RESEARCH ARTICLE

Human T cell derived exosome, natural nano-particles, elicits anti-tumor effect on human solid tumor cells in vitro

Mohammad Saeed Kahrizi [1], Shirin Fattahi [2], Mahdis Kashian [3], Mohammed Adel Jasim[4], Mahdi Heydari [5], Bahareh Tavakoli-Far [6], Mehran Ebrahimi Shah-abadi [7]

[1] Department of Surgery, Alborz University of Medical Sciences, Karaj, Alborz, Iran.
[2] Department of Oral and Maxillofacial Pathology,Dentistry Faculty,Tabriz University of Medical Sciences,Tabriz,Iran.
[3] Department of Obstetrics and Gynecology, Medical college of Iran university, Tehran, Iran.
[4] Anesthesia technology department, Al-Turath University College , Al Mansour, Baghdad, Iraq.
[5] Department of Anatomical Sciences, School of Medicine, Iran University of Medical Sciences, Tehran, Iran.
[6] Dietary Supplements and Probiotic Research Center, Alborz University of Medical Sciences, Karaj, Iran.
[7] Department of surgery, afzalipour hospital, kerman university of medical sciences, kerman, iran.

ARTICLE INFO

Article History:
Received 11 Oct 2022
Accepted 21 Oct 2022
Published 15 Feb 2023

Keywords:
T cell
Exosome
Cancer
Immunotherapy

ABSTRACT

Aims: To evaluate the anti-tumor effect of the T cell-exosome on breast cancer MCF-7, lung cancer A549 and liver carcinoma HepG2 cells.
Methods: Human T cell derived exosomes were isolated from T cell using ultracentrifugation. The expression of the CD9 and CD81 in T cell and T cell-derived exosome, and exosome morphology was assessed using western blotting and TEM image, respectively. The anti-cancer effect of the exosome on cancer cell proliferation was measured using MTT assay. Also, the apoptotic cell percentages in treated cells were assessed by Annexin/PI staining and flowcytometry.
Results: According to results, exosome therapy resulted in a reduction in the viability of the MCF-7, lung cancer A549 and liver carcinoma HepG2 cells within 12, 24, 48 and 72 hours of treatment. As well, exposure with exosomes resulted in an improvement in the apoptosis of the all cell lines within 48 hours of treatment.
Conclusion: Concerning the results, T cell derived exosome could be an effective plan for treating human solid tumors.

How to cite this article
Kahrizi M. S., Fattahi S., Kashian M., Jasim M. A., Heydari M., Tavakoli-Far B., Shah-abadi M. E., Human T cell derived exosome, natural nano-particles, elicits anti-tumor effect on human solid tumor cells in vitro. Nanomed Res J, 2023; 8(1): 89-93. DOI: 10.22034/nmrj.2023.01.008

INTRODUCTION

Different microenvironment parts of human tumors, like soluble mediators, tumor suppressive cells, and amended extracellular matrix (ECM) inspire tumor development and invasion [1, 2]. These mechanisms also may prevent effective antitumor effects of the immune cells. Numerous stromal cells usually are detected in tumor microenvironment (TME) and participate in the estimating clinical outcomes [3]. These cells are largely described by altered molecular mechanism and deregulated signaling pathways [4, 5]. Now, tumor immunotherapy has become an efficient approach for targeting transformed cells. Immunotherapy avoids immune cell dysfunctions and consequently supports the anti-cancer impacts of immune cells [6, 7]. Meanwhile, using immune cells or derivate exosomes has attracted increasing attention. Accumulating proofs have showed that immune cell-derived exosomes facilitate connections between innate and adaptive immunity and thereby inhibit tumor progression [8].

Exosomes are typically secreted by a variety of human cells with 30–150 nm in diameter [9, 10]. They convey biological molecules such

* Corresponding Author Email: *baharehtavakolifar@gmail.com*

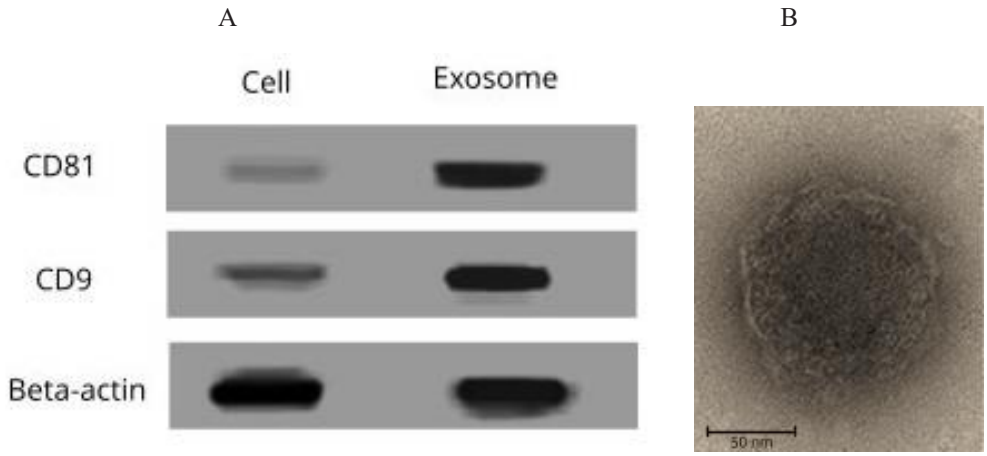

Fig. 1. The western blotting (A) and TEM image (B).

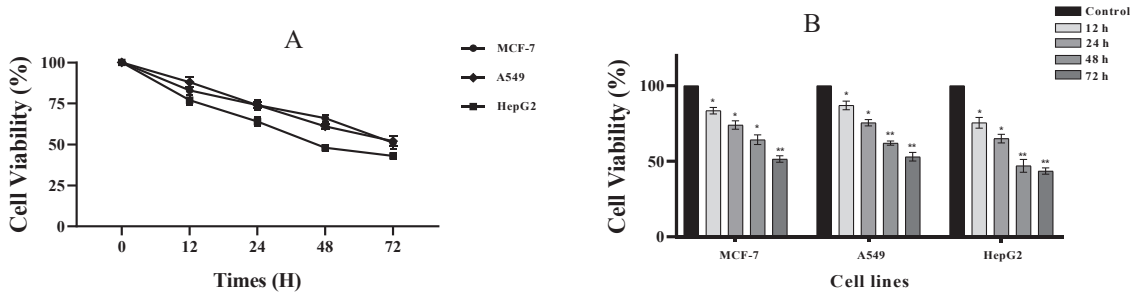

Fig. 2. MTT assay consequences of tumor cell treatment with T cell-derived exosome.

as protein and microRNAs. Through targeting a wide spectrum of procedures, immune cell-exosome prohibit cancer cell progression [11, 12]. Nonetheless, T cell- exosomes acts a dual role sometimes and may target anti-tumor functions of the other immune cells [13]. Reports have exhibited that human immune cells can secret exosomes with either stimulatory and tolerogenic possessions into the TME, conferring the importance of their future application in tumor treatment. In sum, they offer unique strategy for cancer diagnosis and therapy.

Herein, we evaluate the anti-tumor influences of the T cell derived exosomes on human breast cancer cell line MCF-7, lung cancer A549 and liver carcinoma HepG2 cells.

MATERIALS AND METHODS

Cell culture

We used healthy human donor derived blood samples to attain T cell. In brief, PBMCs were primarily procured by employing the centrifugation using the ficoll density gradient. Then, T-cells were procured by magnetic cell sorting (MACS) based on the producer instructions. Isolate T cell then were expanded in RPMI-1640 media containing the 10% FBS, and 1% pen/strep all acquired from Sigma-Aldrich, Germany.

The MCF-7, A549 and HepG2 cells (ATCC), were expanded in DMEM containing the FBS 10% and 1% pen/strep. Then, cancer cells along with the procured T cells were kept in special conditions (5% CO2 at 37 °C).

Exosome isolation

T cell-exosome was acquired from the CM using the MagCapture™ Exosome Isolation Kit with respect to the producer recommendation. The CM was centrifuged by UC at 100,000 × g for 90 min. Then, suspension of the achieved pellet was conducted in PBS and RPMI 1640 medium.

Western blotting

The CD81 and CD9 expression was assessed in T cells and also their exosomes. Cells were lysed using the RIPA buffer (BioLegend, USA) and directed to PVDF. By specific primary and

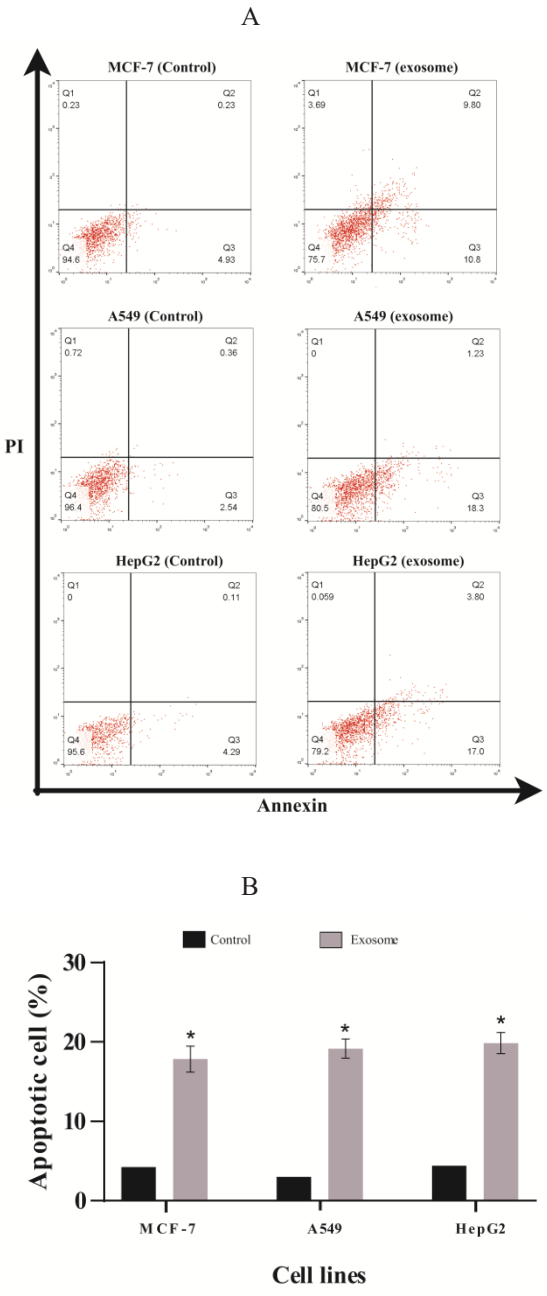

Fig. 3. The apoptosis percentages in MCF-7, HepG2 and A549 cell treated with exosome 100ng/ml within 48 hours of treatment.

secondary antibody obtained from "Abcam, UK", CD81 and CD9 expression was assessed.

MTT assay

To evaluate the anti-tumor impacts of exosome isolated from T cells on MCF-7, A549 and HepG2 cells, firstly 1×10^5 cells (100 µL) were cultured within 96-well plates. Upon, treatment with 100 ng/ml concentrations of exosomes at 12- 72 hours of exposure, 5 µL of 5-10 mg MTT/ml was added to each well. Finally, the wells OD then estimated at 570 nm using ELISA reader.

Flow cytometric analysis of apoptosis

Annexin V Apoptosis Detection Kit with PI (Biolegend, USA) was applied to evaluate the cytotoxicity of exosome on tumor cells apoptosis upon cell incubation with T cell-exosome (100 ng/ml) at 48 hours of exposure. Then, 5 µL of PI and FITC- Annexin-V were used. Finally, the fluorescent emission was detected.

Transmission electron microscopy (TEM)

To analyze the exosome' morphology, the procured exosomes were evaluated by TEM. For negative staining, the exosomes suspension in PBS accomplished and then exosomes put on carbon-coated grids to conduct electron microscopy.

Statistical Analysis

Statistical analysis was performed by GraphPad Prism. The results were presented as means ± SEM from 3 or 4 separate experiments. The statistical differences determined by Student's t-test.

RESULT AND DISCUSSION

Characterizing of Exosome

Western blotting examination was executed to evaluate the expression of CD9 and CD81 on T cell-exosomes and also the morphology of exosomes (Fig. 1 A, B).

T cell-exosome inhibits cancer cells proliferation

Concerning the MTT assay results, exosomes 100 ng/ml decreased the viability of MCF-7, A549 and HepG2 cell lines during 12-72 hours of incubation ($P<0.05$) (Fig. 2). Respecting to outcomes, the exosomes-exerted inhibitory influences on cell viability were more evident within 72 hours of treatment and higher concentration ($P<0.05$) (Fig. 2). Further, the inhibitory effect on HepG2 cells were more evident than other cells, MCF-7 and A549.

The exosomes isolated from induced T-cell may improve the proliferation neighboring immune cells, thus inspiring the anti-tumor effects [14, 15]. Exosomes through the transporting of the microRNAs and other molecules modify the biological process in target cells [16, 17]. Immunological synapses could evoke the

conveyance of exosome among immune cells such as T-cell and APCs [18]. Further, T cell-exosomes can trigger ERK and NF-κB axes in malignant cells thus inspiring tumorigenesis expression [19]. Furthermore, tumor-derived exosome mainly suppress CD8+ T cell activation and induces its apoptosis and exhaustion [20, 21].

T cell-exosome induces MCF-7, HepG2 and A549 cells apoptosis

The apoptosis percentages of all cell lines were assessed following exposure with exosome (100 ng/ml) within 48 h of treatment with by flowcytometry. In this light, exosoms therapy caused a marked increase in the apoptosis percentages of MCF-7, HepG2 and A549 cell within 48 hours of exposure ($P<0.05$) (Figs. 3A, B). The apoptosis percentages in MCF-7 was 19.38±1.88, in HepG2 was 18.84±2.04 and in A549 cell was 21.17±2.06 (Figs. 3A, B).

CONCLUSION

Rendering the achieved outcomes, human T cell derived exosome is capable of exerting anti-tumor effects in vitro. However, study of the proteome of T cell-exosome is required to elucidate the underlying mechanism. Also, evolving the novel potency test is of paramount importance to enable its translation to clinic.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Wu Y, Deng W, Klinke DJ, 2nd. Exosomes: improved methods to characterize their morphology, RNA content, and surface protein biomarkers. Analyst, 2015;140(19):6631-42. https://doi.org/10.1039/C5AN00688K
2. Whiteside T. The tumor microenvironment and its role in promoting tumor growth. Oncogene, 2008;27(45):5904-12. https://doi.org/10.1038/onc.2008.271
3. Masucci MT, Minopoli M, Carriero MV. Tumor associated neutrophils. Their role in tumorigenesis, metastasis, prognosis and therapy. Frontiers in oncology, 2019;9:1146. https://doi.org/10.3389/fonc.2019.01146
4. Guo S, Deng C-X. Effect of stromal cells in tumor microenvironment on metastasis initiation. International journal of biological sciences, 2018;14(14):2083. https://doi.org/10.7150/ijbs.25720
5. Eiro N, Gonzalez LO, Fraile M, Cid S, Schneider J, Vizoso FJ. Breast cancer tumor stroma: cellular components, phenotypic heterogeneity, intercellular communication, prognostic implications and therapeutic opportunities. Cancers, 2019;11(5):664. https://doi.org/10.3390/cancers11050664
6. Xiang X, Wang J, Lu D, Xu X. Targeting tumor-associated macrophages to synergize tumor immunotherapy. Signal transduction and targeted therapy, 2021;6(1):1-12. https://doi.org/10.1038/s41392-021-00484-9
7. Zhao L-P, Zheng R-R, Huang J-Q, Chen X-Y, Deng F-A, Liu Y-B, et al. Self-delivery photo-immune stimulators for photodynamic sensitized tumor immunotherapy. ACS nano, 2020;14(12):17100-13. https://doi.org/10.1021/acsnano.0c06765
8. Xu Z, Zeng S, Gong Z, Yan Y. Exosome-based immunotherapy: a promising approach for cancer treatment. Molecular cancer, 2020;19(1):1-16. https://doi.org/10.1186/s12943-020-01278-3
9. Kouhbanani MAJ, Sadeghipour Y, Sarani M, Sefidgar E, Ilkhani S, Amani AM, et al. The inhibitory role of synthesized Nickel oxide nanoparticles against Hep-G2, MCF-7, and HT-29 cell lines: the inhibitory role of NiO NPs against Hep-G2, MCF-7, and HT-29 cell lines. Green Chemistry Letters and Reviews, 2021;14(3):444-54. https://doi.org/10.1080/17518253.2021.1939435
10. Nasirmoghadas P, Mousakhani A, Behzad F, Beheshtkhoo N, Hassanzadeh A, Nikoo M, et al. Nanoparticles in cancer immunotherapies: An innovative strategy. Biotechnology progress, 2021;37(2):e3070. https://doi.org/10.1002/btpr.3070
11. Zhang X, You Jm, Dong Xj, Wu Y. Administration of mircoRNA‐135b‐reinforced exosomes derived from MSCs ameliorates glucocorticoid‐induced osteonecrosis of femoral head (ONFH) in rats. Journal of cellular and molecular medicine, 2020;24(23):13973-83. https://doi.org/10.1111/jcmm.16006
12. Xie J-y, Wei J-x, Lv L-h, Han Q-f, Yang W-b, Li G-l, et al. Angiopoietin-2 induces angiogenesis via exosomes in human hepatocellular carcinoma. Cell Communication and Signaling, 2020;18(1):1-13. https://doi.org/10.1186/s12964-020-00535-8
13. Zhou Y, Xu H, Xu W, Wang B, Wu H, Tao Y, et al. Exosomes released by human umbilical cord mesenchymal stem cells protect against cisplatin-induced renal oxidative stress and apoptosis in vivo and in vitro. Stem cell research & therapy, 2013;4(2):34. https://doi.org/10.1186/scrt194
14. Tavasolian F, Hosseini AZ, Rashidi M, Soudi S, Abdollahi E, Momtazi-Borojeni AA, et al. The Impact of Immune Cell-derived Exosomes on Immune Response Initiation and Immune System Function. Current pharmaceutical design, 2021;27(2):197-205. https://doi.org/10.2174/18734286MTEyiMTQCy https://doi.org/10.2174/13816128MTEy5MTQmy https://doi.org/10.2174/1381612826666201207221819
15. Yan W, Jiang S. Immune Cell-Derived Exosomes in the Cancer-Immunity Cycle. Trends in cancer, 2020;6(6):506-17. https://doi.org/10.1016/j.trecan.2020.02.013
16. Patwardhan S, Mahadik P, Shetty O, Sen S. ECM stiffness-tuned exosomes drive breast cancer motility through thrombospondin-1. Biomaterials, 2021;279:121185. https://doi.org/10.1016/j.biomaterials.2021.121185
17. Ni C, Fang QQ, Chen WZ, Jiang JX, Jiang Z, Ye J, et al. Breast cancer-derived exosomes transmit lncRNA SNHG16 to induce CD73+γδ1 Treg cells. Signal transduction and targeted therapy, 2020;5(1):41. https://doi.org/10.1038/s41392-020-0129-7
18. Ma F, Vayalil J, Lee G, Wang Y, Peng G. Emerging role of tumor-derived extracellular vesicles in T cell suppression and dysfunction in the tumor microenvironment.

Journal for immunotherapy of cancer, 2021;9(10). https://doi.org/10.1136/jitc-2021-003217
19. Cai Z, Yang F, Yu L, Yu Z, Jiang L, Wang Q, et al. Activated T cell exosomes promote tumor invasion via Fas signaling pathway. Journal of immunology (Baltimore, Md : 1950), 2012;188(12):5954-61. https://doi.org/10.4049/jimmunol.1103466
20. Beccard IJ, Hofmann L, Schroeder JC, Ludwig S, Laban S, Brunner C, et al. Immune suppressive effects of plasma-derived exosome populations in head and neck cancer. Cancers, 2020;12(7):1997. https://doi.org/10.3390/cancers12071997
21. Ye L, Zhang Q, Cheng Y, Chen X, Wang G, Shi M, et al. Tumor-derived exosomal HMGB1 fosters hepatocellular carcinoma immune evasion by promoting TIM-1+ regulatory B cell expansion. Journal for immunotherapy of cancer, 2018;6(1):1-15. https://doi.org/10.1186/s40425-018-0451-6