CYPRUS JOURNAL OF MEDICAL SCIENCES

Review

DOI: 10.5152/cjms.2020.1342

Exosome Mimetic Nanovesicles; Are They Next Best Alternative Therapeutic Approach Combating Cancer?

Cenk Serhan Özverel[1,2]

[1]**Department of Basic Medical Sciences, Near East University School of Dentistry, Nicosia, Cyprus**
[2]**Obesity and Cancer Research Group, DESAM Institute, Near East University, Nicosia, Cyprus**

ORCID iD of the author: C.S.Ö. 0000-0001-9932-4774.

Cite this article as: Özverel CS. Exosome Mimetic Nanovesicles; Are They Next Best Alternative Therapeutic Approach Combating Cancer? Cyprus J Med Sci 2020; 5(4): 361-4.

Cancer is regarded as one of the most dangerous diseases despite the advances in technology and therapeutic strategies against it. The current treatment strategies are ineffective as well as present with various disadvantages, such as drug resistances, ineffective uptake of the therapeutic agents at the tumor site, incompatible delivery of drugs, and immune-rejection, among others. Extracellular vesicles, especially exosome mimetic nanovesicles, have become one of the latest focuses of research in anticancer therapies. The invention of these nano-sized vesicles, which function in cell-to-cell communication, have promoted the development of innovative drug delivery systems due to their cargo-carrying abilities and targeted deliveries. Exosome mimetic nanovesicles have similar surface protein structures to exosomes and offer various important advantages over the exosomes, such as the production yield and isolation protocol. This review aims to summarize the current research studies on exosome mimetic nanovesicles together with their potential in combating cancer in the future.

Keywords: Cancer, cancer therapy, exosomes, nanovesicles

INTRODUCTION

Cancer has been regarded as one of the most dangerous disease affecting people across the world (1). Despite the advancements made in the fields of biotechnology and bioengineering, no effective treatment has yet been established against cancer. Conventional therapeutics, especially chemotherapeutics, are highly known for their abilities of tumoral deoxyribonucleic acid damage, thereby inducing a cell cycle arrest, eventually leading to cell death (2, 3). Although, they are widely being used in the clinical settings, when used aggressively, they offer major drawbacks of chemotherapeutic resistances and damage induction to healthy cells (4). Therefore, these drawbacks direct researchers to search for the next best alternative strategy in terms of effectiveness and reduced cytotoxicity.

Extracellular vesicles secreted between cells are known for their functions in cell-to-cell communication. They are also known for their regulatory activities of the immune system, creating niche for tumor growth, conditioning metastatic sites during tumorigenesis, and facilitating the spread of misfolded proteins in neurodegenerative diseases (5, 6). In addition, these extracellular vesicles are known for their activities of indicating pathogenesis and disease progression owing to their individual protein, peptide, and lipid expression profiles. Furthermore, their ability in cell-to-cell communication enables them to transport signaling molecules and localize at distant tissues, suggesting their potential usage in the development of efficient drug delivery systems (5).

Exosomes and exosome-derived nanovesicles are now considered as the hot topic in drug delivery systems against cancer. Past studies have demonstrated that they possess lower cytotoxicity and higher accuracy when compared to other drug delivery systems and mono-chemotherapies (7, 8).

MATERIAL and METHODS

We conducted aliterature review of studies on exosome mimetic nanovesicles, nanovesicle drug delivery systems, and nanovesicles used in cancer treatment. Data was searched via search engines analyzed widely in terms of the current status and future prospective of nanovesicle-based treatments.

Corresponding Author: Cenk Serhan Özverel
E-mail: cenkozverel@gmail.com

Received: 18.09.2019
Accepted: 20.11.2019

Disadvantages of Conventional Drug Delivery Systems

The development of antitumor strategies has been a huge field of research in the recent years owing to the ineffectiveness of the present conventional methods, including the drug delivery systems. Drug delivery systems have been developed as an efficient approach to induce cell-specific death of the tumor. Some of these conventional drug delivery systems involve the use of liposomes, carbon nanotubes, dendrimers, and gold nanoparticles (9-12). These conventional drug delivery systems present with major drawbacks, for instance, unnecessary release of therapeutic drugs to the neighboring tissues. Another disadvantage of these conventional delivery systems is the adverse immunogenic response as well as their accumulation in the organs such as liver and kidneys (13). Furthermore, the conventional systems fail to accumulate at the tumor site due to their unwanted recognition via the hosts' immune system (14).

Exosomes

Exosomes were first discovered as small vesicles inducing calcification of the long bones in a research study back in the 1960s (15). Later, they were detected in fluids such as blood and semen. In 1987, the term exosome was used for the first time ever in the literature to describe tiny membranous vesicles that are released into the extracellular space via exocytosis (16).

Specific targeting in tumor studies has been a major field with the goal of efficient cell death at lower toxicity in terms of collateral damage (17). During the past decades, several studies have been performed on drug delivery systems against cancer; however, their aforementioned disadvantages make them inefficient. Despite the vast number of studies performed on this subject, only a few of them, especially those on liposomal and polymeric nanoparticle formulations, have achieved the Food and Drug Administration, USA approval and are being used in clinical cancer therapies (11). No clinical trials have reported other drug delivery systems because of the challenges of unwanted distribution and higher toxicity levels (18).

Recently, exosomes have gained a lot of interest as a novel drug delivery system (18). Exosomes are <150 nm in dimensions and hence act as intermediatesin cell-to-cell communication. They can also be produced by almost all mammalian cells, including tumor cells (19). One of the major advantages of exosomes is their capability in transportation of endogenous biological cargos, such as proteins, small ribonucleic acids (sRNAs), and messenger ribonucleic acids (mRNAs) across the cells (20, 21). These capabilities provide advantages such as biocompatibility and decreased immune clearance rates in comparison to those with the conventional drug delivery systems (21). Some of the other advantages of exosomes are their better and longer accumulation at the organs or tumor sites and reduced toxicity levels (20). In addition to these advantages over the conventional systems, they also facilitate their uptake to the target cells due to the presence of surface proteins (22, 23).

Main Points:

- Exosome mimetic nanovesicles are promising candidates against cancer.
- Nanovesicles have exosome-mimicking properties of biocompatibility, easy cargo transportation, low immune clearance rate, and low resultant toxicities.
- Nanovesicles have major advantages over exosomes such as production yield and easy isolation protocol.
- Nanovesicles opened a new era in the drug delivery systems in combination with anticancer therapies.

Exosomes are presently being used to deliver various biological substances as well as chemotherapeutics, including doxorubicin, paclitaxel, curcumin, and some other peptide-based therapeutics, such as *signal transducer and activator of transcription 3* inhibitors, as well as genetic materials, such as small-interfering ribonucleic acids (siRNAs) (23-29). Moreover, they are extremely promising candidates for immunotherapy against cancer. Past studies have demonstrated the potential of dendritic cell-derived exosomes in stimulating patients' immune system against cancer (30). However, these new-era drug delivery systems also present disadvantages, especially in the protocol of isolation and yield obtained. These disadvantages unfortunately limit the usage of exosomes in the drug delivery systems. Exosomes require a huge yield of starting materials, such as cells and culture media, and the protocol takes enormous amount of time, which makes the overall process difficult and expensive to isolate (31).

Several strategies have been investigated in order to increase the production yield of exosomes. Some of these involve lowering of the pH of the culture media, increasing the initial tissue concentration, or prolonging the incubation time during a protocol. However, none of these strategies have been found efficient in increasing either the production yield of exosomes or in decreasing the time consumed in performing isolations (32).

Cell-Derived Exosome Mimetic Nanovesicles

Exosome mimetic nanovesicles are currently being investigated as alternatives to exosomes due to their superior properties over exosomes. They offer advantages over the production and time consumption during isolation. Exosome mimetic nanovesicles are isolated via the application of a physical force across the membranes of nano-scale dimensions. Several methods, including passing the cells through mini-extruders' micro channels or several rounds of centrifugation using custom devices, have been proposed (33-35). Nanovesicles provide advantages in terms of preserving their surface proteins from the parent cells and mimicking exosomal features. These also provide the advantages of decreased clearance rates from the body, targeted delivery of cargo, and efficient uptake mechanisms (36).

Starting from the same initial cell count, nanovesicle production generates a larger number of vesicles when compared to the conventional exosome-isolation protocols. They not only provide larger cell count but also require a shorter period of time for isolation, as short as 72 h (22).

Several studies have been performed on exosome mimetic nanovesicles to further characterize their therapeutic potentials. In a study by Goh et al. (37), nanovesicles derived from monocytes were used in the drug delivery system against cancer cells. They isolated nanovesicles and loaded chemotherapeutic drug doxorubicin to investigate their discriminatory approach between healthy and tumor cells. They found that monocyte-derived nanovesicles target cancerous cells and demonstrate a

clear discrimination toward cancer cells showing their potential in anticancer therapy (37). In another study, nanovesicle-isolated macrophages were loaded with chemotherapeutics, and their anticancer activity was investigated both *in vivo* and *in vitro*. Both *in vivo* and *in vitro* studies demonstrated a promising effect of nanovesicles against tumor cells. Exosomes and nanovesicles were further compared in the same study, and no significant differences were noted in their antitumor activities (32). Chemotherapeutic drug-loaded nanovesicles were investigated in a comparative *in vivo* study, and the data obtained suggested that drug-loaded nanovesicles have better cellular uptake and permeability, resulting in reduced tumor dimensions when compared to liposomal formulations and drug-only treatment (38).

Mesenchymal stem cells (MSCs)-derived exosomes are known for their anticancer activities. Chemotherapeutic agent-loaded MSCs have been determined as great vehicles for delivery. In a study by Kalimuthu et al. (39), the anticancer activities of paclitaxel-loaded exosome mimetic nanovesicles were investigated both *in vivo* and *in vitro*. Our results demonstrated lower cell viability of the breast cancer cells *in vitro* and reduced tumor dimensions *in vivo* (39).

Exosomes are also known for their capabilities in delivering ribonucleic acids (RNAs)such as mRNAs and micro RNAs that alter the phenotype of the target cells. Hence, exosome mimetic nanovesicles can not only be used asnovel drug delivery systems but also for use in various gene therapies due to their potential in carrying genetic materials. RNA interferences possess the ability to selectively attenuate specific genes, making it highly valuable in treating diseases, including cancers that are caused by overexpression of genes (40-42). Lunavat et al. (43) demonstrated the successful uptake of siRNA into nanovesicles and subsequent attenuation of target gene expression. The data suggested that nanovesicles can form a new platform for RNA delivery to successfully target the cell cytoplasm (43).

De-regulations in the cell cycle are the major hallmarks of cancer formation. Disruption of the cell-cycle control kinases (mainly cyclin-dependent kinases [CDKs]) cause the formation of various cancers, including lung, breast, liver, and blood (44-46). Targeting the CDK pathway is one of the major areas focused in cancer-treatment studies. These genetic materials have limited stability and the drug delivery systems are inefficient in relation to the cellular uptakes. Tumor-derived exosome mimetic nanovesicles have been suggested as an alternative method for delivery of these RNAs, which are biocompatible, non-immune reactive, and easily taken up. Yang et al. (47) demonstrated the successful delivery of RNAs in a breast cancer model both *in vivo* and *in vitro* and proved the specific downregulation of CDK4 target genes and induction of cell cycle arrest at the siRNA-delivered study groups (47).

In conclusion, drug delivery systems are important parts of most of the clinical therapeutics practiced in the present time. However, they present with certain drawbacks, such as inefficient immune clearance and uptake mechanisms. Exosomes, which are cell-to-cell communicators, are expressed nearly by all mammalian cells and reportedly possess the capability of better delivery due to their biocompatibility, capability of transportation of endogenous biological cargos, lower immune clearance rates, and lower resultant toxicities. They not only demonstrate their activities in drug delivery systems but also in immunotherapies. Exosome studies have however not moved forward for clinical application owing to their time-consuming isolation techniques and low production yield. Nanovesicles, smaller-diameter exosome mimetics, have therefore opened up a new era in the drug delivery systems in combination with anticancer therapies. These nanovesicles possess exosome-mimicking properties of biocompatibility, easy cargo transportation, low immune clearance rate, and low resultant toxicities and can be produced in a relatively higher quantity. The research field on nanovesicles is progressing quickly since their discovery. Recent studies have demonstrated their enormous potential in cancer therapy. The potential abilities of exosome mimetic nanovesicles in cancer therapy is worth exploring in the future.

Peer-review: Externally peer-reviewed.

Conflict of Interest: Author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

REFERENCES

1. Hanahan D, Weinberg RA. Hallmarks of Cancer: The Next Generation. Cell 2011; 144(5): 646-74. [Crossref]
2. Michod D, Widmann C. DNA-Damage Sensitizers: Potential New Therapeutical Tools to Improve Chemotherapy. Crit Rev Oncol Hematol 2007; 63(2): 160-71. [Crossref]
3. Jackson SP, Bartek J. The DNA-Damage Response in Human Biology and Disease. Nature 2009; 461: 1071-8. [Crossref]
4. Schiff D, Wen PY, van den Bent MJ. Neurological Adverse Effects Caused by Cytotoxic and Targeted Therapies. Nat Rev Clin Oncol 2009; 6(10): 596-603. [Crossref]
5. Jacob A, Richard D J, O'Bryne KJ. EV, Microvesicles/MicroRNAs and Stem Cells in Cancer. In: Mettinger K, Rameshwar P, Kumar V. (eds) Exosomes, Stem Cells and MicroRNA. Adv Exp Med Biol. 2018: 1056.
6. Crenshaw BJ, Sims B, Matthews QL. Biological Function of Exosomes as Diagnostic Markers and Therapeutic Delivery Vehicles in Carcinogenesis and Infectious Diseases. Nanomedicines; Intech Open Limited: London, UK, 2018. [Crossref]
7. Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor Vascular Permeability and the EPR Effect in Macromolecular Therapeutics: A Review. J Control Release 2000; 65(1-2): 271-84. [Crossref]
8. Ferrari M. Nanogeometry: Beyond Drug Delivery. Nat Nanotechnol 2008; 3(3): 131-2. [Crossref]
9. Estanqueiro M, Amaral MH, Conceição J, Sousa Lobo JM. Nanotechnological carriers for cancer chemotherapy: the state of the art. Colloids Surf B Biointerfaces 2015; 126: 631-48. [Crossref]
10. Sajid M I, Jamshaid U, Jamshaid T, Zafar N, Fessi H, Elaissari A. Carbon nanotubes from synthesis to in vivo biomedical applications. Inter J Pharm 2016; 501(1-2): 278-99. [Crossref]
11. Pérez-Herrero E, Fernández-Medarde A. Advanced targeted therapies in cancer: drug nanocarriers, the futurjkbjmnn e of chemotherapy. Eur J Pharm Biopharm 2015; 93: 52-79. [Crossref]
12. Zhu Y, Liao L. Applications of nanoparticles for anticancer drug delivery: a review. J Nanosci Nanotechnol 2015; 15(7): 4753-73. [Crossref]
13. Szebeni J, Storm G. Complement activation as a bioequivalence issue relevant to the development of generic liposomes and other nanoparticulate drugs. Biochem Biophys Res Commun 2015; 468(3): 490-7. [Crossref]

14. Pérez-Herrero E, Fernández-Medarde A. Advanced targeted therapies in cancer: drug nanocarriers, the future of chemotherapy. Eur J Pharm Biopharm 2015; 93: 52-79. [Crossref]
15. Anderson HC. Vesicles associated with calcification in the matrix of epiphyseal cartilage. J Cell Biol 1969; 41(1): 59-72. [Crossref]
16. 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(19): 9412-20.
17. Estanqueiro M, Amaral MH, Conceição J, Sousa Lobo JM. Nanotechnological carriers for cancer chemotherapy: the state of the art. Colloids Surf B Biointerfaces 2015; 126: 631-48. [Crossref]
18. Fanciullino R, Ciccolini J, Milano G. Challenges, expectations and limits for nanoparticles-based therapeutics in cancer: A focus on nano-albumin-bound drugs. Crit Rev Oncol Hematol 2013; 88(3): 504-13. [Crossref]
19. Fais S, Logozzi M, Lugini L, Federici C, Azzarito T, Zarovni N, Chiesi A. Exosomes: the ideal nanovectors for biodelivery. Biol Chem 2013; 394(1): 1-15. [Crossref]
20. Luan X, Sansanaphongpricha K, Myers I, Chen H, Yuan H, Sun D. Engineering exosomes as refned biological nanoplatforms for drug delivery. Acta Pharmacol Sin 2017; 38(6): 754-63. [Crossref]
21. HoodJ L. Post isolation modifcation of exosomes for nanomedicine applications. Nanomedicine 2016; 11(13): 1745-56. [Crossref]
22. Marcus ME, Leonard JN. FedExosomes: Engineering Terapeutic Biological Nanoparticles that Truly Deliver. Pharmaceuticals 2013; 6(5): 659-80. [Crossref]
23. Xitong D, Xiaorong Z. Targeted therapeutic delivery using engineered exosomes and its applications in cardiovascular diseases. Gene 2016; 575: 377-84. [Crossref]
24. Pascucci L, Cocce V, Bonomi A, Ami D, Ceccarelli P, Ciusani E, et al. Paclitaxel is incorporated by mesenchymal stromal cells and released in exosomes that inhibit in vitro tumor growth: A new approach for drug delivery. J Control Release 2014; 192: 262-70. [Crossref]
25. Yang Y, Chen Y, Zhang F, Zhao Q, Zhong H. Increased anti-tumour activity by exosomes derived from doxorubicin-treated tumour cells via heat stress. Int J Hyperthermia 2015; 31(5): 498-506. [Crossref]
26. Sun D, Zhuang X, Xiang X, Liu Y, Zhang S, Liu C, et al. A Novel Nanoparticle Drug Delivery System: The Anti-infammatory Activity of Curcumin Is Enhanced When Encapsulated in Exosomes. Mol Ther 2010; 18(9): 1606-14. [Crossref]
27. Zhuang X, Xiang X, Grizzle W, Sun D, Zhang S, Axtell RC, et al. Treatment of Brain Infammatory Diseases by Delivering Exosome Encapsulated Anti-infammatory Drugs From the Nasal Region to the Brain. Mol Ther 2011; 19(10): 1769-79. [Crossref]
28. Haney MJ, Kiyachko NL, Zhao Y, Gupta R, Plotnikova EG, He Z, et al. Exosomes as drug delivery vehicles for Parkinson's disease therapy. J Control Release 2015; 207: 18-30. [Crossref]
29. Liu Y, Li D, Liu Z, Zhou Y, Chu D, Li X, et al. Targeted exosome-mediated delivery of opioid receptor Mu siRNA for the treatment of morphine relapse. Sci Rep 2015; 5: 17543. [Crossref]
30. Escudier B, Dorval T, Chaput N, Andre F, Caby MP, Novault S, et al. Vaccination of metastaticmelanomapatientswithautologousdendriticcell (DC) derived-exosomes: results of thefirstPhase I clinicaltrial. J Transl Med 2015; 3(1): 10. [Crossref]
31. Téry C, Amigorena S, Raposo G, Clayton A. In Current Protocols in Cell Biology (John Wiley & Sons, Inc., 2001).
32. Ban J-J, Lee M, Im W,Kim M. Low pH increases the yield of exosome isolation. Biochem Biophys Res Commun 2015; 461(1): 76-9. [Crossref]
33. Jang SC, Kim OY, Toon CM, Choi DS, Roh TY, Park J, et al. Bioinspired Exosome-Mimetic Nanovesicles for Targeted Delivery of Chemotherapeutics to Malignant Tumors. ACS Nano 2013; 7: 7698-710. [Crossref]
34. Jo W, Jeong D, Kim J, Cho S, Jang SC, Han C, et al. Microfuidic fabrication of cell-derived nanovesicles as endogenous RNA carriers. Lab on a Chip 2014; 14(7): 1261-9. [Crossref]
35. Jo W, Kim J, Yoon J, Jeong D, Cho S, Jeong H, et al. Large-scale generation of cell-derived nanovesicles. Nanoscale 2014; 6(20): 12056-64. [Crossref]
36. Roth JC, Curiel DT, Pereboeva L. Cell vehicle targeting strategies. Gene Ter 2008; 15(10): 716-29. [Crossref]
37. Goh WJ, Lee CK, Zou S, Woon ECY, Czarny B, Pastorin G. Doxorubicin-loaded cell-derived nanovesicles: an alternative targeted approacj for anti-tumor therapy. Int J Nanomedicine 2017; 12: 2759-67. [Crossref]
38. Ingato D, Edson JA, Zakharian M, Kwon YJ. Cancer Cell-Derived, Drug-Loaded Nanovesicles Induced by Sulphydryl-Blocking for Effective and Safe Cancer Therapy. CS Nano 2018; 129: 9568-77. [Crossref]
39. Kalimuthu S, Gangadaran P, Rajendran RL, ZhuL, Oh JM, Lee HW, et al. A New Approach for Loading Anticancer Drugs IntoMesenchymal Stem Cell-Derived Exosome Mimetics for Cancer Therapy. Front Pharmacol2018; 9: 1116. [Crossref]
40. Hong BS, Cho JH, Kim H, Choi EJ, Rho S, Kim J, et al. Colorectal cancer cell derived microvesicles are enriched in cell cycle-related mRNAs that promote proliferation of endothelial cells. BMC Genomics 2009; 10: 556. [Crossref]
41. Valadi H, Ekstrom K, Bossios A, Sjostrand M, Lee JJ, Lotvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol 2007; 9(6): 654-9. [Crossref]
42. Skog J, Wurdinger T, van Rijn S, Meijer DH, Gainche L, Sena-Esteves M, et al. Glioblastoma microvesicles transport RNA and proteins that promote tumour growth and provide diagnostic biomarkers. Nat Cell Biol 2008; 10(12): 1470-6. [Crossref]
43. Lunavat TR, Jang SC, Nilsson L, Park HT, Repiska G, Lasser C, et al. RNAi delivery by exosome-mimetic nanovesicles - Implications for targeting c-Myc in cancer. Biomaterials 2016; 102: 231-8. [Crossref]
44. Huang C Z, Huang W Z, Zhang G, Tang D L, In vivo study on the effects of curcumin on the expression profiles of anti-tumour genes (VEGF, CyclinDI and CDK4) in liver of rats injected with DEN. Mol Biol Rep 2013; 40(10): 5825-31. [Crossref]
45. Sawai CM, Freund J, Oh P, Ndiaye-Lobry D, Bretz J C, Strikoudis A, Genesca L, et al. Therapeutic targeting of the cyclin D3:CDK4/6 complex in T cell leukemia. Cancer Cell 2012; 22(4): 452-65. [Crossref]
46. Dobashi Y, Goto A, Fukayama M, Abe A, Ooi A. Overexpression of cdk4/cyclin DI, a possible mediator of apoptosis and an indicator of prognosis in human primary lung carcinoma. Int J Cancer 2004; 110(4): 532-41. [Crossref]
47. Yang Z, Xie J, Zhu J, Kang C, Chiang C, Wang X, et al. Functional exosome-mimic for delivery of siRNA to cancer: in vitro and in vivo evaluation. J Control Release 2016; 243: 160-71. [Crossref]