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Extracellular Vesicles and Their Biomedical Applications: An Overview

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1.1 Introduction

Extracellular vesicles (EVs) are small membrane-bound vesicles that are secreted by a variety of cells, including stem cells, immune cells, and cancer cells. These vesicles contain a range of molecules, including lipids, proteins, and nucleic acids, that reflect the cellular and physiological state of the parent cell, and play a variety of physiological and pathological roles in the body. They can act as signaling molecules, carrying bioactive molecules such as proteins, lipids, and nucleic acids, between cells and tissues. They can also act as vehicles for the transfer of genetic material, such as microRNAs, between cells. The biomedical applications of EVs have been widely explored in recent years, and they are emerging as a promising tool for diagnosis, therapy, and drug delivery.

1.2 Biogenesis and Composition of Extracellular Vesicles

EVs are released by cells into the extracellular space and can be classified into three main types based on their biogenesis and size: exosomes, microvesicles, and apoptotic bodies. Exosomes are small vesicles (30–150 nm) that are released by cells through the endosomal pathway, while microvesicles (100–1000 nm) are formed by budding of the plasma membrane. Exosomes and microvesicles are collectively referred to as small EVs. Exosomes are enriched in endosomal markers such as CD63 and Alix, while microvesicles are enriched in plasma membrane markers such as phosphatidylserine and CD31. Apoptotic bodies are referred to as large EVs (1–5 μm)

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that are released by cells undergoing programmed cell death. The biogenesis of each type of EV is distinct and involves the selective packaging of different molecules.

The composition of EVs varies depending on the type of vesicle, the cell of origin, and the physiological or pathological condition. EVs can contain a range of biological molecules, including proteins, nucleic acids (RNA and DNA), lipids, and carbohydrates. Proteins present in EVs include membrane proteins, cytosolic proteins, and extracellular matrix proteins. Nucleic acids in EVs include mRNAs, miRNAs, and long noncoding RNAs. Lipids in EVs include phospholipids, sphingolipids, and cholesterol.

1.3 Biological Functions of Extracellular Vesicles

EVs have been shown to regulate a range of cellular processes, including proliferation, differentiation, and apoptosis. EVs have been shown to play a role in many biological processes, including immune regulation, inflammation, angiogenesis, tissue repair, and cancer progression. They have also been implicated in the pathogenesis of many diseases, including cardiovascular disease, neurological disorders, and infectious diseases. Given their diverse functions, EVs have the potential to be used for a range of biomedical applications, especially in the fields of diagnostics, therapeutics, and regenerative medicine.

In the area of diagnostics, EVs have great potential as diagnostic biomarkers for a variety of diseases. They are stable in biological fluids, and their cargo can reflect the physiological state of the cell of origin, making them attractive targets for disease diagnosis and monitoring. Cancer is one area where EVs have shown particular promise as diagnostic biomarkers. Cancer cells release large numbers of EVs into the circulation, which can be detected in blood samples. These EVs contain specific biomolecules that can be used to detect and monitor the progression of cancer. For example, circulating tumor cells (CTCs) shed EVs that contain proteins and nucleic acids that are specific to the cancer cells. These biomolecules can be used to develop noninvasive diagnostic tests for cancer. In addition, EVs released by cancer cells can provide information about the molecular profile of the tumor, which can be used to develop personalized cancer treatments. EVs have also been explored as diagnostic biomarkers for other diseases, such as cardiovascular disease, neurological disorders, and infectious diseases. For example, EVs released by damaged or diseased heart tissue contain specific proteins and nucleic acids that can be used to diagnose and monitor cardiovascular disease.

Recent research has shown that EVs have enormous potential as therapeutic agents for a variety of diseases, including cancers, neurodegenerative diseases, and cardiovascular disease. Here are some of the key ways that EVs are being investigated for therapeutic use. In cancer therapy, EVs derived from stem cells have been shown to inhibit tumor growth and metastasis in several animal models of cancer. EVs have also been explored as a means of drug delivery, as they can be engineered to carry therapeutic molecules directly to tumor cells. In neurodegenerative diseases, EVs derived from stem cells have shown promise in treating neurodegenerative

diseases such as Parkinson's and Alzheimer's. These EVs contain factors that can protect neurons from damage and promote their survival. In cardiovascular disease, EVs derived from endothelial cells have been shown to improve heart function after a heart attack in animal models. EVs can also carry proteins and genetic materials that promote angiogenesis, which can help to repair damaged blood vessels. In autoimmune diseases, EVs have been shown to play a role in regulating immune responses, and EVs derived from stem cells have been explored as a potential treatment for autoimmune diseases such as multiple sclerosis and rheumatoid arthritis. Overall, EVs offer a promising avenue for the development of novel therapeutics for a wide range of diseases. While there are still many challenges to overcome, including standardization of isolation and characterization techniques, early results suggest that EVs may be a powerful tool in the fight against disease.

EVs have been shown to have tremendous potential in regenerative medicine. EVs are small, membrane-bound structures secreted by cells that contain a variety of biomolecules, including lipids, proteins, and nucleic acids. They play a critical role in intercellular communication and have been shown to have therapeutic effects in a variety of preclinical models of disease. Here are some of the key ways that EVs are being investigated for regenerative medicine. In tissue repair, EVs derived from stem cells have been shown to promote tissue repair in a variety of preclinical models, including wound healing, bone regeneration, and cartilage repair. These EVs contain a variety of growth factors and other molecules that can promote cell proliferation and differentiation, as well as regulate the immune response. In cardiovascular regeneration, EVs have been shown to have beneficial effects on cardiac function following a heart attack in animal models. EVs can carry proteins and genetic material that promote angiogenesis, or the growth of new blood vessels, which can help repair damaged tissue and improve blood flow. In neuroregeneration, EVs have shown promise in promoting neuronal survival and regeneration in preclinical models of neurological disease and injury, including stroke and traumatic brain injury. These EVs can carry neuroprotective factors, and promote the growth and differentiation of new neurons. Overall, EVs offer a promising approach to regenerative medicine, while there are still challenges to overcome, including standardization of isolation and characterization techniques.

1.4 Extracellular Vesicles Isolation and Limitations

EVs have attracted considerable attention as potential biomarkers for various diseases, including cancer, cardiovascular disease, and neurodegenerative disorders. However, the isolation of EVs is a complex and challenging process, and there are several limitations in the manufacturing and clinical translation of EV-based therapeutics.

Isolation of EVs is a crucial step in their study and application. There are several methods for isolating EVs, including ultracentrifugation, size-exclusion chromatography, immunoaffinity capture, and microfluidics-based techniques. Each method has its advantages and limitations in terms of purity, yield, and

scalability. Ultracentrifugation is the most commonly used method for EV isolation, but it can result in coisolation of non-EV contaminants, such as protein aggregates and lipoproteins. Size-exclusion chromatography can provide higher purity, but it is less efficient and requires specialized equipment. Immunoaffinity capture can specifically isolate EVs expressing certain surface markers, but it can result in low yields and requires specific antibodies. Microfluidics-based techniques have the potential for high throughput and precise isolation, but they are still in the development stage and not yet widely used.

There are also some limitations in both manufacturing and clinical translation. One of the major limitations in manufacturing EV-based therapeutics is the variability in EV isolation methods that can lead to variations in the size, content, and purity of EVs. This can impact the efficacy and safety of EV-based therapeutics. Moreover, the scalability of EV isolation methods is also a challenge, as large-scale production of EVs is currently not feasible. Another challenge in manufacturing EV-based therapeutics is the lack of standardized protocols for EV characterization, which can lead to inconsistencies in the reporting of EV-related data. This can make it difficult to compare data between different studies and hinder the development of EV-based therapeutics. As for the clinical translation of EV-based therapeutics is the lack of a regulatory framework for EVs, there is currently no FDA-approved EV-based therapy, and the regulatory pathway for EVs is not well defined. This can make it challenging for researchers and companies to develop and commercialize EV-based therapeutics. Another challenge in clinical translation is the heterogeneity of EVs, which can make it difficult to define a specific population of EVs for therapeutic use. Moreover, the biodistribution and pharmacokinetics of EVs are not well understood, and there is limited data on the safety and efficacy of EV-based therapeutics in humans.

In conclusion, while EVs hold great potential as biomarkers and therapeutics for various diseases, there are several challenges in the isolation, manufacturing, and clinical translation of EV-based products. These challenges highlight the need for standardized protocols, regulatory guidance, and further research to fully harness the potential of EVs for clinical use.