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RESEARCH ARTICLE

Cancer-derived Exosomes Protect Human Neuronal Cell Death in Alzheimer's Disease Model

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ABSTRACT: Neurodegenerative diseases (NDDs) are a group of incurable disorders that cause the loss of neurons. To develop potential treatments against NDDs, mainly the leading Alzheimer's Disease (AD), tumor-derived exosomes (TDEs) are being explored to test their mitigation effect on human neuronal cell death. We hypothesized that TDEs will protect human neuronal cells in the AD model. This study differentiated neuroblastoma cells using 0.5 μM and 5 μM retinoic acid (RA) concentrations. The AD model was established using 5 μM of scopolamine hydrobromide (SH). With the optimal conditions determined, the protective effects of TDEs on neuronal cells were investigated using a transwell model that allowed the exchange of exosomes between neurons and cancer cells. The impact of TDEs on neuronal cells in the AD model was examined by comparing the cell viability of neuronal cells with and without exosomes. Our hypothesis was confirmed as the cell viability of neuronal cells was significantly higher when TDEs were present than when they were absent. This study suggests a practical approach to using highly accessible and cost-effective TDEs to develop potential treatments. Since these exosomes are efficient drug carriers, they can be used to create personalized medicine for patients.

KEYWORDS: Cellular and molecular biology; Molecular Biology; Alzheimer's Disease; Exosome; Cell Viability.

Introduction

Neurodegenerative diseases (NDDs) are a group of neurological disorders that entail the gradual loss of neurons in either the central nervous system (CNS) or peripheral nervous system (PNS). The inability of neural cells to regenerate due to their differentiated nature leads to the collapse of neural networks and the breakdown of communicative circuitry, resulting in severe disabilities and deaths.[1] In a single year, NDDs affected more than 50 million people worldwide, and the incidence is expected to rise as the world's population ages due to an increase in life expectancy.[2] Despite significant efforts to develop effective cures, most NDDs remain incurable.[3]

Alzheimer's disease (AD) is a multifactorial NDD marked by the presence of neuritic plaques and neurofibrillary tangles resulting from the accumulation of amyloid-beta peptide (Aβ) in the affected brain regions. It is a prevalent cause of cognitive impairment that primarily affects individuals in midlife and late life, and its clinical impact is influenced by coexisting neurodegenerative and cerebrovascular conditions like head injuries, vascular diseases, and infections.[4] AD is also known as the leading cause of dementia, characterized by a decline in cognitive abilities and memory.[5] At present, there are around 50 million AD patients worldwide, and the number is expected to double every five years, reaching 152 million by 2050.[6] Yet, there is no cure for AD other than treatments effective only in treating the symptoms.

Tumor-derived exosomes (TDEs) are small membranous microvesicles ranging from 30 nm to 200 nm, found in intracellular multivesicular bodies secreted by most types of cells via exocytosis. These exosomes contain essential genetic materials like DNA, mRNA, microRNA, proteins, and lipids developed from their parent cell, and they serve as signal transducers in intercellular communication.[7] Their high biocompatibility and low immunogenicity due to membrane proteins such as tetraspanin and fibronectin allow efficient transportation of nucleic acids, proteins, and lipids in the interaction between the parent cell and the adjacent cells.[8] TDEs are extensively studied today because they are significant biomarkers for cancer diagnosis and can facilitate tumor cell elimination.[9] TDEs can foster an immunosuppressive environment that hinders tumor cell growth. Moreover, TDEs can be utilized to deliver chemotherapy drugs aimed at oncogenic signaling to exert anti-tumor effects or to modify parent cells to release altered exosomes genetically.[10]

Given the increasing global prevalence of NDDs with limited treatments, this research investigates the effects of TDEs in pathogenesis and their potential role in future therapies. I hypothesize that these exosomes will alleviate cell death associated with NDDs by protecting cells from inflammation and oxidative stress.

Methods

Cell culture and maintenance:

A172 glioblastoma cells and SHSY5Y neuroblastoma cells were cultured in RPMI1640 cell culture media supplemented with FBS and antibiotics. When the cells reached 70-80% confluency, cells were detached from the culture plate using the Trypsin-EDTA cell dissociation buffer and moved to the new culture dish to maintain the cells in healthy condition.

Retinoic acid and scopolamine hydrobromide treatment:

Retinoic acid (RA) and scopolamine hydrobromide (SH) were purchased from Thermofisher. RA and SH stock solu-

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tions were prepared with DMSO solvent. This study used the indicated diluted conditions for RA and SH.

Cell viability analysis and imaging using a fluorescence microscope:

The AO/PI staining method was used to assess cell viability in this study. First, the cell culture medium was removed. Then, a staining solution containing acridine orange (AO) and propidium iodide (PI) was prepared by diluting 100 µg/mL of each in PBS. The staining solution was added to the cells and incubated in the dark at room temperature for 5 minutes. After incubation, cells were analyzed under a Luna-FL cell counting device, where live cells appeared green and dead cells appeared red. The number of live and dead cells was measured, and the percentage of viable cells was calculated. This process was repeated in triplicate to ensure reliable results.

Exosome isolation from the glioblastoma cancer cells:

The cell culture media containing exosomes was collected when the A172 cells reached 80% confluency. Then, the manufacturer provided Total Exosome Isolation Reagent (Invitrogen) instructions to precipitate and enrich the exosome from the collected cell culture media. The cells and debris were removed after the condition media was centrifuged for 30 minutes at 2000 x g speed. To the 10 mL of cultured media, 5 mL total exosome isolation reagent was added. The mixed solution was vortexed to make a homogenous solution. Then, the sample was incubated at 4 ˚C overnight. After the incubation, the samples were centrifuged at 10,000 x g for 1 hour at 4˚C. After aspirating the culture media, the exosome pellet was collected at the bottom of the tube. The exosome pellet was resuspended with the 1mL PBS buffer.

Result and Discussion

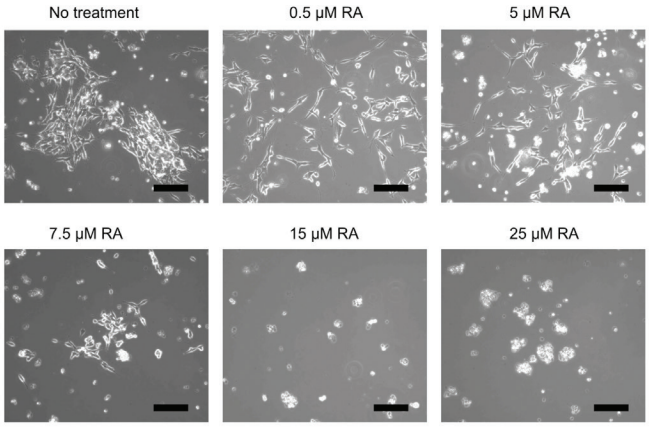

Figure 1: Retinoic acid (RA) treatment changes the cell morphology and differentiates the neuroblastoma SHSY5Y cell line. Different RA concentrations (0 µM, 0.5 µM, 5 µM, 7.5 µM, 15 µM, 25 µM) were incubated for three days. These results suggest that 0.5 µM and 5 µM RA concentrations are optimal for inducing differentiation in SHSY5Y neuroblastoma cells while minimizing toxicity.

Neuroblastoma cells are differentiated into neuronal cells during normal embryonic development using RA. Different RA concentrations were treated to determine the appropriate RA concentration. Six different RA concentrations (0 µM, 0.5 µM, 5 µM, 7.5 µM, 15 µM, 25 µM) induced cell differentiation in neuroblastoma cells. The differentiated cells were incubated for three days, and cell imaging was done to examine the cells (Figure 1). Neuroblastoma cells in the no-treatment group (0 µM) are clustered tightly and grew abundantly in the culture, which yielded the highest cell number among the neuroblastoma cells of different RA concentration groups. No neurites are observed in these neuroblastoma cells. Neuroblastoma cells in the 0.5 µM RA treatment group are differentiated into spindle shapes. These cells are separated individually and grow minor neurites to connect the neuronal cells. Neuroblastoma cells in the 5 µM RA treatment group are differentiated into neuronal cells but have many toxic, round shapes. These cells grew more abundantly than those in the 0.5 µM RA treatment group. Neuroblastoma cells in 7.5 µM, 15 µM, and 25 µM RA concentration groups are primarily found in toxic, round shapes, which signifies that these concentrations create an exceedingly toxic environment for cell differentiation. These results indicate that 0.5 and 5 µM RA concentrations are optimal conditions to induce cell differentiation in neuroblastoma cells. Therefore, 0.5 µM and 5 µM RA concentrations are appropriate conditions to differentiate the neuronal cells from SHSY5Y cells.

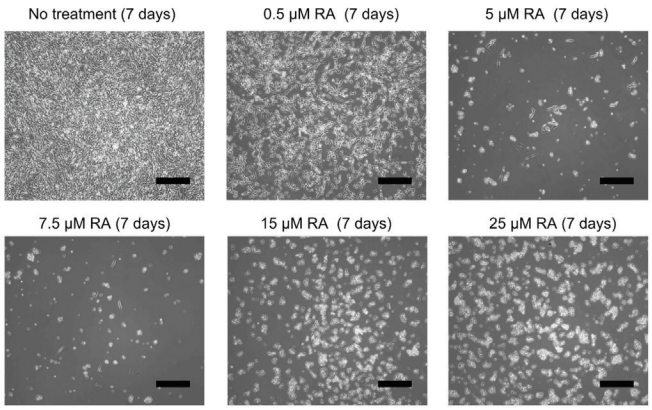

Figure 2: Effect of RA treatment on cell differentiation and neuroblastoma SHSY5Y cell line viability. Different RA concentrations (0 µM, 0.5 µM, 5 µM, 7.5 µM, 15 µM, 25 µM) were incubated for seven days. These findings suggest that a concentration of 0.5 µM RA is optimal for promoting differentiation while maintaining cell viability in SHSY5Y neuroblastoma cells over a seven-day incubation period.

Different RA concentrations are induced to differentiate neuroblastoma cells to observe the changes in cell morphology when RA stays intact with cells for a longer time. Six different RA concentrations (0 µM, 0.5 µM, 5 µM, 7.5 µM, 15 µM, 25 µM) induced cell differentiation in neuroblastoma cells, as shown in Figure 1. The differentiated cells were incubated for seven days, and cell imaging was done to examine the cells. Neuroblastoma cells in the no-treatment group (0 µM) are attached without gaps, and neurites have not fully developed (Figure 2). This group yielded the highest cell line viability among the neuroblastoma cells of different RA concentration groups. When 0.5 µM of RA was induced in neuroblastoma cells for differentiation, cells developed neurites with small gaps between them to connect the cells. The cells developed into spindle shapes, a characteristic of neuronal cells. Neuroblastoma cells in the rest of the RA concentration groups

(5 μM, 7.5 μM, 15 μM, 25 μM) are mainly found in round shapes, which means that these RA concentrations are too toxic for neuroblastoma cells to differentiate and survive. These results indicate that 0.5 μM RA concentration is an optimal condition to induce cell differentiation in neuroblastoma cells. Therefore, 0.5 μM RA concentration is appropriate when cell cultures are incubated for three and seven days.

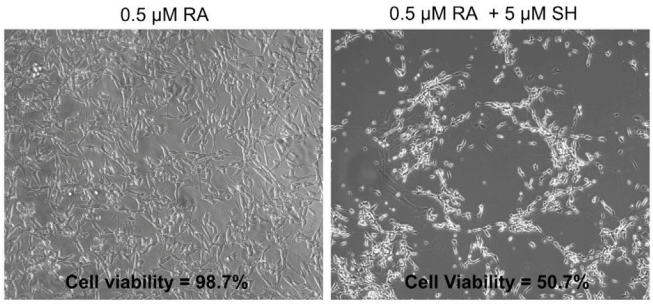

Figure 3: Scopolamine hydrobromide (SH) affects differentiated neuronal SHSY5Y cells' morphology and viability. SH treatment decreases cell viability. These findings establish that a 5 μM SH concentration is optimal for inducing toxicity in neuronal SHSY5Y cells, making it suitable for modeling Alzheimer's disease by reducing cell viability by approximately 50%.

Different concentrations of SH are induced in neuronal SHSY5Y cells to determine the right SH concentration for experimentation. The appropriate SH concentration will decrease the cell viability by nearly 50%. Different concentrations of SH (0 μM, 5 μM) are exposed to differentiated neuronal cells. The cells were incubated for seven days, and cell imaging was done to examine the cells (Figure 3). Differentiated neuronal SHSY5Y cells in the no-treatment group (0 μM) yielded a cell viability of 98.7% because no SH was added to cause toxicity. The cells developed long neurites that confirmed them to be neuronal SHSY5Y cells. However, when 5 μM of SH was added to the cell culture, nearly half of the cells died, with a cell viability of 50.7%. More significant gaps exist between the cells, and some fail to connect through their neurites. These results indicate that 5 μM SH concentration is optimal to induce toxicity in neuronal SHSY5Y cells. Therefore, 5 μM SH concentration will be used as the AD model because it decreases the cell viability by nearly 50%.

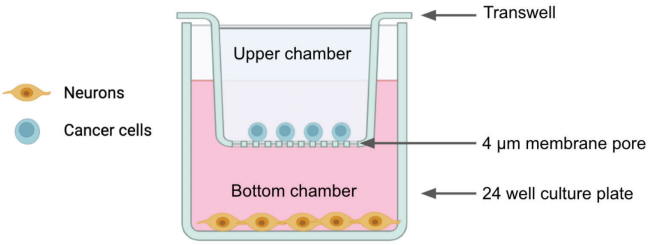

Figure 4: The transwell model was used in this study to separate the neurons and cancer cells and co-cultured. Neurons were cultured in the bottom chamber, and the cancer cells were cultured in the upper chamber. The cells were completely separated since the cancer cells could not move through the transwell. However, since they share the same culture media, molecules smaller than 4 μM can pass through the membrane pore.

Next, the transwell model was designed to create an isolated environment of neurons and cancer cells while allowing exosome exchanges through the membrane pores. By allowing exosomes to pass, we investigated the effects of exosomes produced from the cancer cells on neurons. The cell culture media were shared in the upper and bottom chambers in the transwell model. Therefore, the upper chamber had 4 μM membrane pores on the bottom, allowing exosomes from the cancer cells to pass through (Figure 4). Since the bottom chamber had neurons, the exosomes could pass through the pores and reach neurons. This transwell model was tested in 24 well culture plates.

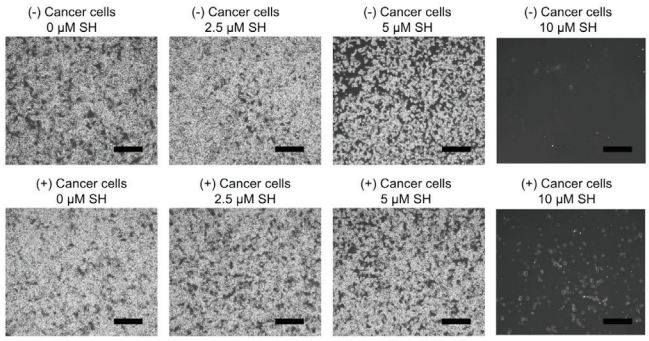

Figure 5: Co-incubation with glioblastoma brain cancer cells' exosome protects the neuronal cells from cell death using the transwell. After the neuronal cells were incubated with the cancer cells for seven days, neuronal cells in the bottom chamber were imaged. The exosomes produced by cancer cells increased the survival rate of neuronal cells in the presence of SH concentrations.

The study investigated the effects of exosomes produced from glioblastoma brain cancer cells on neuronal cells in the SH environment. We hypothesized that the exosomes would have a protective effect on the neuronal cells. Different SH concentrations (0 μM, 2.5 μM, 5 μM, 10 μM) are added to environments with neuronal cells without cancer cells, and this was repeated to those with cancer cells. The transwells are incubated for seven days and imaged. In the transwells of 0 μM SH concentration, there are no notable differences in the survival of neuronal cells between transwells with and without cancer cells. Since there is no stress condition, the neuronal cells are tightly clustered on the bottom of the transwells. There are also no notable differences between transwells with and without cancer cells when 2.5 μM SH concentration was added. Still, fewer neuronal cells survived than the transwells of 0 μM SH concentration. Moving onto transwells with 5 μM SH concentration, the transwell with cancer cells had more neuronal cells surviving than that without cancer cells. Similarly, neuronal cells residing in transwells with 10 μM SH concentration barely survive, but more live cells are found in transwells with cancer cells than those without cancer cells. Therefore, we speculated that the exosomes produced from cancer cells protected the neuronal cells and increased the possibility of neuronal cells surviving through the SH concentrations.

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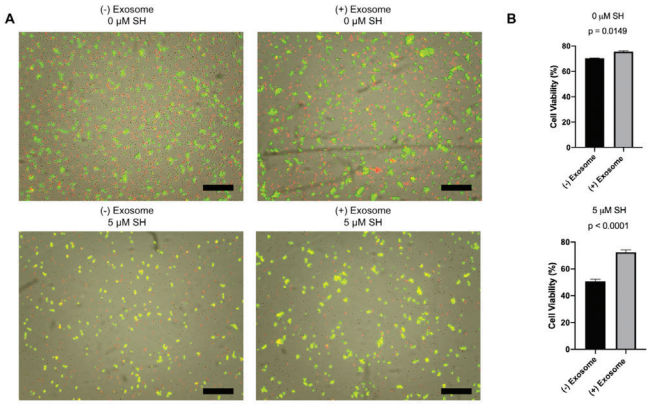

Figure 6: Cancer-derived exosomes protect human brain neuronal cells in both SH minus and SH (A) Image of the live (green) and dead (red) cells after exosome and SH treatment on human brain neuronal cells (B) Quantification of cell viability after exosome and SH treatment on neuronal cells. In the AD treatment model, exosomes derived from cancer protect neuronal cells from apoptosis. The p-value was considered significant if it was less than 0.05, which was calculated using the Student t-test.

The effects of cancer-derived exosomes on neuronal cells treated with SH are studied to test whether the exosomes effectively protect cells. We hypothesized that the cell viability of treatment groups, with and without SH with exosomes, would be higher than those without exosomes. There are four treatment groups: 1) a group without exosome and 0 μM SH concentration, 2) a group without exosome and 5 μM SH concentration, 3) a group with exosome and 0 μM SH concentration 4) a group with exosome and 5 μM SH concentration (Figure 6). The transwells are incubated for seven days, and the cell viability is measured using a cell counting machine. The treatment group without exosome and SH concentration (0μM) yielded a cell viability of 70.3%. This was lower than the cell viability of cells, 75.6% in the environment with the same SH concentration but with exosomes. The treatment group without exosome and SH concentration (5 μM) yielded a cell viability of 50.6%. This was also lower than the cell viability of 72.4% in the environment with the same SH concentration but with exosomes. Cancer-derived exosomes protect neuronal cells from apoptosis in the AD treatment model, SH. Our hypothesis was valid because the cell viability of treatment groups with exosomes was higher than those without exosomes.

■ Conclusion

The study's results confirmed our hypothesis. We determined that an appropriate concentration of RA can differentiate neuroblastoma cells into neuronal cells, while appropriate SH concentrations can be used to create an AD model. The optimal concentration of RA was found to be 0.5μM, and the optimal concentration of SH was found to be either 0 μM or 5 μM. Differentiated neuronal cells were placed in the AD model using such optimal conditions to observe the effects of cancer-derived exosomes on neuronal cell death. The cell viability of neuronal cells when exposed to cancer-derived exosomes was higher than that of neuronal cells alone. Therefore, the study concluded that cancer-derived exosomes protect against human neuronal cell death in the AD model.

It is important to note that this study had some limitations. Our experiment analysis was restricted to only two types of cell lines, namely glioblastoma cells and SHSY5Y neuroblastoma cells. Therefore, further investigation is needed to test more diverse human brain cells to understand better the impact of cancer-derived exosomes on neuronal cells in toxic conditions. Our experiment used *in vitro* models derived from cell differentiation. Since *in vitro* models do not perfectly represent *in vivo*, a mouse experiment should be performed to validate the study's results. Our experiment tested the protective effects of exosomes against SH-dependent brain cell death. Future studies can employ other AD models to observe if the exosomes function similarly to how they did under SH concentrations. Our experiment was restricted to cancer-derived exosomes when testing their effect on the viability of neuronal cells. Future studies can conduct a control experiment with exosomes derived from non-cancerous cells to observe their effect on the viability of neuronal cells. This will emphasize the unique protective effect of cancer-derived exosomes on neuronal cells.

Addressing the variability in exosome composition is important for interpreting experimental results. Factors such as cell source, cancer type, and isolation method may contribute to the variability and reproducibility of our experimental results. Therefore, standardizing exosome isolation and characterization of exosome composition is essential to enhance the reliability of future clinical trials.

This study proposes a potential method to develop AD treatment. Previous studies identified the contents of cancer cell exosomes, including cell proliferating proteins and miRNA. This study discovers the protective effects of exosome treatment on stressed brain cells. Since exosomes are easily accessible and cost-effective, they qualify as suitable resources for developing AD treatment. Exosomes can also easily diffuse to human cells, making exosome treatment effective for AD treatment. Lastly, exosomes are efficient carriers of drugs that can be used to develop personalized medicine to treat cells in a degenerative environment.

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■ References

1. Lamptey, R. N. L.; Chaulagain, B.; Trivedi, R.; Gothwal, A.; Layek, B.; Singh, J. A Review of the Common Neurodegenerative Disorders: Current Therapeutic Approaches and the Potential Role of Nanotherapeutics. *Int. J. Mol. Sci.* **2022**, *23* (3). DOI: 10.3390/ijms23031851.
2. Walker, L.; McAleese, K. E.; Erskine, D.; Attems, J. Neurodegenerative Diseases and Ageing. *Subcell Biochem* **2019**, *91*, 75–106. DOI: 10.1007/978-981-13-3681-2_4.
3. Miller, J. H.; Das, V. Potential for Treatment of Neurodegenerative Diseases with Natural Products or Synthetic Compounds That Stabilize Microtubules. *Curr. Pharm. Des.* **2020**, *26* (35), 4362–4372. DOI: 10.2174/1381612826666200621171302.

4. Wilson, D. M.; Cookson, M. R.; Van Den Bosch, L.; Zetterberg, H.; Holtzman, D. M.; Dewachter, I. Hallmarks of Neurodegenerative Diseases. *Cell* **2023**, *186* (4), 693–714. DOI: 10.1016/j.cell.2022.12.032.
5. Hansson, O. Biomarkers for Neurodegenerative Diseases. *Nat. Med.* **2021**, *27* (6), 954–963. DOI: 10.1038/s41591-021-01382-x.
6. Logroscino, G.; Urso, D.; Savica, R. Descriptive Epidemiology of Neurodegenerative Diseases: What Are the Critical Questions? *Neuroepidemiology* **2022**, *56* (5), 309–318. DOI: 10.1159/000525639.
7. Whiteside, T. L. Tumor-Derived Exosomes and Their Role in Cancer Progression. *Adv. Clin. Chem.* **2016**, *74*, 103–141. DOI: 10.1016/bs.acc.2015.12.005.
8. Nazarenko, I.; Rana, S.; Baumann, A.; McAlear, J.; Hellwig, A.; Trendelenburg, M.; Lochnit, G.; Preissner, K. T.; Zöller, M. Cell Surface Tetraspanin Tspan8 Contributes to Molecular Pathways of Exosome-Induced Endothelial Cell Activation. *Cancer Res.* **2010**, *70* (4), 1668–1678. DOI: 10.1158/0008-5472.CAN-09-2470.
9. Yu, S.; Cao, H.; Shen, B.; Feng, J. Tumor-Derived Exosomes in Cancer Progression and Treatment Failure. *Oncotarget* **2015**, *6* (35), 37151–37168. DOI: 10.18632/oncotarget.6022.
10. Gebeyehu, A.; Kommineni, N.; Meckes, D. G.; Sachdeva, M. S. Role of Exosomes for Delivery of Chemotherapeutic Drugs. *Crit. Rev. Ther. Drug Carrier Syst.* **2021**, *38* (5), 53–97. DOI: 10.1615/CritRevTherDrugCarrierSyst.2021036301.

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