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Original Article

Identification and analysis of exosomes secreted from macrophages extracted by different methods

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Abstract: Exosomes were small vesicles secreted by many cells, and they can play an important role in cell signal transductions. Because the diameter of exosomes is about 30-100 nm, it is so difficult to collection them. In this paper, three kinds of exosomes purifying methods (density gradient ultracentrifugation method, the ultracentrifugation and ultrafiltration method, ExoQuick™ Extraction kit method) were used to collected exosomes in culture supernatants of macrophages. The morphologies of three kinds of exosomes were analyzed by transmission electron microscopy (TEM), and the characteristic molecules such as CD86, LAMP-1, HSP-70 on exosomes were analyzed with Western blot. In addition, the biological activities of exosomes purified by three kinds of methods *in vitro* were analyzed by ELISA and flow cytometry methods. All experimental results show that the purity and quality of exosomes collected by ExoQuick™ extraction kit and ultracentrifugation and ultrafiltration method were better, and they could enhance the expression of MHC-I in macrophages and promote cells to secrete more TNF-α to cause inflammatory response of macrophages. The analysis pointed out that the advantages and disadvantages of the three methods by biological activities or components of exosomes. Therefore, the ultracentrifugation and ultrafiltration method or the ExoQuick™ Extraction kit method were more suitable to be applied in the scientific research.

Keywords: Exosomes, macrophage, TNF-α, MHC-I

Exosomes are small membranous vesicles formed by inward budding of late endosomes resulting in the formation of multivesicular bodies (MVBs) in the cell cytosol [1]. They are subsequently exocytosed to the extracellular space by fusion with the plasma membrane [2]. Exosomes have been shown to be released by several cell types such as dendritic cells [3], B cells [4], T cells [5], and tumor cells [6] among others. The presence of exosomes has also been shown in human body fluids such as plasma [7], bronchoalveolar lavage [8] and malignant effusions [9].

In general, exosomes participate in the transfer of molecules during immune cell-cell communication [10]. Exosomes secreted by antigen-presenting cells are rich in MHC-I/II and co-stimulatory molecules, and could play immune stimulatory roles such as promoting the proliferation of T lymphocytes [11]. *In vivo*, experiments showed dendritic cells with similar functions, can induce immune response or induce immune tolerance. In addition, exosomes derived from different cells could express some same proteins, such as CD80, CD86, ICAM-3, HSP-70, LAMP-1 and so on [12]. According to these characteristics proteins, exosomes may be initially determined, and LAMP-1 can be expressed stably in exosomes, so it was often used as a positive control in exosomes studies [13]. At present, more studies have shown that exosomes carrying tumor antigens could promote antigen-specific T cells activation and tumor rejection *in vivo* [14]. *In vitro*, exosomes could stimulate DCs to produce cytokines IFN-γ, TNF-α to cause inflammatory response, and also can enhance the expression of cell surface molecules in DCs such as MHC-I or MHC-II, which could activate T lymphocytes resulting in cell immunization further [15]. As new acellular vaccines, exosomes in the anti-tumor immunotherapy, the inhibition of graft rejection, autoimmune diseases, and other aspects of the application prospects are of great concerns [16].

Because of the diameter of exosomes is between 30~100 nm, exosomes purifying is quite difficult [17]. At present, exosomes extraction and preparation techniques mainly rely on the traditional method (density gradient ultracentrifugation method), but this method is unable to collect high pure exosomes, which the diameter of vesicles is out of 30~100 nm [18].

Therefore, we need to improve traditional methods or using new methods to purify pure exosomes to meet the needs of exosomes experiments. In this study, three experimental methods, the density gradient ultracentrifugation method, the ultracentrifugation and ultrafiltration method, ExoQuick™ Extraction kit method were utilized to purify exosomes to analyze their vitalities and provide a better or an economic method for exosomes research.

Materials and methods

Materials

The human acute monocytic leukemia cell line THP-1 was purchased from the Center for Type Culture Collection of Wuhan University (Wu Han, China). Phorbol 12-myristate 13-acetate (TPA) was purchased from Sigma-Aldrich Company (USA) and dissolved in DMSO, kept at -20°C and used at a final concentration of 0.1 mM. CD86, LAMP-1, HSP-70 antibodies were purchased from Kuang Bo biotechnology company (Beijing, China). Cocktail protease inhibitors, sucrose, and phosphotungstic acid were all purchased from GenScript biotechnology company (Nanjing, China).

Macrophages cultures

THP-1 cells were cultured in six well plates or flasks at 37°C and under 5% CO_2, in RPMI 1640-GlutaMAX™ containing 10% (v/v) fetal bovine serum, 100 U/mL penicillin, 0.1 mg/mL streptomycin and 0.25 μg/mL amphotericin B. Differentiation of these cells into macrophage-like cells was induced with 0.1 mM TPA for 24 h.

Exosome isolation

Method-1: All procedures were carried out as follow at 4°C [17]. Cell culture supernatants (208 ml) were centrifuged at 18,000 g for 20 min in order to remove cells and cell debris. The supernatants were first centrifuged at 46,300 g for 45 min and finally at 84,000 g for 60 min. Then the pellets were resuspended with 200 μL of 9% sucrose containing protease inhibitors and centrifuged at 100,000 g for 45 min. The exosomes pellets were resuspended with 25 μL of 9% sucrose containing protease inhibitors, stored at -80°C, and exosomes purified by method-1 were named exosomes-1.

Method-2: Exosomes were isolated and purified as described previously [18]. Cell culture supernatants (208 ml) were centrifuged at 1,000 *g* for 10 min, then supernatants were added to ultrafiltration centrifuge tubes of 100,000 MWCO, and were centrifuged at 1,000 *g* for 30 min to obtain the concentrated liquid containing exosomes. Hereafter, the concentrating solution was transferred to sucrose/D_2O density pad (1.210 g/cm^3) of 300 g/L, then was centrifuged at 100,000 *g* for 60 min. The cushion in the tubes (including exosomes) was collected to be diluted with PBS and placed in the ultrafiltration tubes of 100,000 MWCO, then centrifuged at 1,000 *g* for 30 min, washed three times. Finally, exosomes were filtered with 0.22 μm membrane to remove large vesicles , and exosomes pellets were resuspended with 25 μL of 9% sucrose containing protease inhibitors, stored at -80°C. All procedures were carried out at 4°C, and these exosomes were named exosomes-2.

Method-3: Processes as follows were provided by the reference [19], Cell culture supernatants (208 ml) were centrifuged at 3,000 *g* for 15 minutes to remove cells and cell debris, then supernatants were transferred to sterile tubes. The appropriate ExoQuick Exosome Precipitation Solution was added to cell culture supernatants, and tubes were mixed by inverting, then were refrigerated for 30 minutes. ExoQuick/biofluid mixture was centrifuged at 1,500 *g* for 30 minutes, and aspirated supernatant. Spin down residual ExoQuick solution was centrifuged at 1,500 *g* for 5 minutes to remove all traces of fluid by aspiration. Finally, exosomes pellets were resuspend in 1/10 of original volume using nuclease-free water. The exosomes pellets were resuspended with 25 μL of 9% sucrose containing protease inhibitors, stored at -80°C. All procedures were carried out at 4°C and exosomes named exosomes-3.

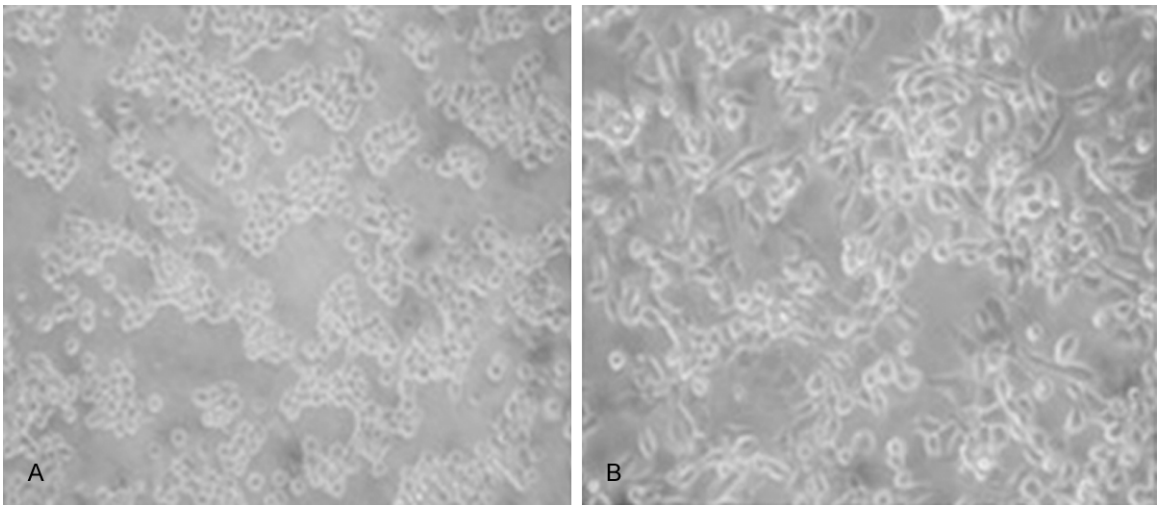

Figure 1. THP-1 cells were cultured and macrophage differentiation (× 40). A: Human monocytic leukemia cell line THP-1; B: THP-1 differentiated into macrophages with 0.1 mM TPA.

Table 1. Amount of exosomes obtained from different methods

Condition	Approximate cell number/Volume of RPMI 1640 media	Concentration (Bradford assay)	Time-consuming
Method-1	4×10^9/208 ml	1.09 μg/μL	170 min
Method-2	4×10^9/208 ml	1.38 μg/μL	130 min
Method-3	4×10^9/208 ml	1.82 μg/μL	50 min

Exosomes identification by TEM

Three kinds of fresh exosomes were stained with 3% phosphotungstic acid for 5 min, placed on a copper grid, dried at 65°C and observed on a Hitachi H-600 transmission electron microscope at 70,000 magnification.

Analysis of exosomes purity

The diameter of exosomes was 30~100 nm, so the diameter of three kinds of exosomes in images of TEM were analyzed. The purities of three kinds of exosomes were analyzed according to statistical data.

Analysis of exosomes collection methods

Exosomes in 208 ml cell culture supernatants were collected by three kinds of collection methods respectively. The whole collecting process of method-1 need 170 min, method-2 needs 130 min, while method-3 only demands 50 min. Exosomes obtained by three kinds of methods were dissolved in 25 μL of 9% sucrose solution containing protease inhibitors, and three kinds of exosomes concentration were 1.09 μg/μL, 1.38 μg/μL and 1.82 μg/μL measured by Bradford method.

Western blot

Proteins of macrophages were extracted with protein extraction kits and the protein concentration was measured by Bradford method; then 50 μg proteins were separated by gel electrophoresis of 10% SDS-PGAE and were transferred to PVDF membrane. Rabbit-anti-mouse antibodies of D86, LAMP-1, HSP-70 were used as the first antibodies, and horseradish peroxidase-labeled were used as secondary antibodies for the immune response.

Cytokine quantification by ELISA

Macrophages were treated with three kinds of exosomes at 20 μg or 40 μg respectively, for 48 h. Aliquots from the cell culture supernatants were used to analyze TNF-α by ELISA, according to the kit manufacturer's instructions. Cytokine concentrations were determined with reference to standard curves.

Flow cytometry analysis of MHC-I

Macrophages were cultured for 48 h treated with three kinds of exosomes at 20 μg or 40 μg respectively, for 48 h, then harvested and

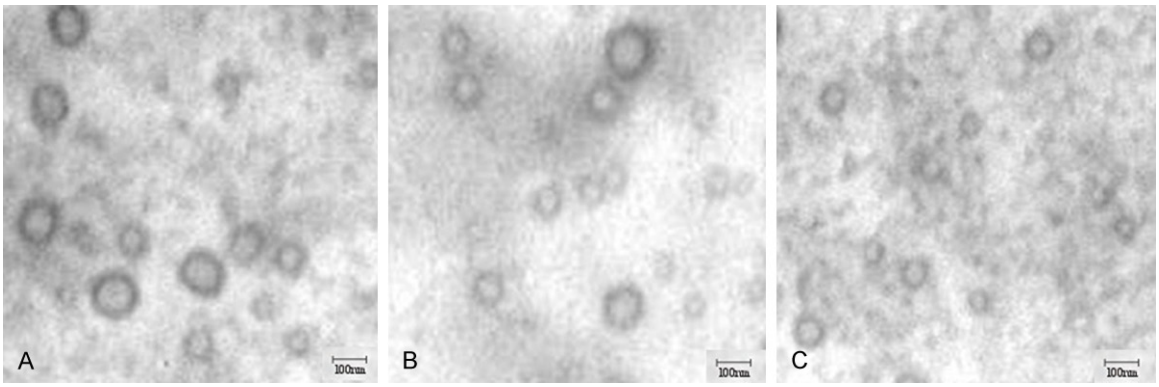

Figure 2. Exosomes as observed by TEM (× 100000). A: Exosomes purified by method-1; B: Exosomes purified by method-2; C: Exosomes purified by method-3.

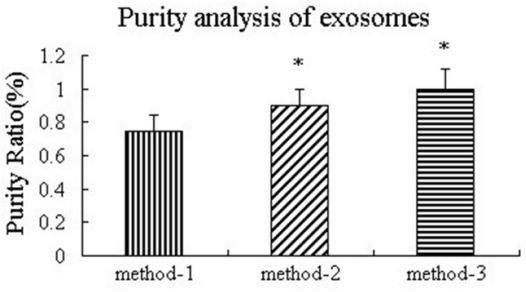

Figure 3. The diameter of exosomes collected by three methods in images of TEM were analyzed by statistical methods. Results are presented as means ± S.D of three independent experiments. All data were analyzed by SPSS 16.0. Tests were 2-sided and *P* values < 0.05 were considered as significant.

washed twice with PBS containing 0.2% BSA. Cells were then incubated with MHC-I-FITC antibody for 30 min in the dark, on ice. After washed twice with PBS, cells were pelleted and resuspended to a concentration of 10^6 cells/mL with PBS-0.2% BSA. Cell analysis and sorting were performed on a FACS (Beckman MOFLO XDP, USA).

Statistical analysis

Within each experiment, triplicates were performed for each condition or group. Data were analyzed by SPSS 16.0 and are presented as means ± SE of at least 3 independent experiments. Differences within each group were subjected to t-test or q-test. Statistically significant differences (*$P \leq 0.05$) between the groups being compared are indicated by asterisks.

Results

THP-1 cells differentiated into macrophages

THP-1 cells were cultured for 48 h, and then THP-1 cells were induced to differentiate into macrophages-like cell with 0.1 mM TPA for 24 h (**Figure 1**).

Analysis of the quality of exosomes obtained from different methods

The quality of exosomes obtained from three different methods was did statistical analysis by three indicators approximate cell number/Volume of RPMI 1640 media, concentration (Bradford assay) and time consuming respectively to judge the advantages and disadvantages of three kinds of separation methods (**Table 1**).

TEM identification of exosomes

Exosomes from macrophages obtained by three kinds of methods revealed a homogenous population of morphologically typical vesicles of 30 to 100 nm diameter (**Figure 2**), similar in appearance and size to those in previous reports.

Analysis of exosomes purity

The diameter of 200 vesicles collected by three methods from images of TEM was analyzed by statistical software (**Figure 3**). The statistical results indicated that the purity of exosomes-3 was the best, but the result of exosomes-1 was unsatisfactory.

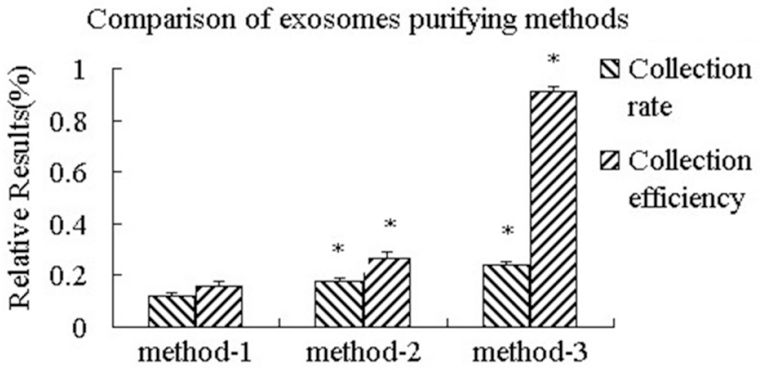

Figure 4. Analysis of exosomes purified by three kinds of methods. Results are presented as means ± S.D of three independent experiments. All data were analyzed by SPSS 16.0. Tests were 2-sided and *P* values < 0.05 were considered as significant.

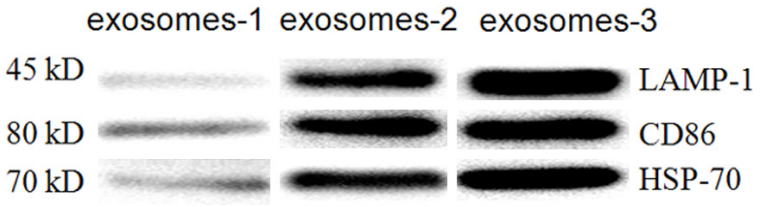

Figure 5. CD86, LAMP-1, HSP-70 expressed in exosomes secreted from macrophages, which were purificated by three kinds of methods and analyzed by Western blot.

Analysis of exosomes collection methods

The collection rate and collection efficiency of three kinds of exosomes purifying methods were analyzed, according to exosomes collection time and the amount of exosomes by statistical software (**Figure 4**). The results showed that the collection rate and collection efficiency of method-3 were the highest, and the following was method-2.

Western blot analysis of CD86, LAMP-1 and HSP-70 in exosomes

CD86, LAMP-1, HSP-70 almost expressed in all kinds of exosomes secreted from different cells, so these three proteins can be used as the characteristic proteins for exosomes research. CD86, LAMP-1 and HSP-70 in exosomes purificated by three kinds of methods were analyzed by Western blot (**Figure 5**). Results revealed that CD86, LAMP-1, HSP-70 in exosomes-3 expressed highest, but they expressed least in exosomes-1.

TNF-α secretion from macrophages treated with exosomes

Macrophages were seeded in a six-well plate and were left untreated or treated with three kinds of exosomes at 20 μg/ml and 40 μg/ml respectively for 48 h. The cell culture supernatants were tested for TNF-α levels by ELISA (**Figure 6**). Macrophages stimulated with exosomes-2 and exosomes-3 secreted more TNF-α.

Flow cytometry analysis of MHC-I in macrophages treated with exosomes

In order to confirm which kind of exosomes could enhance the expression of MHC-I, macrophages seeded in a six-well plate were left untreated or treated with three kinds of exosomes at 20 μg/ml and 40 μg/ml for 48 h, and the expression of MHC-I in macrophages was analyzed by flow cytometry (**Figure 7**). The results showed that MHC-I levels increased higher in macrophages stimulated with exosomes-2 and exosomes-3 than with exosomes-1.

Discussion

Exosomes used to therapy cancer has become a research hotspot, but the exosomes purifying method is a key technology for the immunotherapy [19]. Currently, exosomes extraction methods can be summarized into three categories broadly; 1: density gradient ultracentrifugation method, 2: ultracentrifugation and ultrafiltration binding assay, 3: exosomes extraction kit method. Conventional exosomes extraction methods (method-1) often remove cells and debris by high speed centrifugation and then ultracentrifugated with 9% sucrose solution as the density gradient medium to obtain exosomes [18]. This traditional method is simple, but requires low-temperature high-speed centrifugation and ultracentrifugation. At present, because of the diameter of exosomes was

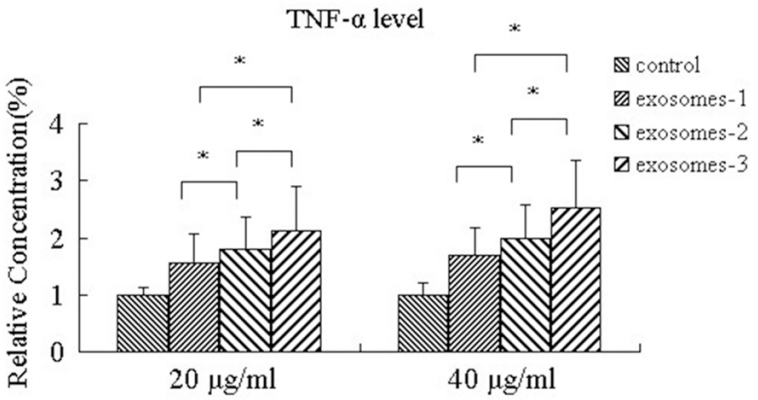

Figure 6. Macrophages were treated with three kinds of exosomes at 20 μg/ml and 40 μg/ml for 48 h and culture supernatants were analyzed for TNF-α by ELISA. Results are presented as means ± S.D of three independent experiments. All data were analyzed by SPSS 16.0. Tests were 2-sided and *P* values < 0.05 were considered as significant.

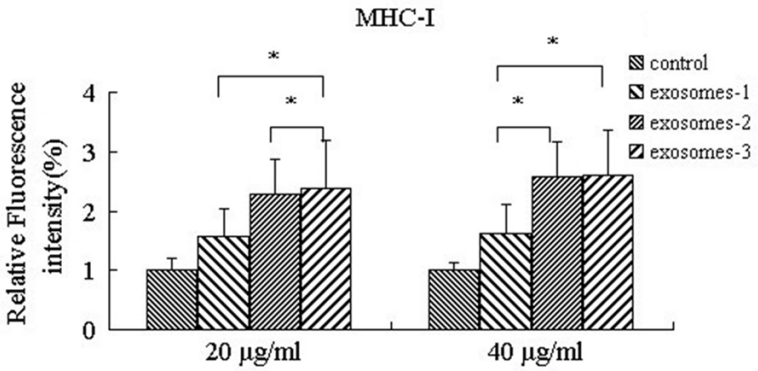

Figure 7. Macrophages were treated with three kinds of exosomes at 20 μg/ml and 40 μg/ml for 48 h, then macrophages were analyzed by flow cytometry for the expression of MHC-I. Results are presented as means ± S.D of three independent experiments. All data were analyzed by SPSS 16.0. Tests were 2-sided and *P* values < 0.05 were considered as significant.

between 30~100 nm, researchers developed membrane filtration technology to separate exosomes, which could expand the research and the application of exosomes effectively. Tubes with 0.1 μm filter membrane can remove components of the diameter which was greater than 100 nm, and the diameter of filter elements which was less than 50 nm could be filtered by 0.05 μm membrane [20]. However, traditional ultra-high-speed density gradient centrifugation and centrifugal filtration membrane were combined in method-2 to prepare exosomes. In this study, results of TEM showed that about 85% of microcapsules obtained by method-1 were exosomes, which the size, morphology and ultrastructure were similar with exosomes, 95% of microcapsules in method-2 were exosomes and exosomes were nearly 100% in method-3, for exosomes were micro-membrane capsule structure, spherical or oval shape, the cavity of vesicles were low electron density components [21]. In addition, all kinds of exosomes purifying data such as time-consuming, quality, contracention were collected to analyze the collection rate and collection efficiency. These three methods results manifest that the purity and efficiency of exosomes extracted by these three ways were still considerable different, which the collection rate and collection efficiency of method-1 was the best, but the worst was method-1.

LAMP-1, CD86, HSP-70 expressed in exosomes could induce the immune response or inflammatory response of macrophages to activate T lymphocytes or mediate information transfer between cells to cells. Characteristics proteins LAMP-1, CD86, HSP-70 in exosomes were analyzed by western blot, and results show that the expressions of LAMP-1, CD86, and HSP-70 in three kinds of exosomes were consistent with their purity and efficiency.

In recent studies, exosomes secreted from *mycoplasma* infected tumor cells could activate the inhibitory B-lymphocytes, to play a role in humoral immunity; in addition B cells can also activate T cells by secreting exosomes [22, 23]. Patients with sarcoidosis, a granulomatous disease of the lung, secrete more exosomes into the BAL fluid, compared with healthy controls, and these exosomes were able to increase the expression of MHC-I/II, CD9 and CD86, as well as increased proinflammatory activity [24]. Mature DCs are characterized by an enhanced expression of type I and II molecules of the MHC and co-stimulatory molecules (CD80, CD86, CD40), which enable them to prime naıve T cells [25]. In addition to their role as APCs, DCs can direct the T cell response

towards Th1 or Th2 phenotypes. Thus, the capacity of DC to produce IL-12, TNF-α and IFN-α that can enhance the secretion of IFN-g by T cells and NK cells contributes to perpetuation of a Th1-biased response [26-28]. In this paper, exosomes purified by three kinds of methods enhanced MHC-I in macrophages to activate T lymphocytes and promote the secretion of TNF-α to enhance the inflammatory effect of macrophage. However, exosomes purified by method-2 and method-3 initiated intensely immunostimulatory effect of macrophage, so these results indicated that the purity of exosomes purified by method-2 and method-3 was better than method-1, therefore could lead to cellular immune effect strongly.

In summary, experimental results manifested that the process of exosomes purified by method-2, 3 were relatively short and simple, and the immunization of their products was better. But the cost of extraction kit is more expensive and is incapable to collect a large number of exosome to meet the need of experiments or clinic therapy. In contrast, the cost-effective of method-2 was the most, and could help the large-scale extraction of exosomes, so it was suitable for large-scale development and application for exosomes vaccine, in future.

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Disclosure of conflict of interest

None.

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References

[1] Schorey JS, Bhatnagar S. Exosome function: from tumor immunology to pathogen biology. Traffic 2008; 9: 871-81.

[2] Vlassov AV, Magdaleno S, Setterquist R, Conrad R. Exosomes: Current knowledge of their composition, biological functions, and diagnostic and therapeutic potentials. Biochim Biophys Acta 2012; 1820: 940-48.

[3] Segura E, Guérin C, Hogg N, Amigorena S, Théry C. CD8+ dendritic cells use LFA-1 to capture MHC-peptide complexes from exosomes in vivo. J Immunol 2007; 179: 1489-96.

[4] Laulagnier K, Motta C, Hamdi S, Roy S, Fauvelle F, Pageaux JF, Kobayashi T, Salles JP, Perret B, Bonnerot C, Record M. Mast cell- and dendritic cell-derived exosomes display a specific lipid composition and an unusual membrane organization. Biochem J 2004; 380: 161-71.

[5] Woodworth JS, Wu Y, Behar SM. Mycobacterium tuberculosis-specific CD8+ T Cells require perforin to kill target cells and provide protection in vivo. J Immunol 2008; 181: 8595-603.

[6] Zech D, Rana S, Büchler MW, Zöller M. Tumor-exosomes and leukocyte activation: an ambivalent crosstalk. Cell Commun Signal 2012; 10: 37.

[7] Keller S, Ridinger J, Rupp AK, Janssen JW, Altevogt P. Body fluid derived exosomes as a novel template for clinical diagnostics. J Transl Med 2011; 9: 86.

[8] Torregrosa Paredes P, Esser J, Admyre C, Nord M, Rahman QK, Lukic A, Rådmark O, Grönneberg R, Grunewald J, Eklund A, Scheynius A, Gabrielsson S. Bronchoalveolar lavage fluid exosomes contribute to cytokine and leukotriene production in allergic asthma. Allergy 2012; 67: 911-19.

[9] Bard MP, Hegmans JP, Hemmes A, Luider TM, Willemsen R, Severijnen LA, van Meerbeeck JP, Burgers SA, Hoogsteden HC, Lambrecht BN. Proteomic analysis of exosomes isolated from human malignant pleural effusions. Am J Respir Cell Mol Biol 2004; 31: 114-21.

[10] Segura E, Guérin C, Hogg N, Amigorena S, Théry C. CD8+ dendritic cells use LFA-1 to capture MHC-peptide complexes from exosomes in vivo. J Immunol 2007; 179: 1489-1496.

[11] Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, Tenza D, Ricciardi-Castagnoli P, Raposo G, Amigorena S. Eradication of established murine tumors using a novel cell-free vaccine: dendritic cell-derived exosomes. Nat Med 1998; 4: 594-600.

[12] Hosseini HM, Fooladi AA, Nourani MR, Ghanezadeh F. The role of exosomes in infectious diseases. Inflamm Allergy Drug Targets 2013; 12: 29-37.

[13] Giri PK, Kruh NA, Dobos KM, Schorey JS. Proteomic analysis identifies highly antigenic proteins on exosomes from *M. tuberculosis*-infected and culture filtrate protein-treated macrophages. Proteomics 2010; 10: 3190-202.

[14] O'Neill SK, Cao Y, Hamel KM, Doodes PD, Hutas G, Finnegan A. Expression of CD80/86 on B cells is essential for autoreactive T cell activation and the development of arthritis. J Immunol 2007; 179: 5109-16.
[15] Gutiérrez-Vázquez C, Villarroya-Beltri C, Mittelbrunn M, Sánchez-Madrid F. Transfer of extracellular vesicles during immune cell-cell interactions. Immunol Rev 2013; 251: 125-42.
[16] Chaput N, Taïeb J, Schartz N, Flament C, Novault S, André F, Zitvogel L. The potential of exosomes in immunotherapy of cancer. Blood Cells Mol Dis 2005; 35: 111-5.
[17] Johnstone RM, Adam M, Hammond JR, Orr L, Turbide C. Vesicle formation during reticulocyte maturation. Association of plasma membrane activities with released vesicles (exosomes). J Biol Chem 1987; 262: 9412-20.
[18] Tang LJ, De Seta F, Odreman F, Venge P, Piva C, Guaschino S, Garcia RC. Proteomic analysis of human cervical-vaginal fluids. J Proteome Res 2007; 6: 2874-83.
[19] Qu JL, Qu XJ, Zhao MF, Teng YE, Zhang Y, Hou KZ, Jiang YH, Yang XH, Liu YP. Gastric cancer exosomes promote tumour cell proliferation through PI3K/Akt and MAPK/ERK activation. J Dig Liver Dis 2009; 41: 875-80.
[20] Taylor DD, Zacharias W, Gercel-Taylor C. Exosome isolation for proteomic analyses and RNA profiling. Methods Mol Biol 2011; 728: 235-46.
[21] Griffiths RE, Heesom KJ, Anstee DJ. Normal prion protein trafficking in cultured humanerythroblasts. Blood 2007; 110: 4518-25.
[22] Wubbolts R, Leckie RS, Veenhuizen PT, Schwarzmann G, Möbius W, Hoernschemeyer J, Slot JW, Geuze HJ, Stoorvogel W. Proteomic and biochemical analyses of human B cell-derived exosomes. Potential implications for their function and multivesicular body formation. J Biol Chem 2003; 278: 10963-72.
[23] Yang C, Chalasani G, Ng YH, Robbins PD. Exosomes released from Mycoplasma infected tumor cells activate inhibitory B cells. PLoS One 2012; 7: e36138.
[24] Qazi KR, Torregrosa Paredes P, Dahlberg B, Grunewald J, Eklund A, Gabrielsson S. Proinflammatory exosomes in bronchoalveolar lavage fluid of patients with sarcoidosis. Thorax 2010; 65: 1016-24.
[25] Steinman RM. Lasker Basic Medical Research Award. Dendritic cells: versatile controllers of the immune system. Nat Med 2007; 13: 1155-9.
[26] Demangel C, Britton WJ. Interaction of dendritic cells with mycobacteria: where the action starts. Immunol Cell Biol 2000; 78: 318-24.
[27] Lande R, Giacomini E, Grassi T, Remoli ME, Iona E, Miettinen M, Julkunen I, Coccia EM. IFN-alpha beta released by Mycobacterium tuberculosis-infected human dendritic cells induces the expression of CXCL10: selective recruitment of NK and activated T cells. J Immunol 2003; 170: 1174-82.
[28] Lanzavecchia A, Sallusto F. Regulation of T cell immunity by dendritic cells. Cell 2001; 106: 263-66.