancer cell-type tropism on the *in vivo* uptake of cancer cell-derived exosomes, exosomes were PEGylated by the post-insertion method [38] to improve their circulation half-lives by reducing their interactions with the cells of mononuclear phagocyte system (MPS) [43–45]. Exosomes were mixed with different ratios of PEGylated lipid-based micelles, 1:10, 1:50 and 1:100 (μg exosome protein:μg PEGylated lipid). After PEGylation, no significant change in the diameter of exosome particle was detected (Table 1). Since it is well-recognized that PEGylation can significantly reduce the cellular binding/uptake of PEGylated nanoparticles [46], the effect of PEGylation of the exosome surface on exosome uptake by autologous (donor) cells *in vitro* was determined by studying the percentage of exosomes taken up by the cells. As expected, PEGylation significantly compromised the *in vitro* uptake of C26-Exos by donor C26 cancer cells. The percentage uptake inhibition of C26-Exos by PEGylation was 79.81%, 83.95% and 82.77% at exosome protein:PEGylated lipid ratios of 1:10, 1:50 and 1:100, respectively (Fig. 2A). Similar results were observed for the combination of PEGylated B16BL16-Exos and B16BL6 cells; the percentage uptake inhibition by PEGylation was 89.43%, 94.43% and 90.36%, at exosome protein:PEGylated lipid ratios of 1:10, 1:50 and 1:100, respectively (Fig. 2B). In this study, the percentage of exosome uptake inhibition imparted *in vitro* by PEGylation was employed as an indicator of the PEGylation process efficiency. Although different amounts of PEGylated lipid-based micelles appeared to have no significant difference in exosome uptake inhibition *in vitro*, the ratio (1:50) produced the highest uptake inhibition with both exosome types. Therefore, we selected the ratio (1:50) for *in vivo* studies. In addition, PEGylation significantly increased concentrations of exosomes in the blood circulation (Supplementary Fig. 1). These provide evidence that the PEGylation of exosomes has been achieved under our experimental condition.

To study tumor accumulation of exosomes, exosomes were modified with PEG_{2000}-DSPE at a ratio of 1:50 (μg exosome protein:μg PEGylated lipid) and were injected intravenously into C26 tumor-bearing mice. The C26 tumor model was selected due to its high vascular permeability [47–49]. To exclude background fluorescence, tumor-bearing mice were scanned for any fluorescence before exosome injection. No fluorescence was detected in the untreated tumor-bearing mice (Supplementary Fig. 2). Naked C26-Exos were accumulated primarily in liver at 4 h, and were still detected in liver up to 72 h, after which they could no longer be detected (Fig. 3A). Naked C26-Exos did not show any detectable accumulation in the region of the tumor (Fig. 3A). PEGylated C26-Exos were also detected primarily in liver. Interestingly, they could be detected in the region of the tumor beginning at 8 h post-injection and could still be detected at 96 h (Fig. 3A and C). Naked B16BL6-Exos were detected in liver by 4 h after injection and appeared to peak at 24–48 h, and then gradually decreased (Fig. 3B). They did not show any detectable accumulation in tumor tissue (Fig. 3B). PEGylated B16BL6-Exos were also detected in tumors, but to a lesser degree, with peak levels at 48–72 h (Fig. 3B and C). As shown in Fig. 3C, at every time point, the tumor accumulation of PEGylated C26-Exos was higher than that of PEGylated B16BL6-Exos. As anticipated, PEGylation of exosomes resulted in tumor accumulation of cancer cell-derived exosomes within tumor tissue, although uptake by cells *in vitro* was significantly reduced (Fig. 2A and B). It appears that autologous exosomes (C26-Exos) tend to preferentially accumulate in donor C26

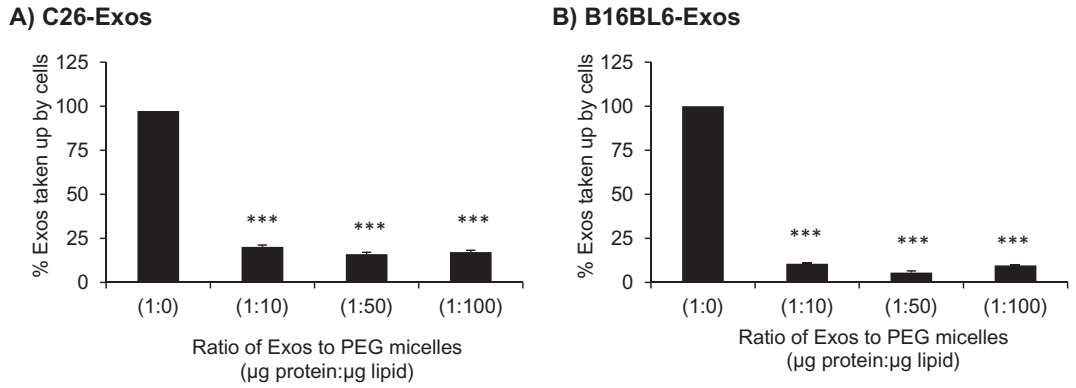

Fig. 2. The effect of PEGylation on *in vitro* uptake of exosomes. PKH67-labeled exosomes (C26-Exos and B16BL6-Exos) were PEGylated by the post-insertion method at three different PEGylated lipid ratios. The PEGylated exosomes were then incubated with autologous (donor) cells. After 4 h-incubation, cells were harvested and analyzed using a flow cytometer. All data represent the mean ± SD. One way ANOVA test (Dunnett's test) was applied. *** $p < 0.001$.

tumor tissue, but likely not via interaction with the tumor cells themselves.

3.3. *In vivo* uptake of PEGylated exosomes by tumor cells and tumor-associated cells

To gain more insight into exosome tumor accumulation, tumor tissue was collected and the level of DiR-labeled exosomes was evaluated *ex vivo*. At 24 hr post-injection, no fluorescent signal was detected in tumors treated with either type of naked exosomes, indicating no tumor accumulation (Fig. 4A). The C26 tumor, treated with either PEGylated autologous C26-Exos or PEGylated allogeneic B16BL6-Exos, showed increased fluorescence indicating accumulated exosomes at time points between 24 and 96 h (Fig. 4A). The signal intensity was higher for PEGylated C26-Exos than for PEGylated B16BL6-Exos in C26 tumor tissue (Fig. 4A and B). These observations were consistent with the result observed in Fig. 3, indicating that autologous C26-Exos tend to accumulate in donor C26 tumor tissue *in vivo* even if their surface was decorated with PEG.

To confirm whether PEGylated exosomes were taken up by tumor cells following accumulation into tumor tissue, the uptake of exosomes by tumor cells was investigated. PEGylated DiI-labeled exosomes were intravenously injected into C26 tumor-bearing mice. PEGylated autologous C26-Exos were significantly taken up by donor C26 tumor cells relative to PEGylated allogeneic B16BL6-Exos at 24 h post-injection (Fig. 5A). Approximately 1.5% of C26 tumor cells (1.5×10^4 cell per 10^6 cell) in the tumor tissue had taken up the PEGylated autologous C26-Exos, which was ten-times lower than the *in vitro* uptake amount (16.0%) (Fig. 2). In addition, the ability of certain tumor-infiltrating immune cells to accumulate PEGylated exosomes in tumor tissue was studied because tumor tissue is composed not only of cancer cells but also of immune cells such as B cells, T cells, tumor associated macrophages (TAMs) and dendritic cells (DC) [50]. PEGylated C26-Exos had increased uptake by TAMs (Fig. 5B) and T cells (Fig. 5C) compared to

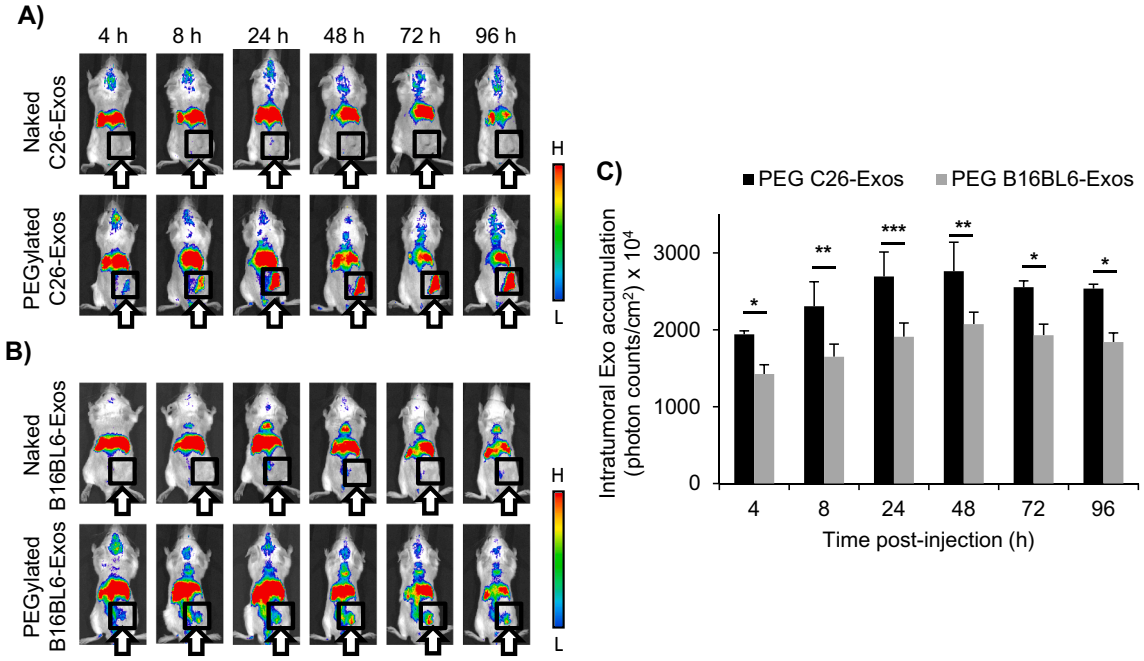

Fig. 3. *In vivo* tumor accumulation of exosomes. Four types of DiR-labeled exosomes, (A) naked C26-Exos and PEGylated C26-Exos, and (B) naked B16BL6-Exos and PEGylated B16BL6-Exos, were intravenously injected into C26 tumor-bearing mice. At selected time points post-injection, the biodistribution of exosomes, along with tumor accumulation, were monitored using IVIS. The arrow head in each picture indicates the tumor region. (C) Fluorescence intensity of accumulated exosomes in the tumor region was measured as photon counts/cm^2. The data (C) represent the mean ± SD. One way ANOVA test (Tukey's test) was applied. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

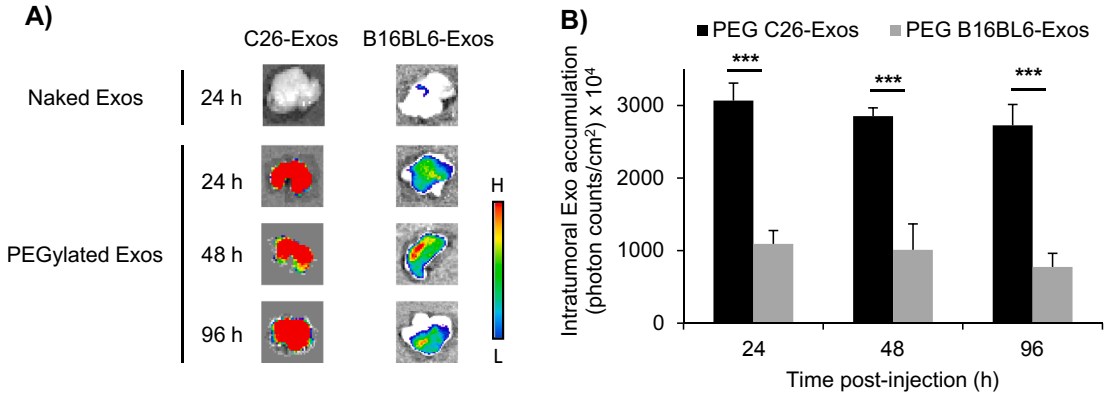

Fig. 4. *Ex vivo* evaluation of tumor accumulation of PEGylated exosomes. DiR-labeled exosomes (PEGylated C26-Exos and PEGylated B16BL6-Exos) were intravenously injected into C26 tumor-bearing BALB/c mice. At 24, 48, 96 h post-injection, the mice were sacrificed and tumors were collected. (A) Exosomes accumulated in the collected tumors were evaluated using IVIS. (B) Fluorescence intensity of accumulated exosomes in collected tumor was measured as photon counts/cm^2. All data (B) represent the mean ± SD. One way ANOVA test (Tukey's test) was applied. *** $p < 0.001$.

B16BL6-Exos and also compared to autologous tumor cells. In DC (Fig. 5D) and B cells (Fig. 5E), there was no significant difference in uptake between PEGylated C26-Exos and PEGylated B16BL6-Exos. These results indicate that autologous exosomes (C26-Exos) were taken up by C26 cancer cells, as well as by TAM and T cells, resulting in cell-type tropism in tumor accumulation of exosomes (Figs. 3 and 4).

4. Discussion

In the current study, we showed higher overall tumor accumulation of autologous exosomes (C26-Exos) compared to allogeneic exosomes (B16BL6-Exos) in C26 tumor tissue *in vivo* (Figs. 3 and 4). *In vitro* exosome uptake study also indicated that C26-Exos were preferentially taken up by donor cancer cells (C26) compared to allogeneic cancer cells (B16BL6) (Fig. 1). These results suggest that there clearly exists cancer cell-type tropism on tumor accumulation of exosomes. Interestingly, the enhanced tumor accumulation resulted from the uptake by not only cancer cells but also by tumor-associated immune cells (mainly TAM and T cells) to an even greater degree (Fig. 5). This indicates that cancer cell-type tropism between donor cells and autologous exosomes is one cause of increased tumor accumulation of autologous exosomes, but accumulation by immune cells also plays a major role.

It is well recognized that systemically administered exosomes exhibit very short circulation times [19,30], presumably due to their

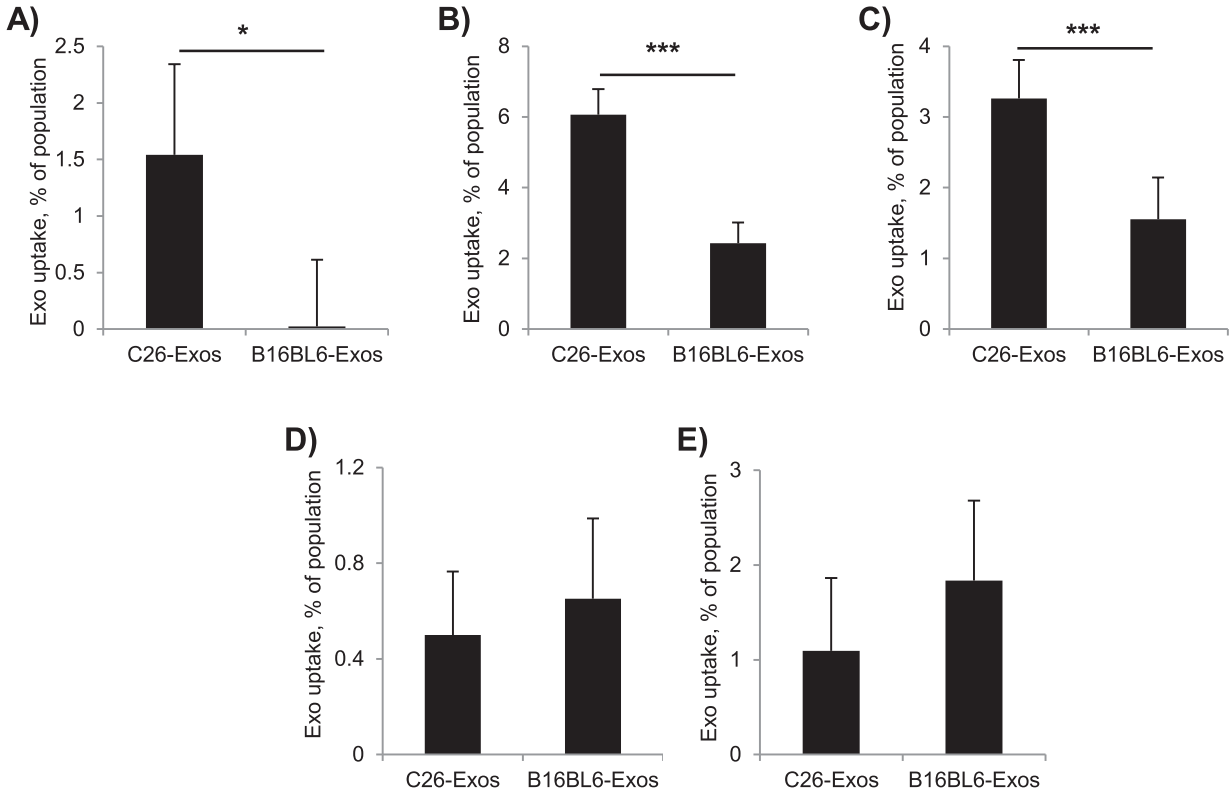

Fig. 5. *In vivo* uptake of PEGylated exosomes by tumor cells and tumor-associated immune cells. DiI-labeled exosomes (PEGylated C26-Exos and PEGylated B16BL6-Exos) were intravenously injected into C26 tumor-bearing BALB/c mice. At 24 h post-injection, tumors were harvested. The uptake of DiI-labeled exosomes by tumor cells (A), TAMs (B), T cells (C), DCs (D) and B cells (E) was analyzed using a flow cytometer. All data represent the mean ± SD. An unpaired *t* test was applied. * $p < 0.05$. ***$p < 0.001$.

recognition by the cells of MPS, which could limit exosome accumulation in target tissues such as solid tumors. In the current study, in an attempt to increase their blood circulation time and thereby increase their tumor accumulation via the enhanced permeability and retention (EPR) effect [44,45], the exosomes were PEGylated, which gives them a chance to interact with tumor and tumor-associated cells. Indeed, PEGylation of exosomes resulted in a detectable increase in tumor accumulation of both autologous and allogeneic exosomes in C26 tumor tissue compared to naked exosomes (Figs. 3 and 4). It appears that this increased selective tumor accumulation of autologous exosomes (C26-Exos) is cell-mediated, and made possible by the increased tumor accumulation of PEGylated exosomes via the EPR effect (Fig. 3).

Interestingly, the tumor-associated immune cells (mainly TAM and T cells) in the C26 tumor tissues took up C26-Exos to a greater degree than C26 tumor cells (Fig. 5). This indicates that accumulation by immune cells also plays a major role in cancer cell-type tropism. It is believed that cancer cells tend to release more exosomes than healthy cells, which may be due to an enhanced growth rate or as a result of stimulation in response to stressful conditions. So, the tumor-associated immune cells should be continuously exposed to the exosomes during the tumor progression and might be sensitized to “host” cancer cells. Many papers reported that tumor-derived exosomes play an important role in the transfer of immunosuppressive and immunostimulatory molecules to tumor-associated immune cells [51–55]. If the tumor-derived exosomes facilitate crosstalk between tumors and the host immune, preferred uptake of exosomes derived from “host” cancer cells might play a role in this.

Nevertheless, one question arises about exosome uptake in solid tumors. It is well-known that PEG on the surface of particles can inhibit the interaction of the particles with cells [46]. Notably, in this study, PEGylation of exosomes substantially inhibited the cellular uptake of exosomes *in vitro* (Fig. 2). However, PEGylation increased the EPR effect, so after accumulation, if dissociation of PEGylated lipid from exosomes had occurred, then exosomes could associate with the cells directly, as was seen for naked exosomes (Fig. 1). Parr et al. [56] have reported that a primary factor for retention of the PEG coating is the lipid anchor and DSPE is one of the better lipid anchors. The *in vitro* half-life of the exchange rate of $mPEG_{1900}$-DSPE out of the bilayer is reported to be 70 h at 37 °C [57]. In meantime, stimuli-triggered de-PEGylation strategies are extensively developed by attaching PEG molecules on surface of nanoparticles through labile bonds that are sensitive to the unique features of tumor microenvironment, such as low pH, overexpressed enzymes, and altered redox potential [58–60]. Introduction of modifications that control the kinetics of PEG-dissociation from exosomes might further improve cancer cell-oriented exosome uptake *in vivo*.

Solid tumors are characterized by leaky blood vessels and lack of functional lymphatics. Consequently, nanoparticles with diameters up to 250 nm (the size range of exosomes is 160–210 nm in diameter (Table 1)) are prone to extravasation across the abnormal endothelial lining and are efficiently retained in tumors over time [61]. However, PEGylated liposomes do not readily interact with tumor cells and they are thought to be retained in the tumor stroma [62] and subsequently eliminated from the tissue without internalization by the tumor cells. We have previously shown that PEGylated liposomes (100 nm in diameter), including entrapped oxaliplatin, accumulated in C26 tumor tissue *in vivo*; oxaliplatin concentration reached the maximum level at 24 h after injection, and then rapidly decreased [63]. Many papers have reported that cancer cell-derived exosomes express various membrane proteins available to interact with the membrane receptors of target cells [26,40,64–68]. Hence, the longer retention of PEGylated exosomes by tumor tissue (Figs. 3 and 4) may be due to the binding of these exosomes to cells in the tumor tissue, relative to PEGylated liposomes. However, experiments to identify exosome surface proteins responsible for preferential uptake of autologous exosomes by donor tumor cells are in progress in our laboratory. In addition, the PEG layer of PEGylated exosomes can be further decorated with targeting ligands via chemical modification or post-insertion technique for more specific applications [38,69,70]. Currently, there is an increased interest in the use of exosome-based drug delivery for the use of these naturally-occurring nanoparticles in various therapeutic approaches for different types of diseases [22,71]. Our findings might pave the way for a deployment of exosomes as drug delivery vehicles, especially for cancer therapy.

5. Conclusion

In the current study, we showed that cell-type tropism lead to ‘homing’ of cancer cell-derived exosomes and their preferential uptake by the donor cancer cells and tumor-associated immune cells in solid tumors. PEGylation of exosomes increased their tumor accumulation *in vivo* and cell-type tropism extended their accumulation and retention within tumor tissue. The study implies that exosomes are naturally programmed to selectively interact with specific cell types or tissues.

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Declaration of Competing Interest

No potential conflicts of interest were disclosed.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejpb.2019.10.005.

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