Cellular and Molecular Biology

Review Article Open Access

Trends and Challenges in Exosome-Based Anticancer Therapy: Roles and Strategies in the Tumor Microenvironment

Sang Bum Kim*

Department of Pharmacy, Sahmyook University, Seoul, South Korea

Abstract

The multiple forms of cancer, which comprises over 200 different forms, remains a leading cause of death globally, with existing treatments encountering challenges in achieving satisfactory responses. While immunotherapy shows promise, it has low response rates and necessitates alternative or complementary strategies. Classical chemotherapy, which is widely used, is limited by significant side effects and efficacy constraints. Hence, novel therapies that leverage existing anticancer treatments, while addressing their limitations, are urgently needed. Exosomes, nano-sized vesicles (50–150 nm), play crucial roles in intercellular communication by transporting lipids, proteins, and nucleic acids derived from parent cells. This review explores the potential of exosomes in anticancer drug development, focusing on their roles in the tumor microenvironment, and discusses the challenges and strategies for drug development. Exosomes have been implicated in the tumor microenvironment by modulating cancer growth, metastasis, and drug resistance.

They mediate metabolic reprogramming in cancer cells, promote angiogenesis, and facilitate immune evasion, thereby contributing to tumor progression. However, exosomes exhibit antitumor functions by activating tumor-specific immune responses and suppressing cancer growth. Efforts to harness exosomes for drug development face several challenges, including exosome heterogeneity, standard isolation methods, mass production, and purification. Strategies to address these challenges include refining exosome isolation techniques based on size and biomarker profiles, advancing large-scale production technologies, and optimizing purification methods. In conclusion, despite the hurdles in exosome research and drug development, recent advancements in our understanding of exosome biology and technology offer promising avenues for anticancer therapy. Future research focusing on refining exosome-based therapies and overcoming existing challenges holds great potential for improving cancer treatment outcomes

Keywords: Cancer therapy; Drug delivery system; Drug development; Exosome, Exosome-based therapy

Introduction

Cancer is a formidable global health challenge, claiming countless lives each year, encompassing over 200 different types. Despite advancements, current cancer treatments often yield suboptimal responses. Immunotherapy, known for its efficacy in regulating the immune system to suppress cancer, has shown promise [1]. However, low response rates underscore the need for alternative or complementary strategies. Classical chemotherapy, which is widely employed, is hampered by significant side effects and limited efficacy. Thus, there is a pressing demand for novel therapies that can complement existing anticancer treatments and address their shortcomings. Exosomes, nano-sized vesicles typically ranging from 50–150 nm, are essential mediators of intercellular communication. Composed of a lipid bilayer, exosomes transport lipids, proteins, and nucleic acids derived from parent cells, exerting profound effects on target cells and modulating cellular metabolism. This lipid bilayer not only protects the contents of the exosomes but also facilitates their absorption by recipient cells [2,3]. Consequently, their potential in cancer therapy lies in their direct use as therapeutic agents or as drug delivery systems. Although cancer cells can secrete various extracellular vesicles, including exosomes, the development of technology to selectively purify active exosomes is imperative [4]. Given the diversity in size and content, an ideal exosome isolation system should consider both size and biomarker profiles. Recent research highlighting the variations in exosome content based on size emphasizes the necessity of refining exosome purification techniques. Although size-based Tangential Flow Filtration (TFF) and size-exclusion chromatography methods have been developed for exosome separation, achieving complete homogeneity remains a challenge. In this mini-review, we examine the roles of exosomes in the tumor microenvironment, exploring both their pro-tumor and antitumor functions, and discuss the implications of exosome-based drug development [5].

Literature Review

Pro and anti-tumorigenic functions in the tumor microenvironment

Tumors intricately modulate their microenvironment, collectively termed the tumor microenvironment, to foster optimal conditions for growth and metastasis. Predominantly comprised of cancer cells, this milieu is also influenced by tumor derived exosomes, which play pivotal roles in promoting cancer growth, metastasis, and drug resistance. Exosomes exert their effects directly on cancer cells or by modulating the immune system, creating an environment conducive to cancer progression. Direct interactions between tumor-derived exosomes and cancer cells induce significant metabolic reprogramming, which is conducive to cell proliferation. These exosomes deliver active substances to the target cells, thereby regulating cellular metabolism. For instance, the delivery of oncogenic proteins, such as *EGFRvIII* mRNA, *via* exosomes can activate signaling pathways such as Mitogen-Activated Protein Kinase (MAPK) and AKT, promoting cell proliferation [6]. Moreover, tumor-derived exosomes can suppress oxidative phosphorylation and enhance glycolysis to regulate energy metabolism reactions, thereby facilitating cell proliferation by suppressing the tumor suppressor gene, *p53* [7,8].

***Corresponding author:** Sang Bum Kim, Department of Pharmacy, Sahmyook University, Seoul, South Korea; E-mail: sbk@syu.ac.kr

Received: 08-Apr-2024, Manuscript No. CMB-24-131819; **Editor assigned:** 10-Apr-2024, Pre QC No. CMB-24-131819 (PQ); **Reviewed:** 24-Apr-2024, QC No.CMB-24-131819; **Revised:** 02-May-2024, Manuscript No. CMB-24-131819 (R); **Published:** 09-May-2024, DOI:10.4172/1165-158X.24.S1.002.

Citation: Kim SB (2024) Trends and Challenges in Exosome-Based Anticancer Therapy: Roles and Strategies in the Tumor Microenvironment. Cell Mol Biol S1:002.

Copyright: © 2024 Kim SB. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Tumor metastasis, a critical determinant of cancer mortality, involves the spread and growth of tumor cells in distant organs *via* blood and lymphatic vessels. This intricate process necessitates angiogenesis, immune evasion, and the establishment of a pre-metastatic niche. Exosomes play crucial roles in cancer metastasis, as evidenced by their involvement in various metastasis mechanisms. For instance, tumor-derived exosomes containing Vascular Endothelial Growth Factor (VEGF) promote angiogenesis, a crucial step in metastatic progression [9]. Additionally, exosomes derived from patients with metastatic melanoma have been implicated in reducing T-cell responses *via* Programmed Death-Ligand 1 (PD-L1), thereby facilitating immunosuppression and aiding metastasis [10].

These findings underscore the multifaceted roles of exosomes within the tumor microenvironment, where they orchestrate both pro and anti-tumorigenic processes. Elucidating the precise mechanisms underlying these functions is essential for devising targeted therapeutic interventions to disrupt tumor progression and metastasis. Drug resistance is a critical factor that contributes to cancer mortality, and numerous studies have consistently demonstrated its association with exosomes. MiRNAs carried by tumor-derived exosomes can promote drug resistance by modulating the expression of ABC transporters [11]. Furthermore, the delivery of anticancer drugs through exosomes reduces their efflux from cancer cells, thereby mitigating drug resistance (Reference required). Tumor-derived exosomes not only facilitate cancer growth, metastasis, and mortality but also exhibit antitumor functions. These exosomes can activate tumor-specific immune responses, primarily mediated by tumor-related antigens and immune stimulants.

Consequently, tumor-derived exosomes have the potential to be used as anticancer vaccines. For instance, treatment with hepatocellular carcinoma-derived exosomes decreases tumor growth and enhances the activation of T lymphocytes surrounding the tumor [12]. These findings suggest the feasibility of developing Dendritic Cell (DC) vaccines using tumor-derived exosomes. Furthermore, immune cells within the cancer microenvironment secrete exosomes that contribute to cancer suppression. DC-derived exosomes, which contain major histocompatibility complex molecules and co-stimulatory molecules necessary for T-cell activation, have shown efficacy in inducing T-cell-dependent anticancer responses and tumor eradication [13]. In contrast, T-cell-derived exosomes exhibit direct cytotoxic effects on cancer cells and induce anticancer responses by activating other T-cells.

Activated CD8 T-cell-derived exosomes exert anticancer effects by directly targeting malignant tumors and activating the surrounding T-cells. For instance, Interleukin-12 (IL-12) treated activated CD8 T-cell exosomes promote the production of interferon γ and granzyme B by activating adjacent CD8 T-cells [14]. Notably, unlike PD-1 carried by tumor-derived exosomes, T-cell-derived exosomal PD-1 enhances the survival rate of patients with triple-negative breast cancer by alleviating immune suppression through PD-1 and PD-L1 binding [15]. These findings underscore the potential for developing anticancer vaccines and drugs utilizing exosomes derived from immune cells, which play a pivotal role in anticancer immune activity.

Exosome-based drug development: Drug delivery, secretion inhibition, and removal technology

The development of anticancer drugs using exosomes can be categorized into three methods;

- Direct elimination of cancer cells using exosomes as delivery vehicles.
- Utilization of exosomes as source materials for anticancer vaccines.
- Removal of exosomes that facilitate cancer growth and metastasis.

Exosomes serve as efficient drug delivery vehicles owing to their safety, cargo protection, and efficient cellular uptake. The lipid bilayer of exosomes, which is identical to that of the cell membrane, provides sufficient protection for the cargo. Additionally, the safety and low immunogenicity of exosomes during blood transfusion have been confirmed. Despite the presence of 109–1010 exosomes/mL in blood, they do not cause problems during the transfusion process. Cargo loading into exosome delivery vehicles, can occur either during exosome production or through post-production methods, where drugs are injected into purified exosomes. Pre-production drug processing in the parent cell is a common method for loading drugs during exosome production. Cargo loading into exosomes typically involves techniques such as electrophoresis and sonication. These methods have been shown to effectively load chemicals, proteins, and nucleic acids into exosomes [16,17].

Notably, exosome-loaded doxorubicin showed a 40% reduction in cardiac toxicity while maintaining its anticancer effect [18]. Moreover, certain anti-cancer drugs exhibit improved anticancer effects when loaded into exosomes. For example, paclitaxel-loaded exosomes have demonstrated enhanced efficacy against prostate cancer [19]. Consequently, the use of exosomes as delivery vehicles for chemotherapeutic agents exhibits increased efficacy and reduced toxicity. Given that more than 50% of existing anticancer drugs are plant-derived substances, utilizing plant-derived exosomes for delivery could yield novel combination therapies. The inhibition of tumor-derived exosome secretion, which plays a critical role in tumor progression, metastasis, and drug resistance, holds promise as a novel anticancer strategy. Among the methods used to block exosome secretion, one molecular biological approach involves the inhibition of Rab27a, which is essential for exosome secretion. In mouse experiments involving melanoma, the suppression of Rab27a led to a reduction in metastasis, confirming its therapeutic potential [20].

Furthermore, the recently reported US Food and Drug Administration-approved antibiotic sulfisoxazole, which inhibits exosome secretion, has demonstrated efficacy in suppressing tumor progression and metastasis [21]. Additionally, the development of a blood filtration system, the Aethlon ADAPT™ system, capable of separating and removing exosomes, represents another promising approach [22]. Although further research is needed to refine the process of removing tumor-derived exosomes, inhibiting their secretion has emerged as a viable strategy for cancer treatment. The anticancer efficacy of tumor-derived exosomes has been validated using cancer vaccines. Vaccines suppress cancer by activating the immune system against specific antigens. Cancer cell-specific antigens and immune adjuvants are required for this purpose. Tumor-derived exosomes act in the early stages of the cancer immune cycle by facilitating immune activation along with the presentation of cancer cell-specific antigens [23].

The efficacy of cancer vaccines containing exosomes was first reported in 2001 [24]. Tumor-derived exosomes can activate innate immune cells because they contain Damage-Associated Molecular Patterns (DAMPs) such as Heat Shock Protein (HSP70). Clinical trials on tumor-derived exosomes are ongoing (NCT02657460 and NCT01854866). However, because tumor-derived exosomes tend to aid cancer growth, their safety needs to be assessed through additional

research. Furthermore, immune cell-derived exosomes are also used as cancer vaccines, and activation of antitumor immunity by DC-derived exosomes has been reported [25].

Challenges in exosome-based drug development: Isolation methods, large-scale production, and purification quality control

Several challenges related to exosome heterogeneity, standard isolation methods, mass production, purification, and quality control must be addressed for successful exosome-based drug development. Exosomes, generally defined as bio-derived vesicles between 50 and 150 nm in size, exhibit considerable heterogeneity within this range. Moreover, cell-derived vesicles of varying sizes are present along with exosomes, posing a significant challenge for drug development that requires homogeneity. A recent study by Kalluri et al., categorized exosomes into three groups based on size and analyzed the differences in marker proteins and exosome content across these sizes [26].

Therefore, a dual approach is necessary to address exosomal heterogeneity. First, exosomes need to be purified by size classification, followed by the isolation of active exosomes using specific marker proteins. Size-based purification methods include TFF, which employs size filters and size-exclusion chromatography. These techniques enhance the homogeneity of isolated exosomes and facilitate their application in drug development. The TFF method enables the classification of exosomes in 10 nm increments using filters and offers automated purification capabilities [27]. ExoQuick, a commercially available method, purifies exosomes using markers, thereby contributing to the production of a homogeneous exosome population by incorporating additional active markers.

Discussion

A significant challenge in producing and purifying exosomes for clinical trials, which is pivotal for drug development, is the advancement of large-scale culture and purification technologies. The cost associated with purifying animal cell-derived exosomes remains prohibitive [28]. To address this, studies have investigated exosome production without using fetal bovine serum during culture to potentially reduce costs [29]. Furthermore, developing novel cell lines capable of enhancing exosome secretion through genetic manipulation is crucial to overcome limited exosome secretion from animal cells. Harnessing plant-derived exosomes is a viable alternative. Plant stem cell cultures are cost-effective, support prolonged culture periods similar to those of animal cells, and yield exosome-like vesicles. Moreover, plant-derived exosomes offer economic viability and enable ample vesicle production, addressing the economic constraints associated with production using animal cell cultures.

Furthermore, optimization of exosome purification methods is of paramount importance. An ideal purification approach involves sequential filtration-based recovery, followed by size chromatography-based technology. Thus, this purification system is optimal for large-scale exosome purification [30]. Therefore, for drug development research utilizing exosomes, the following process would be ideal: First, to ascertain the characteristics of active exosomes, they must be identified through size and marker-based purification. Subsequently, homogeneously active exosomes can be purified using ultrafiltration, size-based chromatography, and marker-based purification techniques. Additionally, alternative approaches include; Developing animal cells with enhanced exosome secretion through genetic manipulation; Using plant-derived exosomes.

Conclusion

Exosomes have emerged as a focal point of global research, extending beyond drug development to encompass fields like cosmetics. The advancement of anticancer drugs using exosomes requires further research on the development of anticancer-active exosomes; the utilization of exosomes as anticancer drug carriers; and the use of exosomes as anticancer vaccines. This review presents a new potential strategy for anticancer treatment. Despite the need for additional research owing to the pro-tumor properties of certain exosomes and the challenges in exosome production, isolation, and purification, recent advancements in understanding exosome secretion mechanisms, plant-derived exosomes, and exosome purification technologies for clinical trials have shown promise for drug development.

Conflict of interests

The authors declare that they have no conflict of interest.

Acknowledgments

This work was supported by the National Research Foundation of Korea (NRF) Grant funded by the Korean Government (MSIT) (Grant Nos. 2022R1C1C1013481).

References

1. Waldman AD, Fritz JM, Lenardo MJ (2020) A guide to cancer immunotherapy: From T cell basic science to clinical practice. Nat Rev Immunol 20:651-668.
2. Lopez-Verrilli MA (2013) Exosomes: Mediators of communication in eukaryotes. Biol Res 46:5-11.
3. Dai J, Su Y, Zhong S, Cong L, Liu B, et al. (2020) Exosomes: Key players in cancer and potential therapeutic strategy. Signal Transduct Target Ther 5:145.
4. Doyle LM, Wang MZ (2019) Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. Cells 8:727.
5. Kim SB (2022) Function and therapeutic development of exosomes for cancer therapy. Arch Pharm Res 45:295-308.
6. Al-Nedawi K, Meehan B, Micallef J, Lhotak V, May L, et al. (2008) Intercellular transfer of the oncogenic receptor EGFRvIII by microvesicles derived from tumour cells. Nat Cell Biol 10:619-624.
7. Zhao H, Yang L, Baddour J, Achreja A, Bernard V, et al. (2016) Tumor microenvironment derived exosomes pleiotropically modulate cancer cell metabolism. Elife 5:e10250.
8. Soldevilla B, Rodríguez M, San Millán C, García V, Fernandez-Perianez R, et al. (2014) Tumor-derived exosomes are enriched in ΔNp73, which promotes oncogenic potential in acceptor cells and correlates with patient survival. Hum Mol Genet 23:467-478.
9. Ludwig N, Whiteside TL (2018) Potential roles of tumor-derived exosomes in angiogenesis. Expert Opin Ther Targets 22:409-417.
10. Chen G, Huang AC, Zhang W, Zhang G, Wu M, et al (2018) Exosomal PD-L1 contributes to immunosuppression and is associated with anti-PD-1 response. Nature 560:382-386.
11. Guo QR, Wang H, Yan YD, Liu Y, Su CY, et al. (2020 The role of exosomal microRNA in cancer drug resistance. Front Oncol 10:472.
12. Rao Q, Zuo B, Lu Z, Gao X, You A, et al. (2016) Tumor-derived exosomes elicit tumor suppression in murine hepatocellular carcinoma models and humans *in vitro*. Hepatology 64:456-472.
13. Zitvogel L, Regnault A, Lozier A, Wolfers J, Flament C, et al. (1998) Eradication of established murine tumors using a novel cell-free vaccine: Dendritic cell derived exosomes. Nat Med 4:594-600.
14. Li L, Jay SM, Wang Y, Wu SW, Xiao Z (2017) IL-12 stimulates CTLs to secrete exosomes capable of activating bystander CD8+ T cells. Sci Rep 7:13365.

15. Qiu Y, Yang Y, Yang R, Liu C, Hsu JM, et al. (2021) Activated T cell-derived exosomal PD-1 attenuates PD-L1-induced immune dysfunction in triple-negative breast cancer. Oncogene. 40:4992-5001.

16. Walker S, Busatto S, Pham A, Tian M, Suh A, et al. (2019) Extracellular vesicle-based drug delivery systems for cancer treatment. Theranostics 9:8001.

17. Chen YS, Lin EY, Chiou TW, Harn HJ (2020) Exosomes in clinical trial and their production in compliance with good manufacturing practice. Ci Ji Yi Xue Za Zhi 32:113-120.

18. Toffoli G, Hadla M, Corona G, Caligiuri I, Palazzolo S, et al. (2015) Exosomal doxorubicin reduces the cardiac toxicity of doxorubicin. Nanomedicine 10:2963-2971.

19. Saari H, Lázaro-Ibáñez E, Viitala T, Vuorimaa-Laukkanen E, Siljander P, et al. (2015) Microvesicle-and exosome-mediated drug delivery enhances the cytotoxicity of Paclitaxel in autologous prostate cancer cells. J Control Release 220:727-737.

20. Li J, Chen J, Wang S, Li P, Zheng C et al. (2019) Blockage of transferred exosome-shuttled miR-494 inhibits melanoma growth and metastasis. J Cell Physiol 234(9):15763-15774.

21. Im EJ, Lee CH, Moon PG, Rangaswamy GG, Lee B, et al. (2019) Sulfisoxazole inhibits the secretion of small extracellular vesicles by targeting the endothelin receptor A. Nat Commun 10:1387.

22. Marleau AM, Chen CS, Joyce JA, Tullis RH (2012) Exosome removal as a therapeutic adjuvant in cancer. J Transl Med 10:1-2.

23. Dutta A (2021) Exosomes-based cell-free cancer therapy: A novel strategy for targeted therapy. Immunological Medicine 44:116-23.

24. Wolfers J, Lozier A, Raposo G, Regnault A, Théry C, et al. (2001) Tumor-derived exosomes are a source of shared tumor rejection antigens for CTL cross-priming. Nat Med 7:297-303.

25. Pitt JM, André F, Amigorena S, Soria JC, Eggermont A, et al. (2016) Dendritic cell-derived exosomes for cancer therapy. J Clin Invest 126(4):1224-1232.

26. Kalluri R, LeBleu VS (2020). The biology, function, and biomedical applications of exosomes. Science 367:eaau697.

27. Bari E, Perteghella S, Catenacci L, Sorlini M, Croce S, et al. (2019) Freeze-dried and GMP-compliant pharmaceuticals containing exosomes for acellular mesenchymal stromal cell immunomodulant therapy. Nanomedicine 14:753-765.

28. Isasi R, Rahimzadeh V, Charlebois K (2016) Uncertainty and innovation: Understanding the role of cell-based manufacturing facilities in shaping regulatory and commercialization environments. Appl Transl Genom 11:27-39.

29. Kordelas L, Rebmann V, Ludwig AK, Radtke S, Ruesing J, et al. (2014) MSC-derived exosomes: a novel tool to treat therapy-refractory graft-*versus*-host disease. Leukemia 28:970-973.

30. Nordin JZ, Lee Y, Vader P, Mäger I, Johansson HJ, et al. (2015) Ultrafiltration with size-exclusion liquid chromatography for high yield isolation of extracellular vesicles preserving intact biophysical and functional properties. Nanomedicine 11:879-983.