Age-related changes in the proteostasis network in the brain of the naked mole-rat: Implications promoting healthy longevity

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place to prevent and eliminate protein misfolding, and promotes degradation of unwanted and damaged organelles and proteins.

2.5. Sypro Ruby and Pro-Q Diamond staining

of the Swiss-Prot database by SEQUEST (Proteome Discoverer v1.4, Thermo Scientific) was used to interrogate the data files of each sample. At least two high-confidence peptide matches were used for protein identification where the false discovery rate is <1%. Proteins that were matched with the same peptides were reported as one protein group. Protein data reported from these analyses include: the SwissProt accession number, the percentage of the protein sequence identified by matching peptides, the number of peptide sequences detected by the MS/MS analysis, the confidence score of the protein, and the expected molecular weight and predicted isoelectric point (pI).

2.9. Immunoprecipitation and Western blotting

2.9.1. Immunoprecipitation (IP)

Individual NMR brain homogenates (250 µg) were suspended in 500 µL of IP buffer [0.05% NP-40, aproprotin 5 µg/mL, leupeptin 4 µg/mL, pepstatin 4 µg/mL, and phosphatase inhibitor cocktail 10 µg/mL] in a phosphate buffer solution, pH 8 [8 M NaCl, 0.2 M KCl, 1.44 M Na_2HPO_4, and 0.24 M KH_2PO_4

significantly decreased from the intermediate to the old age group ($p = 0.0007$). Additionally, there was a significant decrease from the intermediate to the oldest age group ($p < 0.0001$). The p-Akt/Akt ratio was decreased from the intermediate age group to the old age group ($p = 0.031$). Similar to the other proteins in the PI3K/Akt/mTOR axis, the p-Akt/Akt ratio increases from the youngest to the intermediate age group (data trended toward significance). There were no significant changes in the protein levels of mTOR; however the p-mTOR/mTOR ratio follows a corresponding trend to that of p-PI3K, in which there is a significant increase from the early to intermediate age group ($p = 0.013$) and a significant decrease from the intermediate age group to the old and oldest age groups ($p = 0.0009$) and ($p = 0.0013$), respectively.

We further analyzed the quantitative index of autophagy, the LC3-II/LC3-I ratio in all age groups. The LC3 ratio showed no significant changes with age (

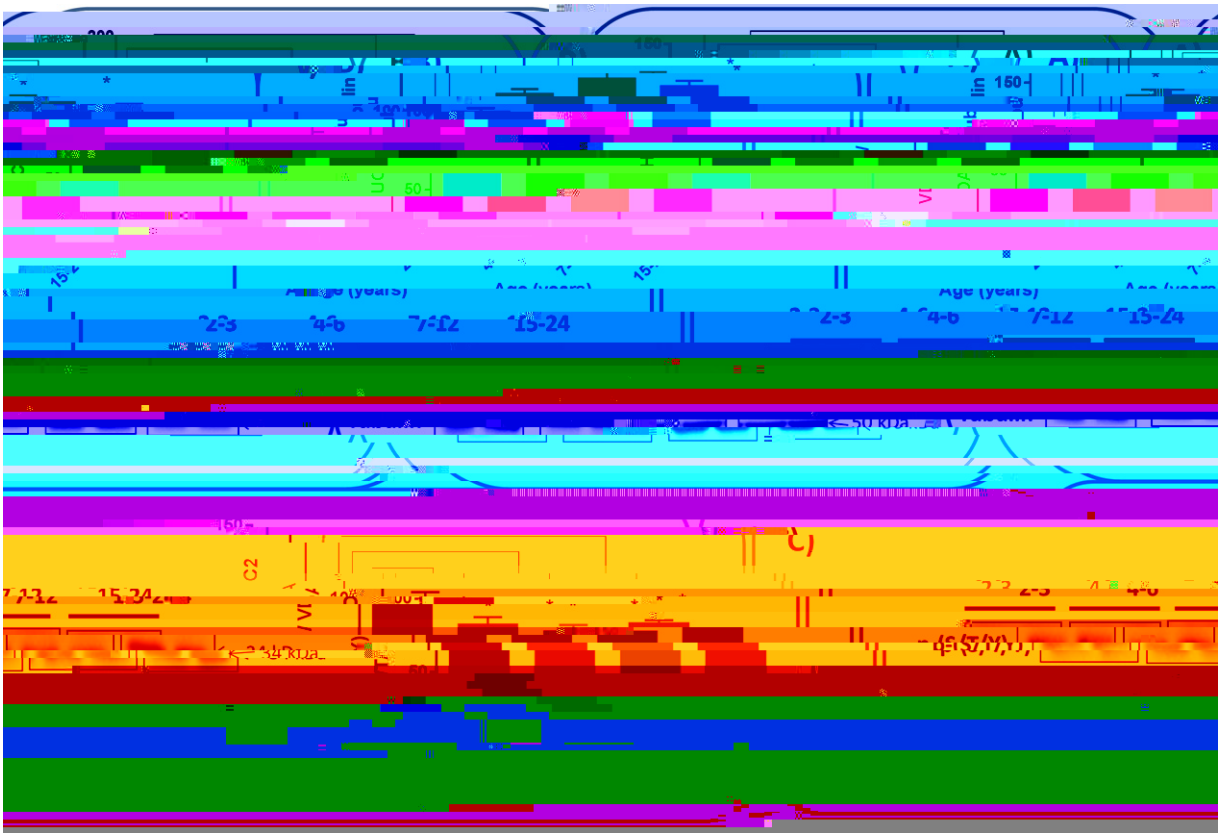

Fig. 5. Western blot and corresponding bar graph representations from the validation experiments of the changes in the protein levels of (A) Ub carboxy-term

4. Discussion

Not only are NMRs the longest-lived rodent, but they also maintain an extended health span. This extraordinary salubrious lifespan has been attributed to, in part, by mechanisms that contribute to maintaining proteostasis [7]. Processes that promote sustained cellular homeostasis, such as unfolded protein response and proteasome and autophagy pathways, remove damaged or unwanted proteins, macromolecules and organelles which can be cytotoxic and lead to neuronal death [42]. Additionally, these proteostasis systems play a critical role in maintaining health by modulating protein levels in response to fluctuating physiological environments [42]. Previous studies have shown that NMRs exhibit a more robust proteostasis as

UBE1 were found to be increased compared with the youngest age group. UBE1 not only catalyzes the first step in the Ub–proteasomal pathway, but it is also essential for the protein ubiquitinylation that

[60] R.M. Hofmann, C.M. Pickart, Noncanonical MMS2-encoded ubiquitin-conjugating enzyme functions in assembly of novel polyubiquitin chains for DNA repair, Cell 96 (1999) 645–653.
[61]