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Cell type-specific and common characteristics of exosomes derived from mouse cell lines: Yield, physicochemical properties, and pharmacokinetics

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ABSTRACT

Exosomes are small membrane vesicles secreted from cells and are expected to be used as drug delivery systems. Important characteristics of exosomes, such as yield, physicochemical properties, and pharmacokinetics, may be different among different cell types. However, there is limited information about the effect of cell type on these characteristics. In the present study, we evaluated these characteristics of exosomes derived from five different types of mouse cell lines: B16BL6 murine melanoma cells, C2C12 murine myoblast cells, NIH3T3 murine fibroblasts cells, MAEC murine aortic endothelial cells, and RAW264.7 murine macrophage-like cells. Exosomes were collected using a differential ultracentrifugation method. The exosomes collected from all the cell types were negatively charged globular vesicles with a diameter of approximately 100 nm. C2C12 and RAW264.7 cells produced more exosomes than the other types of cells. The exosomes were labeled with a fusion protein of *Gaussia* luciferase and lactadherin to evaluate their pharmacokinetics. After intravenous injection into mice, all the exosomes rapidly disappeared from the systemic circulation and mainly distributed to the liver. In conclusion, the exosome yield was significantly different among the cell types, and all the exosomes evaluated in this study showed comparable physicochemical and pharmacokinetic properties.

1. Introduction

Exosomes are small membrane vesicles with a diameter of 30–120 nm that are secreted from various types of cells (Johnsen et al., 2014; Vlassov et al., 2012). Since the discovery that exosomes act as intercellular communication tools by transferring their cargo, including proteins and nucleic acids, to the cells that uptake exosomes, they have attracted much attention, and the roles of exosomes in physiological events, such as tumor metastasis and immune response, have been vigorously investigated (Bobrie et al., 2011; Iero et al., 2008; Théry et al., 2009). In addition to the physiological roles of exosomes, the possibility of the development of exosome-based drug delivery systems (DDS) has been demonstrated by several studies in which exosomes were used to efficiently deliver proteins and nucleic acids to specific types of target cells (Alvarez-Erviti et al., 2011; Tian et al., 2014).

The yield of exomes and the physicochemical properties that affect their pharmacokinetics, such as particle size and surface charge, may vary with the type of exosome-producing cell. Because these factors are expected to greatly influence the therapeutic efficacy of exosomes, it is necessary to select appropriate types of exosome-producing cells for the development of exosome-based DDS. Moreover, the exosome yield is an important factor for the development of exosome-based DDS, and the yield may vary among different types of cells. However, little information is available about how the yield, physicochemical properties, and pharmacokinetics of exosomes depend on the cell type.

In the present study, five different types of murine cell lines, which represent whole body of mouse, were selected as model exosome-producing cells: B16BL6 melanoma cells, C2C12 myoblast cells, NIH3T3 fibroblasts cells, MAEC aortic endothelial cells, and RAW264.7 macrophage-like cells. B16BL6 cell line was selected because we reported the pharmacokinetics of B16BL6-derived exosomes in our previous study. We also selected other 4 types of normal, not tumor, cell lines, because these cells were easily transfected and produced gLuc-LA-modified exosomes. We collected exosomes from these types of cells and evaluated the exosome yield by measuring protein amount and particle number. We then investigated the particle size and zeta potential of these exosomes. To evaluate the pharmacokinetics of exosomes after an intravenous injection into mice, a fusion protein of *Gaussia* luciferase

Abbreviations: AUC, area under the curve; CL, clearance; DDS, drug delivery systems; FBS, fetal bovine serum; GLuc, *Gaussia* luciferase; HRP, horseradish peroxidase; LA, lactadherin; MSC, mesenchymal stem cells; PBS, phosphate-buffered saline; PS, phosphatidylserine; PSG, penicillin/streptomycin/L-glutamine; TEM, transmission electron microscopy.

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(gLuc) and lactadherin (LA), gLuc-LA, was used to label the exosomes with gLuc (Takahashi et al., 2013). The time course of the serum exosome concentration was examined by measuring the gLuc activity after an intravenous injection of gLuc-LA-labeled exosomes, and the biodistribution of the labeled exosomes was visualized using *in vivo* imaging.

2. Material and methods

2.1. Cell culture

B16BL6 cells were obtained from the Cancer Chemotherapy Center of the Japanese Foundation for Cancer Research. C2C12, NIH3T3, and RAW264.7 cells were purchased from the American Type Culture Collection. MAEC cells were a gift from Professor Ichiro Saito (Department of Pathology, Tsurumi University School of Dental Medicine, Yokohama, Japan). B16BL6 cells, C2C12 cells, and NIH3T3 cells were cultured in Dulbecco's modified Eagle's minimum essential medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin/L-glutamine (PSG). RAW264.7 cells were cultured in Roswell Park Memorial Institute medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 10% FBS and PSG. MAEC cells were cultured in medium 199 (Gibco, Grand Island, NY, USA) supplement with 10% FBS and PSG. Cells were cultured at 37 °C in humidified air containing 5% CO_2.

2.2. Collection of exosomes

Culture media used for exosome collection were prepared by ultracentrifugation at 100,000 $\times g$ for 2 h to remove FBS-derived exosomes. To reach approximately 80% confluency after 24 h of incubation, cells were seeded into 15-cm dishes at the following numbers: 8×10^6 cells for B16BL6 and C2C12, 7×10^6 cells for NIH3T3, 5×10^6 cells for MAEC, and 2×10^7 cells for RAW264.7. Twenty-four hours after cell seeding, the medium was replaced with exosome-depleted medium and incubation was resumed for another 24 h. At the time of harvesting exosomes, the confluency of cells was almost 100% for all the cases examined. Exosomes in the supernatant were purified using a previously described procedure (Takahashi et al., 2013). In brief, cell debris and large vesicles were cleared from the supernatant by sequential centrifugation and filtration using a 0.2 μm filter. Subsequently, the supernatant was subjected to ultracentrifugation at 100,000 $\times g$ for 1 h to sediment the exosomes. The exosomes were washed twice with phosphate-buffered saline (PBS). The amount of collected exosomes was estimated by measuring the protein concentration using the Quick Start Bradford protein assay (BioRad, Hercules, CA,

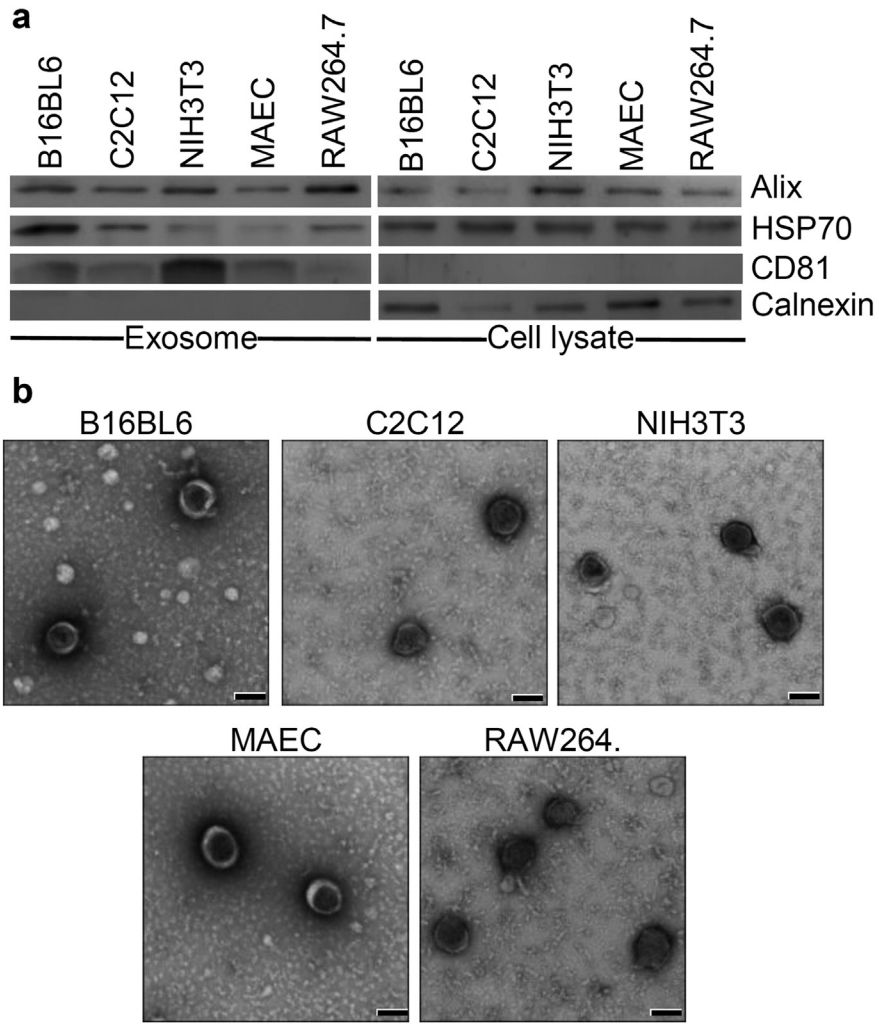

Fig. 1. Collection of exosomes from five different cell types. (a) Western blotting analysis of the Alix, HSP70, CD81, and calnexin present in the exosomes and cell lysates derived from B16BL6, C2C12, NIH3T3, MAEC, and RAW264.7 cells. (b) Transmission electron microscopy images of exosomes derived from B16BL6, C2C12, NIH3T3, MAEC, and RAW264.7 cells. Scale bar = 100 nm.