

Proteomic Clarification of Neuroprotection from Aggresome-prone Proteins

Hyun Jin Jung¹, Seungeun Yeo², Jaemyung Jang¹, Soonbong Baek¹ and Youngshik Choe¹

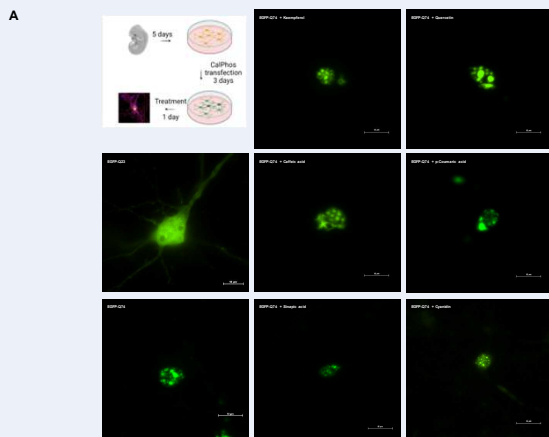
¹Department of Neural Development and Disease, Korea Brain Research Institute, 41062 Daegu, Republic of Korea

²Gut-Brain System Regulation Korean Medicine Research Center, Dongshin University, 58245 Naju, Republic of Korea

Abstract

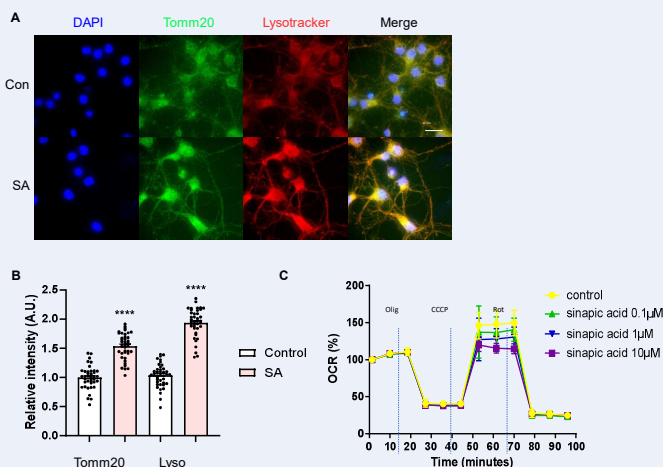
Huntington's disease (HD) is a progressive neurodegenerative disorder marked by neuronal degeneration and the accumulation of intracellular inclusions containing mutant huntingtin (mHTT) protein. The disaggregation of misfolded, aggregation-prone proteins is essential for sustaining neuronal integrity. In this study, we investigated the molecular pathways governing the segregation of intracellular protein aggregates enriched with polyglutamine (polyQ) expansions within huntingtin proteins. A naturally derived bioactive compound demonstrated the capacity to limit the formation and propagation of aggregation foci. To elucidate the underlying proteostatic mechanisms, we performed an interactome analysis of the protein aggregates, which revealed that aggresome-associated chaperones, including Hsc70, operate downstream of aggregate segregation. Treatment with the compound promoted proteomic homeostasis in neuronal cells, in part by remodeling pathways linked to extracellular vesicle release. These findings suggest that modulation of the proteome and protein quality control machinery by certain bioactive agents may contribute to alleviating proteopathic burden in post-mitotic neurons.

Results-1



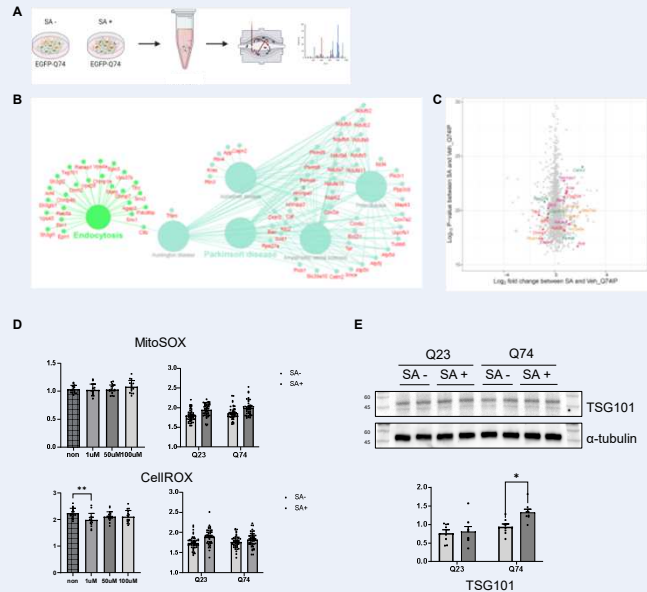
Disaggregation effects of antioxidants A. Sinapic acid reduced the size of EGFP-Q74 aggregates. Mouse cortical primary neurons were transfected with EGFP-fusion HTT proteins such as EGFP-Q23 or EGFP-Q74 using a calcium phosphate transfection method. Antioxidants (1 μM) were treated on the primary neurons for 24 hr and the size of EGFP-Q74 aggregates were measured using a 1 μm scale bar in the ImageJ software. Fluorescence microscopy of representative neuronal cells expressing EGFP-Q74. B. A plot depicts the number of EGFP aggregates. Results were analyzed using 2-way ANOVA followed by Dunnett's multiple comparisons test.

Results-2



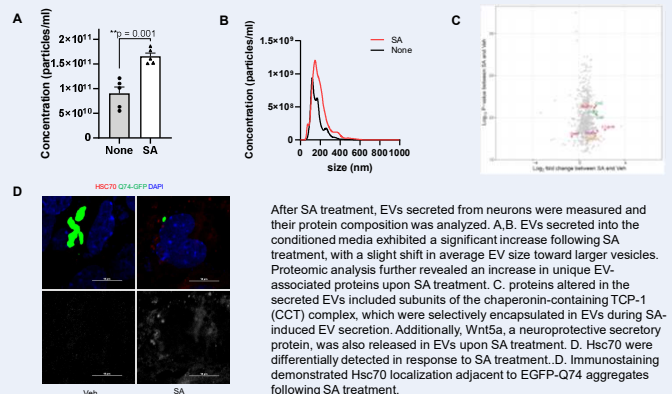
Effect of SA on neuronal mitochondria and lysosome functions A. Neurons were treated with SA for 30 min and fixed to stain Tom20 and LysoTracker. A. Fluorescence images were used to measure the signal intensity of Tom20 and LysoTracker which represent specific signals subtracted with background signals. B. A plot depicts the signal intensity measured from fluorescence images. C. Measurement of the mitochondrial oxygen consumption rates using Seahorse kits. SA dose-dependently reduced the total mitochondrial respiratory capacity, without affecting basal oxygen consumption.

Results-3



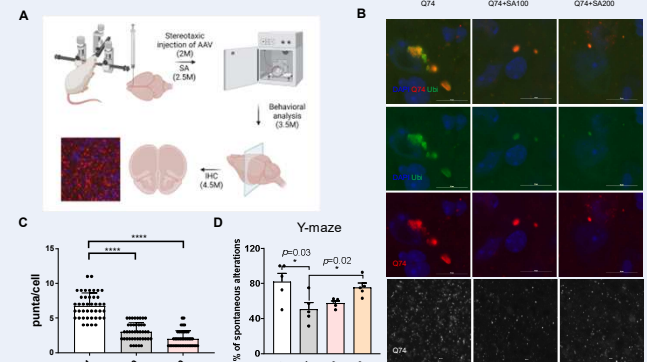
Proteomic profiling of SA-treated neurons showed activation of secretory pathways. A. Schematic drawing for proteomic analysis of SA-treated neurons. B. Enrichment of significantly regulated signaling pathways by SA treatment B. Scatter plot shows differentially expressed proteins involved in the signaling pathways shown in B. D. SA-mediated changes of reactive oxygen species in the mitochondria (MitoSOX) and the whole cells (CellROX) SA E. Alteration of exosome-related TSG101 by SA treatment in neurons expressing aggregation-prone HTT-Q74

Results-4



After SA treatment, EVs secreted from neurons were measured and their protein composition was analyzed. A. B. EVs secreted into the conditioned media exhibited a significant increase following SA treatment, with a slight shift in average EV size toward larger vesicles. Proteomic analysis further revealed an increase in unique EV-associated proteins upon SA treatment. C. Proteins altered in the secreted EVs included subunits of the chaperonin-containing TCP-1 (CCT) complex, which were selectively encapsulated in EVs during SA-induced EV secretion. Additionally, Wnt5a, a neuroprotective secretory protein, was also released in EVs upon SA treatment. D. Hsc70 were differentially detected in response to SA treatment. D. Immunostaining demonstrated Hsc70 localization adjacent to EGFP-Q74 aggregates following SA treatment.

Results-5



Clearance of RFP-Q74 and recovery of RFP-Q74-induced memory impairment by SA in vivo. A. Schematic illustration about the workflow of an in vivo experiment. B. Representative immunofluorescence images of mPFC regions. RFP-Q74 expressing neurons were co-stained for Ubiquitin and DAPI was used to visualize neuronal nuclei. Perinuclear RFP-Q74 puncta were reduced by SA treatment. C. Quantification of the number of RFP-Q74 puncta per cell in the mPFC region of mouse brain. D. Mice treated with 200 mg/kg SA exhibited a significant recovery in spontaneous alternation in the Y-maze test, while the 100 mg/kg group showed a trend toward improvement, though not statistically significant.

Acknowledgement

This research was funded by the KBRI basic research program (25-BR-03-05, 25-BR-02-01), the Ministry of Trade, Industry and Energy (No. 2410006472), and the Ministry of Science, ICT & Future Planning (No. 2022R1A5A202954611).