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Application of Exosomes in the Diagnosis and Treatment of Neurodegenerative Diseases

Haotian Yin

Nanjing No.13 High School, 14,Xijiadatang, Xuanwu District, Jiangsu, China

7779975@qq.com

Abstract. As the aging population becomes a growing concern in modern society, the incidence of neurodegenerative diseases, characterized by the loss of neurons in the brain and spinal cord, has been steadily rising. An increasing number of individuals are afflicted by these conditions, yet effective solutions remain elusive. Current medical approaches to neurodegenerative diseases are far from ideal, with treatment options primarily focused on attempting targeted therapy through medications. However, these treatments are only capable of alleviating symptoms and cannot provide a cure. Over the past few decades, advancements in science and technology have paved the way for the discovery of novel drugs, antibodies, vaccines, and stem cells, all of which have laid a solid foundation for the successful treatment of neurodegenerative diseases. This paper aims to discuss what neurodegenerative diseases are, the discovery of exosomes, and the relationship between exosomes and the diagnosis and treatment of neurodegenerative diseases.

Keywords: Exosomes, Neurodegenerative diseases, Isolation and identification, Early diagnosis, Treatment.

1. Introduction

1.1. Neurodegenerative Diseases

Neurodegenerative diseases are a group of disorders caused by the loss of neurons and their myelin sheaths, which progressively worsen over time, leading to neurological dysfunction[1]. The hallmark of neurodegenerative diseases is the degeneration or dysfunction of neuronal structures, with common conditions including Alzheimer's disease, Parkinson's disease, and stroke[2]. The symptoms vary depending on the type of disease and the affected area of the nervous system. Common symptoms include memory decline, cognitive impairment, motor retardation, balance disorders, speech difficulties, and sleep disturbances[3]. These symptoms tend to worsen over time, severely affecting patients' daily lives and independence. Currently, there is no cure for neurodegenerative diseases, and treatments are primarily focused on symptom relief, improving quality of life, and slowing disease progression.

1.2. Alzheimer's Disease

Alzheimer's disease (AD) is a typical neurodegenerative disease, with a complex etiology involving multiple factors such as genetics, environment, and lifestyle. This complexity is one of the main reasons why there is currently no cure for Alzheimer's disease[4]. The key pathological features include the formation of β-amyloid plaques in the brain and neurofibrillary tangles caused by the excessive

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phosphorylation of tau protein. These pathological changes result in neuronal damage and death, as well as a decrease in acetylcholine release, which gradually leads to cognitive decline and memory loss. Although the exact pathogenesis of Alzheimer's disease is not fully understood, research suggests that inflammation, oxidative stress, cholesterol metabolism abnormalities, and neurotransmitter system dysregulation may play significant roles in the progression of the disease[5]. Moreover, neurons are terminally differentiated cells, meaning that their damage and death are irreversible. The blood-brain barrier (BBB) further complicates treatment, as it prevents over 95% of small-molecule drugs and almost all large-molecule drugs from entering the brain. This barrier is a major obstacle to treating Alzheimer's disease through tau protein inhibition or by reducing β-amyloid plaques[6].

Lecanemab has emerged as a potential option for alleviating Alzheimer's disease. It is a humanized monoclonal antibody specifically designed to bind to and clear β-amyloid (Aβ) aggregates in the brain, particularly soluble Aβ protofibrils and oligomers. By targeting these structures, lecanemab aims to reduce the Aβ burden in the brain, thereby slowing the pathological progression of Alzheimer's disease. In a randomized, double-blind, phase IIb clinical trial, lecanemab demonstrated efficacy in reducing amyloid plaques in the brains of Alzheimer's patients at various doses and dosing regimens. Notably, the biweekly administration of 10 mg/kg showed early and sustained activity, with a more significant reduction in amyloid plaques compared to the placebo group. However, lecanemab is not without limitations. One of the concerns with its use is the potential side effect of amyloid-related imaging abnormalities (ARIA). Before administering the drug, amyloid testing is required, and brain MRI monitoring is necessary during treatment to observe any occurrence of ARIA.

1.3. Parkinson's Disease

The causes of Parkinson's disease (PD) include the degeneration of dopaminergic neurons, genetic factors, exposure to environmental toxins, aging, and head trauma. Since the gradual loss and death of dopaminergic neurons are irreversible, Parkinson's disease cannot be fully cured by medication[7]. Levodopa is one of the most effective drugs in the treatment of Parkinson's disease, as it can cross the blood-brain barrier and be converted into dopamine in the brain, replenishing the reduced dopamine levels caused by Parkinson's disease. Dopamine is a critical neurotransmitter essential for regulating motor functions. Levodopa is commonly used in combination with a decarboxylase inhibitor, such as carbidopa, to reduce the peripheral metabolism of levodopa, increase its availability in the brain, and improve its efficacy while minimizing side effects. However, long-term use of levodopa may lead to complications such as diminished efficacy, symptom fluctuations, and motor disorders. Some reports suggest that high doses of levodopa used over an extended period may accelerate disease progression. In addition, levodopa can cause adverse reactions, such as nausea, vomiting, and dizziness[8]. Amantadine, originally an antiviral drug, is also used in the treatment of Parkinson's disease, particularly in the early stages of the disease. Its mechanism of action includes promoting the release of dopamine in the striatum and possibly affecting dopamine reuptake. Amantadine can be used alone or in combination with levodopa and other antiparkinsonian drugs to improve motor symptoms such as tremors, muscle rigidity, and bradykinesia. Potential side effects of amantadine include irritability, ankle edema, livedo reticularis (a skin condition), and psychiatric symptoms. Patients with liver or kidney dysfunction or epilepsy should use it with caution, and it is contraindicated for breastfeeding women[9].

1.4. Stroke

The causes of stroke primarily include vascular abnormalities, blood component disorders, heart disease, and lifestyle factors. Stroke can be categorized into two main types: ischemic and hemorrhagic. Ischemic stroke occurs due to the obstruction of cerebral blood vessels, leading to brain tissue ischemia and hypoxia, while hemorrhagic stroke results from the rupture of blood vessels, causing intracerebral hemorrhage. The lack of an effective cure for stroke stems from the complexity of its pathological processes and the narrow therapeutic window[10]. For ischemic stroke, the primary drug treatment is tissue plasminogen activator (tPA), which can dissolve clots and restore blood flow to the brain. However, this treatment is time-sensitive, typically effective only within 4.5 hours of stroke onset, and

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carries the risk of causing intracranial hemorrhage. Even if the clot is successfully dissolved, brain tissue may suffer further damage during reperfusion after ischemia, a process for which no effective drug currently exists to fully prevent or treat this reperfusion injury. The treatment of hemorrhagic stroke is even more complex, primarily relying on symptomatic treatment and surgical intervention to control bleeding and reduce intracranial pressure. Once brain tissue has undergone necrosis, no medication can restore its function, so treatment focuses on preventing further brain damage and promoting the recovery of neurological functions[11].

In the search for solutions to the challenges posed by these neurodegenerative diseases, attention has gradually shifted to a substance capable of transporting small molecules. Some experts speculate that this substance might be harnessed for effective targeted therapy, and this substance is exosomes.

2. Exosomes

The discovery of exosomes dates back to 1983, when Rose M. Johnstone's research team at McGill University in Canada first identified these small vesicles in the supernatant of cultured sheep reticulocytes. Initially, these vesicles were considered "cellular waste" produced by metabolism. However, in 1987, Johnstone named these vesicles "exosomes." As research progressed, scientists gradually recognized the crucial role of exosomes in intercellular communication. They are not only present in red blood cells but are also widely found in various body fluids, participating in a range of biological processes such as immune responses, antigen presentation, and cell migration. In 2013, research on the regulatory mechanisms of vesicle transport, including exosomes, earned James E. Rothman, Randy W. Schekman, and Thomas C. Südhof the Nobel Prize in Physiology or Medicine, further highlighting the significance of exosomes in the scientific community [12].

2.1. What Are Exosomes?

Exosomes are small vesicle-like structures secreted by living cells, with a diameter ranging from 30 to 150 nanometers, and they possess a lipid bilayer membrane [13]. Exosomes primarily originate from multivesicular endosomes (MVEs) inside the cell. These MVEs fuse with the cell membrane through exocytosis, releasing vesicles containing cellular proteins, lipids, DNA, RNA, and other molecules into the extracellular environment. Exosomes participate in various biological processes, including immune responses, antigen presentation, cell differentiation, tumor growth, and invasion. They play a role in intercellular communication and material exchange, influencing cellular physiological states and are closely associated with the development and progression of various diseases [14].

2.2. Exosome Source Cells

Exosomes can be derived from various cell types, including immune cells, neurons, epithelial cells, endothelial cells, cancer cells, and stem cells (such as embryonic and mesenchymal stem cells). In previous studies, specific cell types are often selected for exosome extraction depending on the research purpose and application. For example, exosomes derived from mesenchymal stem cells (MSCs) have been widely studied and applied due to their rich bioactive components and ability to promote tissue repair [15]. Additionally, exosomes derived from all blood sources are commonly used, as they can be easily obtained through simple blood draws and contain a wealth of biomarker information. The extraction of exosomes from specific cell sources is primarily driven by the fact that these exosomes may carry specific molecular signals valuable for disease diagnosis, treatment, and research. For instance, in the diagnosis of Alzheimer's disease (AD), neurons and other brain cells in AD patients secrete exosomes containing pathological molecules into the bloodstream. By analyzing the molecular composition of these exosomes, AD biomarkers can be detected. Research has shown that the levels of Aβ42, T-tau, and P-tau181 in exosomes are significantly higher in AD patients than in healthy controls, making these molecules potential biomarkers for the early diagnosis of AD.

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2.3. Methods for Exosome Isolation

2.3.1. Ultracentrifugation. Ultracentrifugation is one of the standard methods for isolating exosomes. It relies on centrifugal force to remove cell debris, proteins, and small particles, ultimately yielding exosome samples. The specific steps involve using different centrifugation speeds to remove particles of varying sizes, with the final exosome pellet obtained after several centrifugation rounds. This process may include steps such as centrifugation at 300×g, 2000×g, 10000×g, and 100000×g to progressively remove larger impurities and purify the exosomes.

2.3.2. Density Gradient Centrifugation. Density gradient centrifugation combines ultracentrifugation with a density gradient to improve exosome purity. By establishing a gradient with sucrose or other density media during centrifugation, exosomes can be positioned within the gradient according to their density, achieving more efficient separation.

2.3.3. Polymer Precipitation. Polymer precipitation uses high-molecular-weight polymers, such as polyethylene glycol (PEG), to alter the solubility and dispersibility of exosomes, allowing them to precipitate under lower centrifugal forces. This method is simple to operate and does not require an ultracentrifuge, but it may co-precipitate non-vesicular contaminants.

2.3.4. Ultrafiltration. Ultrafiltration employs membranes to separate particles based on molecular size, allowing the isolation of exosomes from a sample. This method is convenient and efficient for exosome enrichment, though it may cause exosome loss and membrane clogging.

2.3.5. Magnetic Bead-Based Specific Capture. Magnetic bead-based specific capture uses specific proteins on the surface of exosomes as separation markers. Antibody-coated magnetic beads bind to exosomes, enabling their specific enrichment. This method can yield highly pure exosomes while preserving their membrane structure without significant damage.

2.4. Methods for Exosome Identification

2.4.1. Transmission Electron Microscopy (TEM). Transmission electron microscopy (TEM) utilizes a high-energy electron beam to pass through a sample, producing images based on the interaction between the electrons and the sample, allowing observation of the sample's ultrastructure. In exosome identification, TEM provides high-resolution images that reveal the bilayer membrane structure and morphological characteristics of exosomes. Negative staining is commonly employed to enhance the contrast of exosomes, making them easier to observe under the microscope.

2.4.2. Western Blot. Western blot is a protein detection technique that uses specific antibodies to identify proteins within a sample. In the identification of exosomes, Western blot is employed to detect exosome-specific protein markers, such as CD63, TSG101, and Alix. The presence of these markers helps confirm that exosomes are present in the sample.

2.4.3. Exosome Tracing. Exosome tracing involves labeling exosomes with fluorescent markers or radioactive isotopes to track their distribution and behavior within biological systems. This method is particularly useful when studying the biological functions of exosomes and their potential clinical applications.

After successfully extracting and isolating exosomes, experts have conducted a series of evaluations and studies, ultimately determining that exosomes can be used as a tool for the diagnosis and treatment of neurodegenerative diseases. The following sections will focus on how exosomes are employed in the diagnosis and treatment of these conditions.

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3. Exosomes and the Diagnosis of Neurodegenerative Diseases

The diagnosis of neurodegenerative diseases typically involves a combination of clinical evaluations, medical history, imaging studies, and biomarker testing. Physicians begin by carefully assessing the patient's medical history and observing clinical manifestations. These diseases often share common clinical features, such as memory decline, cognitive impairment, and motor dysfunction. Based on the patient's symptoms, onset, and family history, physicians can make an initial assessment of the likely type of neurodegenerative disease. Imaging studies play a crucial role in diagnosis. Magnetic resonance imaging (MRI) can help doctors detect structural abnormalities in the brain, such as hippocampal atrophy in Alzheimer's disease patients. Positron emission tomography (PET) can also be used to assess metabolic activity and protein deposition in the brain[16]. Neuropsychological evaluations may also be considered, involving standardized tests that assess memory, attention, language, and executive functions. These tests help in determining whether a patient may be suffering from a neurodegenerative condition. Blood tests, in the context of neurodegenerative disease diagnosis, focus on detecting biomarkers in the blood. These biomarkers are proteins or other molecules released by damaged or dying nerve cells through exosomes into the bloodstream. By analyzing the levels of these biomarkers, doctors can gain insights into the health of the nervous system[17].

Phosphorylated tau protein (p-tau) detection is a blood test used to assist in the diagnosis of Alzheimer's disease. This test analyzes specific forms of tau protein in the blood to identify Alzheimer's disease patients, as tau protein becomes abnormally phosphorylated in the brain and forms neurofibrillary tangles. The process begins by collecting a blood sample from the patient, typically using anticoagulant tubes to prevent blood clotting. The blood sample may then be centrifuged to separate plasma and undergo necessary pre-processing to prepare for immunoassay analysis. A detection plate or microbeads coated with specific antibodies are then used to bind to the phosphorylated tau protein present in the blood sample. In enzyme-linked immunosorbent assay (ELISA), the sample is added to a microplate containing capture antibodies, followed by the addition of detection antibodies, which are usually linked to enzyme markers. The enzyme reaction causes a color change, allowing quantification. In the Single Molecule Array (Simoa) technique, tiny magnetic beads and a highly sensitive detection system are used to quantify extremely low concentrations of p-tau. The color change from the enzyme reaction or the fluorescent signal from the magnetic beads is read by the instrument, which converts it into a p-tau concentration reading. Finally, the test results are compared against normal reference ranges or Alzheimer's disease-specific thresholds to assist doctors in making a diagnosis[18]. With the latest advances in research and development, the sensitivity and specificity of p-tau detection have steadily improved, making it a promising blood biomarker. This advancement helps increase the accessibility of Alzheimer's disease diagnosis globally.

Similarly, exosomes can be used for diagnosing Parkinson's disease. A typical example involves detecting α-synuclein in exosomes derived from plasma to diagnose Parkinson's disease. Abnormal aggregation of α-synuclein in exosomes is considered a hallmark of Parkinson's disease, and its aberrant expression in the early stages of the disease may serve as a key biomarker for early diagnosis and disease monitoring. Researchers have optimized exosome isolation and detection techniques to enhance the sensitivity and specificity of α-synuclein detection, providing a new non-invasive approach to diagnosing Parkinson's disease. Techniques such as ultracentrifugation, size-exclusion chromatography, and magnetic bead capture can effectively enrich exosomes from plasma. Subsequently, methods like immunoblotting or ELISA can be used to quantitatively analyze α-synuclein levels[19].

A detailed example of exosome-based stroke diagnosis, particularly ischemic stroke, involves the detection of microRNA (miRNA). miRNA is a class of small non-coding RNA molecules that play a key role in intercellular communication, especially in the progression of neurodegenerative diseases and brain injuries. Specific miRNAs present in exosomes, such as miR-124, miR-146a, and miR-9, have been studied as biomarkers for stroke diagnosis. To perform the diagnostic test, a blood sample is collected from suspected stroke patients, typically using EDTA anticoagulant tubes. After the sample is left to stand at room temperature, it is centrifuged to remove cellular components. Techniques such as ultracentrifugation or size-exclusion chromatography are then used to purify exosomes from the plasma.

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This process may involve multiple rounds of centrifugation to ensure the separation of exosomes from cellular debris. Next, commercial RNA extraction kits, such as TRIzol, are used to extract total RNA from the exosomes. The extracted RNA is reverse-transcribed into complementary DNA (cDNA), followed by real-time quantitative PCR (qPCR) to detect and quantify the expression levels of specific miRNAs. The expression levels of miRNAs are then compared between the stroke patient group and a healthy control group to identify miRNAs with significant differential expression. The data is normalized using the 2^-ΔΔCt method to assess the relative expression levels of the miRNAs. Furthermore, receiver operating characteristic (ROC) curve analysis is performed to determine the diagnostic threshold and evaluate the sensitivity and specificity of the miRNA as a biomarker for stroke. For example, specific miRNAs in the plasma exosomes of stroke patients show significant alterations compared to healthy controls. High expression of miR-146a may be associated with inflammatory responses in cerebral blood vessels, while downregulation of miR-9 might correlate with neuronal damage. These findings not only aid in the early diagnosis of stroke but also provide new insights into the pathological mechanisms of stroke[20].e

4. Exosome-Based Treatment of Neurodegenerative Diseases

Once a patient has been diagnosed with a neurodegenerative disease following the relevant diagnostic steps, experts will evaluate whether the patient is suitable for exosome-based therapy. This evaluation includes a comprehensive assessment of the patient's cognitive function, ability to perform daily activities, and any potential risk factors. Based on the individual differences of the patient, an appropriate exosome treatment plan is selected. For instance, in a clinical trial conducted by Professor Gang Wang's team at Ruijin Hospital, affiliated with Shanghai Jiao Tong University School of Medicine, mesenchymal stem cell (MSC)-derived exosomes were administered using a nasal spray[21]. The exosome dosage and treatment course were determined based on data and experiences from clinical trials. In the aforementioned trial, patients were divided into low-dose, medium-dose, and high-dose groups to identify the optimal therapeutic effect. Based on the patient's response to treatment and monitoring results, experts may adjust the treatment plan, including modifying the dosage or frequency of treatment to achieve the best therapeutic outcome. In recent clinical trials, nasal administration of MSC-derived exosomes for the treatment of Alzheimer's disease has been shown to be safe, with cognitive improvements and reduced hippocampal atrophy observed in the medium-dose group. These results offer new hope for the treatment of Alzheimer's disease and lay the foundation for further clinical research. In practical applications, experts use the latest research findings to guide treatment decisions, tailoring therapies to the specific needs and responses of each patient.

In a notable example of exosome therapy for Alzheimer's disease, Professor Gang Wang's team at Ruijin Hospital, affiliated with Shanghai Jiao Tong University School of Medicine, used mesenchymal stem cell (MSC)-derived exosomes in their treatment approach. MSC-derived exosomes were selected due to their ability to carry a variety of regulatory molecules, such as proteins and RNA, which are believed to protect neuronal cells and promote neuroregeneration. These exosomes are considered advantageous due to their low immunogenicity and ease of acquisition. The exosomes are extracted from stem cells using specific cultivation and isolation techniques to ensure their purity and biological activity. The exosomes were administered via a nasal spray, a delivery method chosen to bypass the blood-brain barrier and act directly on the brain. The nasal spray was carefully designed, taking into account the size and surface characteristics of the exosomes to enhance their effective delivery. In the clinical trial, patients were assigned to different dosage groups to evaluate the safety and efficacy of various doses. Before and after the treatment, patients underwent comprehensive safety evaluations, including biochemical tests and clinical observations, to ensure the treatment's safety. To assess the treatment's efficacy, several cognitive function assessment scales were used, such as the Alzheimer's Disease Assessment Scale-Cognitive Subscale (ADAS-cog), the Mini-Mental State Examination (MMSE), and the Montreal Cognitive Assessment-B (MoCA-B). Additionally, the Activities of Daily Living scale (ADCS-ADL) was employed to measure the patients' abilities in daily tasks, providing further insights into the therapy's impact.

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In recent years, exosomes have demonstrated significant potential as therapeutic carriers for Parkinson's disease. Stem cell-derived exosomes, engineered to carry specific small interfering RNA (siRNA) or microRNA (miRNA), are designed to inhibit the abnormal aggregation of α-synuclein (α-synuclein). Exosomes are extracted from human umbilical mesenchymal stem cells, and siRNA or miRNA is loaded into the exosomes using lipid transfection, electroporation, or other biotechnological methods. These RNA molecules can specifically bind to and degrade α-synuclein mRNA, thereby reducing its expression. Using a Parkinson's disease mouse model, the engineered exosomes are administered via tail vein injection. Researchers monitor the biodistribution of the exosomes and their impact on α-synuclein expression and related pathological features. Behavioral tests (such as the rotarod test) and histological analyses (such as immunohistochemistry) are used to assess the therapeutic effects of exosome treatment on Parkinson's symptoms. Studies have shown that engineered exosomes can effectively cross the blood-brain barrier, reach the brain, reduce α-synuclein accumulation, and improve motor dysfunction and pathological features in Parkinson's disease mouse models. This provides a potential non-invasive therapeutic strategy for Parkinson's disease patients. Using exosomes as therapeutic carriers not only enhances drug targeting and bioavailability but also reduces systemic side effects, offering a new avenue for Parkinson's disease treatment. However, research in this area is still in its early stages, and more preclinical and clinical studies are needed to verify its safety and efficacy.

Using stem cell-derived exosomes loaded with miRNA, such as miR-133b, which can inhibit inflammation and promote angiogenesis, represents an ideal treatment approach for mitigating neurological damage following a stroke. In this process, exosomes are extracted from mesenchymal stem cells, and miR-133b is efficiently loaded into the exosomes using bioengineering techniques to enhance their therapeutic effects. In studies utilizing rat or mouse models of ischemic stroke, engineered exosomes are administered via tail vein injection. Research shows that these exosomes can effectively target and deliver their contents to the brain's damaged areas. Behavioral tests (such as neurological function scoring) and histological analyses (including immunohistochemistry and TTC staining) are then used to evaluate the effects of exosome treatment on post-stroke recovery, specifically in terms of neurological function and the size of the damaged area. Further analyses using techniques like qPCR and Western blotting reveal how miR-133b affects the expression of genes and proteins associated with neuroprotection and angiogenesis after exosome therapy. The results show significant improvements in animals treated with engineered exosomes compared to control groups, with enhanced neurological recovery and reduced brain damage[22]. The delivery of miR-133b not only suppresses inflammatory responses but also promotes angiogenesis and the survival of neural cells [23].This research offers a new strategy for stroke treatment, utilizing exosome-mediated miRNA delivery to reduce brain damage and potentially promote neural repair and functional recovery. However, clinical translation of this approach still requires further in-depth studies, including safety assessments and large-scale clinical trials.

5. Conclusion

In summary, exosomes have shown unprecedented potential in the diagnosis and treatment of neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease. As natural tools for intercellular communication, exosomes can serve as carriers of disease biomarkers, offering the possibility of early diagnosis. Additionally, they can act as efficient delivery vehicles for therapeutic molecules, directly targeting diseased neurons to promote neuroprotection and repair. From a diagnostic perspective, specific miRNAs, proteins, and lipids within exosomes provide non-invasive detection methods for neurodegenerative diseases, aiding in early detection and monitoring. On the therapeutic side, engineered exosomes can be loaded with therapeutic cargo (such as miRNA, siRNA, or neurotrophic factors) to precisely regulate pathological molecular processes, reduce inflammation, promote neural regeneration, and improve neurological function. Although current research primarily focuses on animal models and in vitro experiments, and challenges such as the standardization of exosome production, their fate within the body, and long-term safety remain to be addressed, exosomes undoubtedly offer new hope for precision medicine in neurodegenerative diseases. As more preclinical

and clinical studies are conducted, exosomes are expected to become an innovative, effective, and safe tool for the diagnosis and treatment of these diseases in the future.

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