Heat shock-induced exosomes derived from neural stem cells confer marked neuroprotection against oxidative stress and amyloid-β caused neurotoxicity

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Research Article

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Abstract

Alzheimer's disease (AD) is the most prevalent neurodegenerative disease and a leading cause of dementia. Although the amyloid-β (Aβ) peptide is deemed a crucial driver of AD, there are no effective therapeutics available to treat Aβ-caused neurotoxicity. Exosomes are extracellular vesicles with a size range of 30 – 150 nanometers in diameter. Stem cell-derived exosomes are a potential therapeutic in AD, while exosomes isolated from normal stem cell cultures generally have a low yield. Here, we studied the exosomes secreted by the rat neural stem cells in the presence of heat shock (HS) stimulus. Nanoparticle tracking analysis confirmed HS-derived exosomes exhibit significantly higher concentration and larger diameter in comparison to the non-heat shock (NHS)-derived exosomes. Mass spectrometric studies of exosomal proteins reveal that HS-derived exosomes contained fewer diverse proteins than NHS-derived exosomes. GO enrichment analysis of the proteins suggested that the top two biological functions of the proteins in HS-derived exosomes are involved in the negative regulation of apoptotic process and positive modulation of DNA repair. Importantly, the therapeutic efficacy of the NHS- and HS-derived exosomes were tested in a cell culture model of AD: HS-derived exosomes exhibited greater neuroprotection against not only oxidative stress but also amyloid-β (Aβ) induced neurotoxicity compared to NHS-derived exosomes. These data indicate that in response to HS, neural stem cells increase exosome production and alter exosome morphology and cargo to confer better neuroprotection against oxidative stress and Aβ caused neurotoxicity, suggesting that HS-induced exosomes from neural stem cells can be a therapeutic reagent for AD and possibly other neurological disorders.

Introduction

Alzheimer's disease (AD) is a prevalent neurodegenerative disorder and the most common cause of dementia in the elderly. The disease is associated with two defining neuropathological characteristics: the accumulation of extracellular senile plaques and intracellular neurofibrillary tangles. By the year 2060, 13.8 million Americans over the age of 65 are projected to be diagnosed with the disease [1]. Despite this, current therapeutics for this disorder are not able to effectively attenuate or prevent the progression of the disease [2].

Exosomes are 30–150 nanometers in diameter extracellular vesicles that are secreted from all cell types and found in various biological fluids [3]. These vesicles exhibit favorable characteristics as a therapeutic reagent: low immunogenicity and toxicity, ability to cross the blood-brain barrier (BBB), and biodegradability and contain neuroprotective molecules [4, 5]. Previous studies have demonstrated that exosomes are a diagnostic tool and potential therapeutic reagent in cancer immunotherapy, ischemic stroke, diabetes and cardiovascular diseases [6]. Exosome biogenesis develops from the inward-budding of the early endosome to form multivesicular bodies that consist of intraluminal vesicles. Upon fusion with the plasma membrane, the intraluminal vesicles are released as exosomes [7]. As a result, exosomal content reflects their donor cells, making them a favorable therapeutic for AD and other diseases [8, 9].

Even though exosomes exhibit advantageous characteristics, the low yield from cultured cells, mostly stem cells, has limited their application as a therapeutic reagent. For instance, the yield of exosomes from culture media is usually less than 1 µg of exosomal protein from 1 mL of media [10]. To overcome this hurdle, the present study aims to determine whether the highly proliferating rat neural stem cell, HC2S2 [11], can increase the yield of exosomes in response to heat shock (HS) and whether HS-induced exosomes confer neuroprotection against oxidative stress and Aβ caused neurotoxicity in neuronal cell cultures.

Materials And Methods

Cell culture

The HC2S2 rat neural stem cell line that was described previously [12,13] was utilized for the isolation of exosomes. Cell culture petri-dishes (10 cm) were coated with Matrigel for 2 hours at room temperature prior to use. Cells were cultured in DMEM/F12 supplemented with N2 medium and 20 ng/ml basic fibroblast growth factor. Cells were grown at 37°C in 5% CO_2 to 80 – 85% confluency before passage.

Heat-shock of HC2S2 cells

Once cells reached 80% confluency, media was collected and utilized for non-heat shock (NHS) exosome isolation. Then, fresh media was added, and cells were incubated at 42°C in 5% CO_2 for three hours to stimulate HS conditions [14]. Subsequently, cells were returned to 37°C in 5% CO_2 for 21 hours, and media was then collected for isolation of exosomes from heat shock-derived cells.

Exosome isolation

Exosomes were isolated and purified using a sucrose gradient ultracentrifugation method adapted from our previously described protocols [15,16]. The cell culture media was collected by centrifugation at 300 x g for 20 minutes to remove cellular debris. The supernatant was centrifuged at 1,000 x g for 15 minutes to remove apoptotic bodies. The supernatant was then pooled and ultracentrifuged at 100,000 x g for 90 minutes in the Beckman SW41Ti Rotor using Beckman Coulter ultracentrifuge tubes. For fractionation, the supernatant was discarded, and the resulting pellets (crude exosomes) were resuspended in 0.32 M sucrose and added to the top of a sucrose gradient with the following concentrations of sucrose in 5 mM HEPES dissolved in dH_2O with a pH of 7.4: 0.72 M, 1.16 M, 1.58 M and 2.0 M. The sucrose gradient was then ultracentrifuged at 100,000 x g for 16 hours. Next, ten 1 mL fractions were collected using a fractionation machine and each individual fraction was added to 1X PBS and further centrifuged at 100,000 x g for 60 minutes. The supernatant was discarded, and the pellets were resuspended in 1X PBS and stored at -80°C until use. All centrifugation steps were carried out at 4°C.

Exosome characterization

Nanoparticle Tracking Analysis (NTA) was conducted to assess the concentration and size of exosomes collected from HS- and NHS-derived cells. The NanoSight NS300 system utilized 100 µg of exosomal protein diluted in 1X PBS for comparison between the size and concentration of exosomes isolated from the two conditions (Malvern Instruments). Transmission Electron Microscopy (TEM) was conducted to further validate the morphology of the collected samples adapted from a previously described protocol [17]. Briefly, NHS and HS-derived exosomes were resuspended in water containing 0.2% paraformaldehyde (PFA). Samples (10 microliters each) were prepared by drop-casting on a graphine-based grid and then 1% phosphotungstic acid was applied as a contrast agent. Samples were imaged using the FE-TECNAI-G2 transmission electron microscope (FEI) with an exposure of 1.0 sec and magnification of SA 59,000.

Western blot analysis was conducted on the 10 isolated fractions to determine the presence of known exosomal markers. Proteins from each fraction were separated onto 12% Bis-Tris gels and transferred to polyvinylidene difluoride membranes. Antibodies against HSP-90 and Flotillin-1 were used to characterize the isolated fractions.

Mass spectrometric analysis of exosomal proteins

The exosomal proteins were separated by SDS-PAGE, and the gel was stained with Coomassie G250. Each gel line corresponding to each replicate/group was cut into small slices before being subjected to destaining and in-gel digestion as previously described [18,19,16]. The tryptic peptides were analyzed in an Easy nLC 1200 using a trapping and desalting online trap column (300 µm x 20 mm Acclaim PepMap C18 100Å (Thermo Scientific), and were separated by an Acclaim PepMap RSLC 2 µm, 75 µm x 15 cm, nanoViper (Thermo Scientific) coupled to nanoESI QExactive Plus Orbitrap HR/MA mass spectrometer as previously reported [20,18]. The ions were analyzed in positive MS ion mode (m/z 300 −1800) with 70,000 resolution (m/z 200) after accumulation with target ions to 1×10^6 value based on predictive AGC. The MS/MS ions selection was set at m/z 65-1800 to 1×10^5 counts. The spectrum was deconvoluted and analyzed using the Mascot Distiller v2.6 (www.matrixscience.com) and Discovery and Proteome Discoverer v2.1 (Thermo Scientific). A mascot generic format list (MGF format) was generated to identify +1 or multiple charged precursor ions from the MS data file.

Mascot server v2.7.0.1 (www.matrix-science.com, UK) in MS/MS ion search mode (local licenses) was applied to conduct peptide matches (peptide masses and sequence tags) and protein searches against SwissProt 2021_04 (565,928 sequences; 204,173,280 residues) using taxonomy filter for *Rattus norvegicus*. The protein redundancy that appeared at the database under different GI and accession numbers was limited to Human. All the proteins identified in the current study were found in these domains.

Bioinformatic analysis of NHS and HS exosomal proteins

The DAVID functional annotation tool (https://david.ncifcrf.gov/summary.jsp) was utilized to perform gene ontology (GO) and pathway analysis of significant interactors. The UNIPROT IDs for each protein

were converted to the official gene ID with *Rattus norvegicus* selected as the species. The default settings for thresholds of 2 genes per term and an EASE threshold of 0.1 were utilized for the analysis. The p-value was converted to the -log(p). The Venny 2.1 software (https://bioinfogp.cnb.csic.es/tools/venny/) was utilized to determine differences in GO terms and pathways between NHS and HS exosomal proteins.

Treatment of neuronal cultures with NHS- or HS-derived exosomes in the presence of oxidative stress or Aβ peptide

Mouse neuronal cells obtained from the Coriell Institute for Medical Research were cultured in 12-well plates in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotics as previous described [21]. The cells were co-treated with 250 μM H_2O_2 in the presence of 2 μg/ml of crude NHS- or HS-derived exosomes for 8 h. Alternatively, neuronal cultures were treated with 250 nM of $A\beta_{1-42}$ peptide that we previously used [22] in the presence of 4 μg/ml of purified NHS or HS-exosomes (pooled fraction 3-8) for 24 h. Following the treatments, the cells were collected for assessment of cell viability by measuring ATP levels using an ATP assay kit as previously described [23].

Statistical analysis

All numerical data are presented as mean ± SD. For comparison between NHS- and HS-derived exosomes in terms of concentration and diameter, a student's t test was utilized. All other analyses were carried out using a one-way ANOVA followed by a Tukey's post hoc test. Statistical significance was accepted when $p < 0.05$. For statistical analysis and graphical displays, GraphPad Prism Statistical Software version 8.2.1 was utilized.

Results

HS-derived exosomes exhibited increased concentration and diameter compared to NHS-derived exosomes

Exosomes were isolated from the HC2S2 neural stem cells incubated at 37°C (NHS) or 42°C (HS). Exosomes were then subjected to characterization via WB, TEM and NTA to determine protein levels of known exosomal markers, morphology, size and concentration of the particles, respectively. WB analysis revealed the isolation of exosomes based on two known exosomal markers from NHS- (**Fig. 1A**) and HS-derived cells (**Fig. 1B**). Using TEM, we observed that the exosomes isolated from both NHS- (**Fig. 1C**) and HS-derived cells (**Fig. 1D**) exhibited characteristics consistent with membrane-bound vesicles. NTA determined that NHS-derived exosomes exhibited an average diameter of 111.3 nanometers with an average concentration of 2.63×10^{10} particles/mL (**Fig. 1E**). Further, NTA determined that HS-derived exosomes displayed an average diameter of 129.2 nanometers and an average concentration of 3.46×10^{11} particles/mL (**Fig. 1F**). HS-derived exosomes exhibited a statistically significant increase in diameter in comparison to the NHS-derived exosomes ($p < 0.05$; data analyzed by a student's t test) (**Fig. 1G**). HS-derived exosomes also exhibited a statistically significant increase in concentration in comparison to

NHS-derived exosomes ($p < 0.0001$; data analyzed by a student's t test). The concentration of HS-derived exosomes are approximately 13-times higher than NHS-derived exosomes (**Fig. 1H**). Consequently, the yield of exosomes from the HS-induced cell cultures was 269.36 µg of total exosomal protein from 1 ml medium, which is dramatically higher than the yield from NHS treated cell culture (27.3 µg/ ml). Thus, we have isolated and characterized exosomes from two treatment groups from neural stem cells: cells exposed to HS conditions and cells exposed to normal conditions. Hence, the neural stem cells utilized in this study produced a significantly higher concentration and an increase in diameter of exosomes in response to HS stress compared to the NHS condition.

HS-derived exosomes contained fewer diverse proteins than NHS-derived exosomes but were enriched in anti-apoptotic and DNA repair proteins

Mass spectrometric analysis of exosomal proteins revealed that HS-derived exosomes contained 418 different proteins, which is much fewer than 706 proteins included in NHS exosomes (**Fig. 2A**). Gene Ontology (GO) analysis of NHS and HS derived exosomal proteins identified differential molecular functions. Intriguingly, the top two biological functions for HS-derived exosomal proteins were involved in negative regulation of apoptotic processes and modulation of DNA repair (**Fig. 2C, Table 1 & 2**). On the other hand, the NHS-derived exosomes contained proteins responsible for protein transport and cell-cell adhesion (**Fig. 2B**). These data suggest that HS-derived exosomes contain different proteins that may have differential functions when compared to NHS derived exosomes.

HS-derived exosomes exhibited a marked neuroprotection against oxidative stress and Aβ-induced neurotoxicity

To determine whether HS derived exosomes play a differential role in protection of neuronal cells under conditions of stress, such as oxidative stress, we co-treated a neuronal culture with 250 µM of H_2O_2 and 2 µg/ml of NHS- or HS-derived exosomes. Previous data has indicated that H_2O_2 induces neuronal apoptosis [24]. As shown in **Fig. 3A**, even crude HS-derived exosomes could significantly suppress H_2O_2 induced cell death. It is worth noting that NHS-derived exosomes were also able to suppress H_2O_2 induced cell death with a lower efficacy. Additionally, we also tested the effect of the purified HS- and NHS-derived exosomes on Aβ-induced neurotoxicity due to studies highlighting the role of Aβ in inducing neuronal apoptosis [25]. In the absence of NHS- or HS-derived exosomes, Aβ caused a marked decrease in neuronal viability (**Fig. 3B**). Both NHS- and HS-derived exosomes were also able to reverse Aβ induced neurotoxicity, although HS-derived exosomes exhibited the potential to confer greater neuroprotection against Aβ-induced neurotoxicity compared to NHS-derived exosomes (**Fig. 3B**).

Discussion

Owing to their favorable characteristics of being able to cross the BBB and reflect the content of their donor cells, exosomes derived from neural stem cells are a promising therapeutic for AD. However, previous studies have highlighted the low yield of exosomes following isolation. Here, we demonstrated

that exosomes isolated from cells under HS conditions exhibit a significant increase in diameter and concentration in comparison to exosomes isolated from cells under physiological conditions. Previous studies have shown that exosomes isolated from HS conditions increased the quantity of exosomes in comparison to NHS conditions in B-lymphoblastoid cells [14]. Of note, this study utilized the bicinchoninic acid assay to determine the change in exosomal proteins following HS. Our study utilized NTA, a well-accepted approach for exosome characterization, to determine the specific concentration and size of exosomes in solution; however, it remains unclear how HS alters the size and quantity of exosomes, despite previous studies suggesting that heat shock proteins may play a role in modulation of exosome biogenesis [26,27].

Although HS causes the neural stem cells to secret dramatically more exosomes, the protein diversity of HS-derived exosomes was much lower than NHS-derived exosomes. However, our bioinformatics data indicated that these fewer diverse HS exosomal proteins were enriched in anti-apoptotic and DNA repair proteins, suggesting that these samples may provide therapeutic efficacy. Importantly, our cell culture results validated the role of the HS-derived exosomes in providing marked neuroprotection against H_2O_2-induced oxidative stress and Aβ-caused neurotoxicity, both of which have been reported to trigger neuronal cell death via apoptotic manner [24,25]. Given the fact that apoptosis plays a key role in the progression of AD and several other neurologic disorders as revealed by studies on animal models and cell lines [28], HS-derived exosomes from neural stem cell and possibly other stem cell cultures could be an invaluable therapeutic reagent to treat these diseases.

In conclusion, this study shows promising results in utilizing HS-derived exosomes as a therapeutic agent to treat oxidative stress- and Aβ-induced neurotoxicity, due to their ability to significantly enhance the production of exosomes, enriched anti-apoptotic components, and ability to cause marked neuroprotection against oxidative stress and Aβ caused neurotoxicity. These results provide useful information for the consideration of HS-derived exosomes from other stem cells, which would allow investigators to overcome the low yield of exosomes seen in previous studies. HS-derived exosomes from other stem cells could be a therapeutic agent to treat AD and possibly other neurological diseases. Future studies are necessary to elucidate the mechanism underlying the increase in concentration, size and composition of HS-derived exosomes.

Declarations

Ethics Approval Not applicable.

Consent to participate Not applicable.

Consent for publication Not applicable.

Availability of Data and Materials The data sets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Competing Interests The authors declare no competing interests.

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Author Contributions Christa C. Huber andHongmin Wang contributed to the study conception and design. Experiments and data analysis were performed by Christa C. Huber, Eduardo A. Callegari, Maria D. Paez, Svetlana Romanova. The first draft of the manuscript was written by Christa C. Huber. All authors read and approved the final manuscript.

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Compliance with Ethical Standards

Disclosure of Potential Conflicts of Interest Not applicable.

Research involving Human Participants and/or Animals Not applicable.

Informed Consent Not applicable.

References

1. 2021 Alzheimer's disease facts and figures (2021) Alzheimers Dement 17(3):327–406. doi:10.1002/alz.12328
2. Se Thoe E, Fauzi A, Tang YQ, Chamyuang S, Chia AYY (2021) A review on advances of treatment modalities for Alzheimer's disease. Life Sci 276:119129. doi:10.1016/j.lfs.2021.119129
3. Doyle LM, Wang MZ (2019) Overview of Extracellular Vesicles, Their Origin, Composition, Purpose, and Methods for Exosome Isolation and Analysis. Cells 8(7). doi:10.3390/cells8070727
4. Tian T, Zhang HX, He CP, Fan S, Zhu YL, Qi C, Huang NP, Xiao ZD, Lu ZH, Tannous BA, Gao J (2018) Surface functionalized exosomes as targeted drug delivery vehicles for cerebral ischemia therapy. Biomaterials 150:137–149. doi:10.1016/j.biomaterials.2017.10.012
5. Xu M, Feng T, Liu B, Qiu F, Xu Y, Zhao Y, Zheng Y (2021) Engineered exosomes: desirable target-tracking characteristics for cerebrovascular and neurodegenerative disease therapies. Theranostics 11(18):8926–8944. doi:10.7150/thno.62330
6. Lener T, Gimona M, Aigner L, Borger V, Buzas E, Camussi G, Chaput N, Chatterjee D, Court FA, Del Portillo HA, O'Driscoll L, Fais S, Falcon-Perez JM, Felderhoff-Mueser U, Fraile L, Gho YS, Gorgens A, Gupta RC, Hendrix A, Hermann DM, Hill AF, Hochberg F, Horn PA, de Kleijn D, Kordelas L, Kramer BW, Kramer-Albers EM, Laner-Plamberger S, Laitinen S, Leonardi T, Lorenowicz MJ, Lim SK, Lotvall J, Maguire CA, Marcilla A, Nazarenko I, Ochiya T, Patel T, Pedersen S, Pocsfalvi G, Pluchino S, Quesenberry P, Reischl IG, Rivera FJ, Sanzenbacher R, Schallmoser K, Slaper-Cortenbach I, Strunk D,

Tonn T, Vader P, van Balkom BW, Wauben M, Andaloussi SE, Thery C, Rohde E, Giebel B (2015) Applying extracellular vesicles based therapeutics in clinical trials - an ISEV position paper. J Extracell Vesicles 4:30087. doi:10.3402/jev.v4.30087

7. Zhang Y, Liu Y, Liu H, Tang WH (2019) Exosomes: biogenesis, biologic function and clinical potential. Cell Biosci 9:19. doi:10.1186/s13578-019-0282-2
8. Guo M, Yin Z, Chen F, Lei P (2020) Mesenchymal stem cell-derived exosome: a promising alternative in the therapy of Alzheimer's disease. Alzheimers Res Ther 12(1):109. doi:10.1186/s13195-020-00670-x
9. Urbanelli L, Buratta S, Sagini K, Ferrara G, Lanni M, Emiliani C (2015) Exosome-based strategies for Diagnosis and Therapy. Recent Pat CNS Drug Discov 10(1):10–27
10. Yamashita T, Takahashi Y, Takakura Y (2018) Possibility of Exosome-Based Therapeutics and Challenges in Production of Exosomes Eligible for Therapeutic Application. Biol Pharm Bull 41(6):835–842. doi:10.1248/bpb.b18-00133
11. Hoshimaru M, Ray J, Sah DW, Gage FH (1996) Differentiation of the immortalized adult neuronal progenitor cell line HC2S2 into neurons by regulatable suppression of the v-myc oncogene. Proc Natl Acad Sci U S A 93(4):1518–1523. doi:10.1073/pnas.93.4.1518
12. Dong G, Ferguson JM, Duling AJ, Nicholas RG, Zhang D, Rezvani K, Fang S, Monteiro MJ, Li S, Li XJ, Wang H (2011) Modeling pathogenesis of Huntington's disease with inducible neuroprogenitor cells. Cell Mol Neurobiol 31(5):737–747. doi:10.1007/s10571-011-9679-0
13. Hoshimaru M, Ray J, Sah DWY, Gage FH (1996) Differentiation of the immortalized adult neuronal progenitor cell line HC2S2 into neurons by regulatable suppression of the v-myc oncogene. P Natl Acad Sci USA 93(4):1518–1523. doi:DOI 10.1073/pnas.93.4.1518
14. Clayton A, Turkes A, Navabi H, Mason MD, Tabi Z (2005) Induction of heat shock proteins in B-cell exosomes. J Cell Sci 118(Pt 16):3631–3638. doi:10.1242/jcs.02494
15. Liu Y, Huber CC, Wang H (2020) Disrupted blood-brain barrier in 5xFAD mouse model of Alzheimer's disease can be mimicked and repaired in vitro with neural stem cell-derived exosomes. Biochem Biophys Res Commun. doi:10.1016/j.bbrc.2020.02.074
16. Liu Y, Subedi K, Baride A, Romanova S, Callegari E, Huber CC, Wang X, Wang H (2021) Peripherally misfolded proteins exacerbate ischemic stroke-induced neuroinflammation and brain injury. J Neuroinflammation 18(1):29. doi:10.1186/s12974-021-02081-7
17. van der Pol E, Coumans FA, Grootemaat AE, Gardiner C, Sargent IL, Harrison P, Sturk A, van Leeuwen TG, Nieuwland R (2014) Particle size distribution of exosomes and microvesicles determined by transmission electron microscopy, flow cytometry, nanoparticle tracking analysis, and resistive pulse sensing. J Thromb Haemost 12(7):1182–1192. doi:10.1111/jth.12602
18. Dong G, Callegari E, Gloeckner CJ, Ueffing M, Wang H (2012) Mass spectrometric identification of novel posttranslational modification sites in Huntingtin. Proteomics 12(12):2060–2064. doi:10.1002/pmic.201100380

19. Dong G, Callegari EA, Gloeckner CJ, Ueffing M, Wang H (2012) Prothymosin-alpha interacts with mutant huntingtin and suppresses its cytotoxicity in cell culture. J Biol Chem 287(2):1279–1289. doi:10.1074/jbc.M111.294280
20. Liu Y, Min JW, Feng S, Subedi K, Qiao F, Mammenga E, Callegari E, Wang H (2020) Therapeutic Role of a Cysteine Precursor, OTC, in Ischemic Stroke Is Mediated by Improved Proteostasis in Mice. Transl Stroke Res 11(1):147–160. doi:10.1007/s12975-019-00707-w
21. Liu Y, Qiao F, Wang H (2017) Enhanced Proteostasis in Post-ischemic Stroke Mouse Brains by Ubiquilin-1 Promotes Functional Recovery. Cell Mol Neurobiol 37(7):1325–1329. doi:10.1007/s10571-016-0451-3
22. Liu Y, Huber CC, Wang H (2020) Disrupted blood-brain barrier in 5xFAD mouse model of Alzheimer's disease can be mimicked and repaired in vitro with neural stem cell-derived exosomes. Biochem Biophys Res Commun S0006-291X(20)30342-9. 10.1016/j.bbrc.2020.02.074
23. Liu Y, Lu L, Hettinger CL, Dong G, Zhang D, Rezvani K, Wang X, Wang H (2014) Ubiquilin-1 protects cells from oxidative stress and ischemic stroke caused tissue injury in mice. J Neurosci 34(8):2813–2821. doi:10.1523/JNEUROSCI.3541-13.2014
24. Chen L, Liu L, Yin J, Luo Y, Huang S (2009) Hydrogen peroxide-induced neuronal apoptosis is associated with inhibition of protein phosphatase 2A and 5, leading to activation of MAPK pathway. Int J Biochem Cell Biol 41(6):1284–1295. doi:10.1016/j.biocel.2008.10.029
25. Yao M, Nguyen TV, Pike CJ (2005) Beta-amyloid-induced neuronal apoptosis involves c-Jun N-terminal kinase-dependent downregulation of Bcl-w. J Neurosci 25(5):1149–1158. doi:10.1523/JNEUROSCI.4736-04.2005
26. Liu Y, Qiao F, Wang H (2016) Enhanced Proteostasis in Post-ischemic Stroke Mouse Brains by Ubiquilin-1 Promotes Functional Recovery. Cell Mol Neurobiol. doi:10.1007/s10571-016-0451-3
27. Feng Y, Huang W, Meng W, Jegga AG, Wang Y, Cai W, Kim HW, Pasha Z, Wen Z, Rao F, Modi RM, Yu X, Ashraf M (2014) Heat shock improves Sca-1 + stem cell survival and directs ischemic cardiomyocytes toward a prosurvival phenotype via exosomal transfer: a critical role for HSF1/miR-34a/HSP70 pathway. Stem Cells 32(2):462–472. doi:10.1002/stem.1571
28. Radi E, Formichi P, Battisti C, Federico A (2014) Apoptosis and oxidative stress in neurodegenerative diseases. J Alzheimers Dis 42(Suppl 3):S125–152. doi:10.3233/JAD-132738

Tables

Table 1 and 2 are available in the Supplemental Files section.

Figures

Figure 1

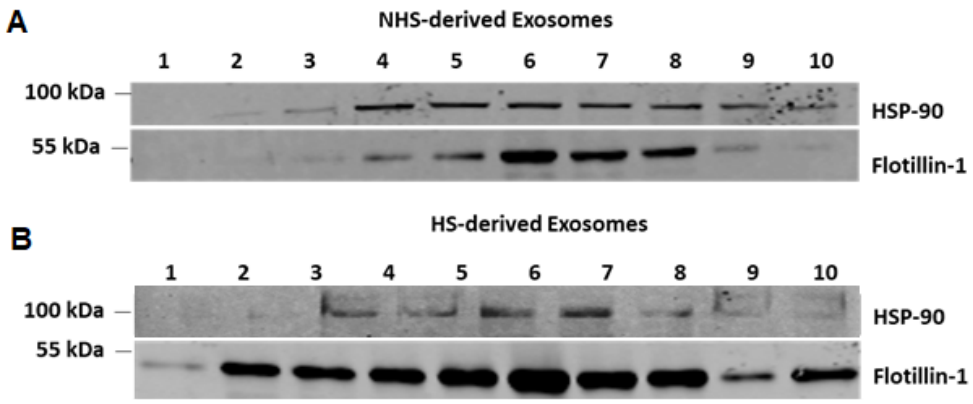

Figure 1

HS-derived exosomes exhibit increased diameter and concentration compared to NHS-derived exosomes. Western blot analysis of known exosomal markers in 10 isolated fractions: NHS (**A**) and HS (**B**). Transmission electron microscopy highlighting the morphology of NHS- (**C**) and HS-derived exosomes (**D**). Nanoparticle tracking analysis to determine the concentration and average size of the particles in solution: NHS- (**E**) and HS-derived exosomes (**F**). Size (**G**) and concentration (**H**) comparison between

NHS- and HS-derived exosomes analyzed from the NTA. Data were shown as mean ± SEM; n = 5, * p < 0.05; *** p < 0.0001 (Student's t test).

Figure 2

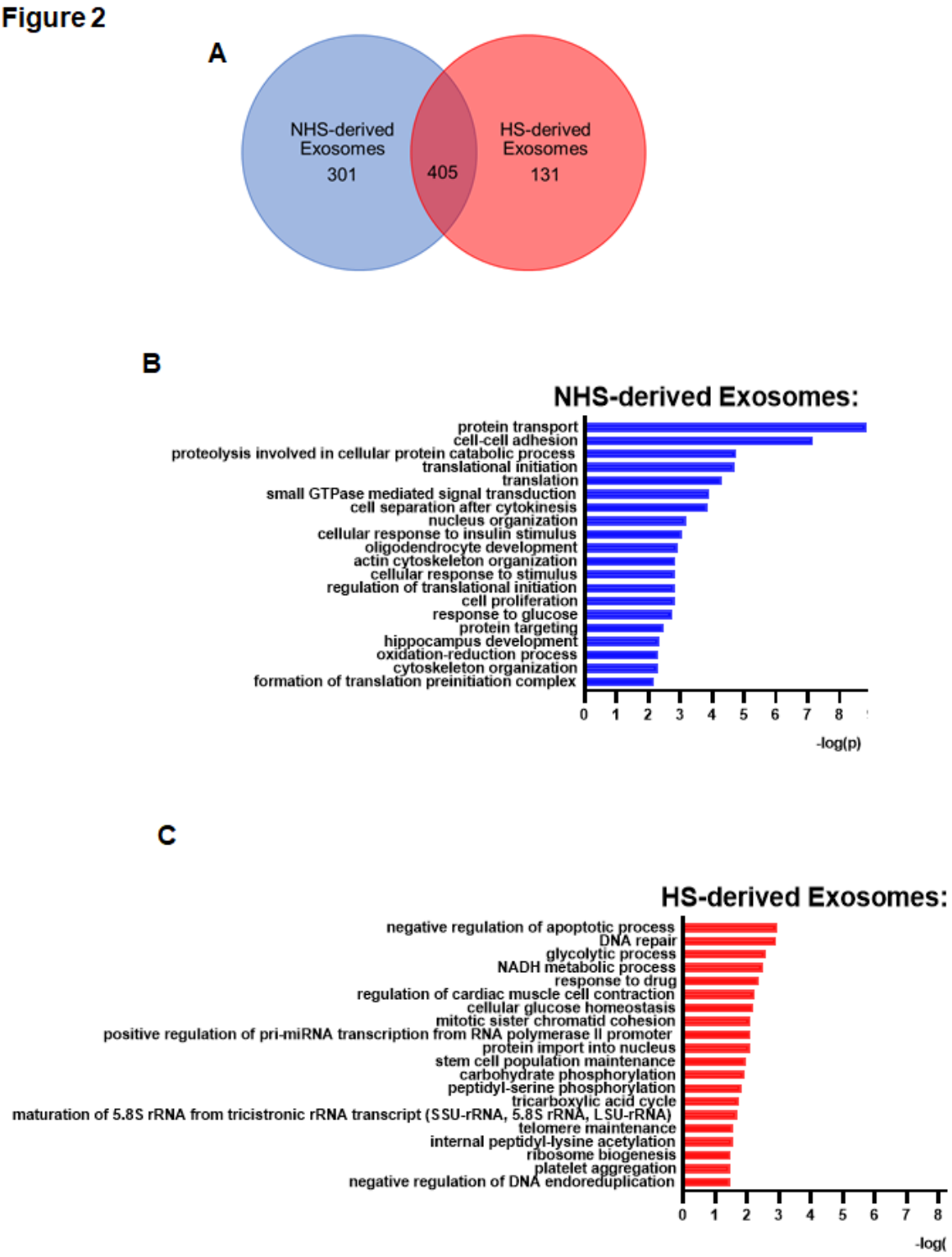

Figure 2

HS-derived exosomes are enriched in anti-apoptotic and DNA repair proteins. Mass spectrometric analysis of HS- and NHS-derived exosomal proteins (**A**). GO enrichment analysis of biological functions

for proteins unique to NHS- (**B**) and HS-derived exosomes (**C**).

Figure 3

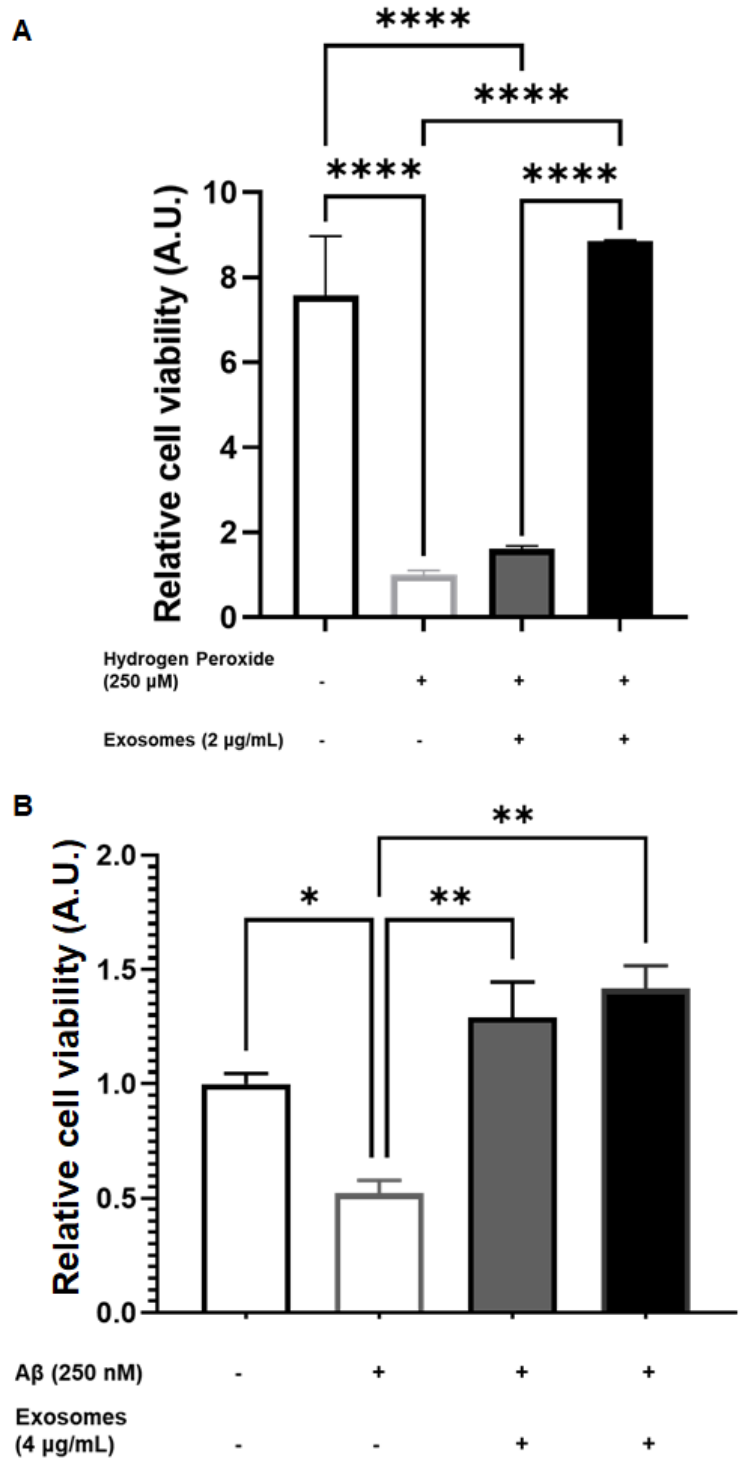

Figure 3

Treatment of neuronal cells with HS-induced exosomes confers greater neuroprotection against oxidative stress and Aβ-induced neurotoxicity compared to NHS-derived exosomes. A. Neuronal cells were treated with 250 µM H_2O_2 in presence of 2 µg/ml of NHS or HS-exosomes. After 8 h following the treatments,

cells were harvested for viability analysis. **B.** Neuronal cell cultures were treated with indicated conditions for 24 h before cell viability was assessed. Data are presented as Mean ± SD; n = 3-6. *p<0.05; ***p < 0.001; **** p < 0.0001 (one-way ANOVA and Tukey's post hoc test).

Supplementary Files

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- Table1and2.pptx