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Transgenic Mice Have Proven Successful in Combating Neurogenerative Diseases

Sultonova Dildor Bakhshilloyevna
Assistant, Department of Biochemistry of the Bukhara State Medical Institute named after Abu Ali Ibn Sino, Uzbekistan

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Abstract: A study investigating the role of cathepsin D (CTSD) in neuroprotection has provided compelling evidence of its critical importance in preventing neurodegenerative processes.
The central idea of the work is that overexpression of CTSD can compensate for the deficiency of this enzyme caused by genetic knockout, thereby preventing the development of lethal consequences and the accumulation of amyloid plaques characteristic of neurodegenerative diseases such as Alzheimer's disease.
Lysosomes, intracellular organelles, play a key role in the degradation of proteins and other cellular components, thereby ensuring cellular homeostasis. Cathepsins, including CTSD, are the most important lysosomal proteases responsible for the breakdown of various substrates, including toxic protein aggregates. CTSD deficiency in humans leads to the development of neurolipofuscinosis (NCL), a group of severe inherited neurodegenerative diseases characterized by the accumulation of lipofuscin in nerve cells and progressive neuronal damage. This disease demonstrates a direct link between lysosomal protease deficiency and the development of neurodegeneration.

Keywords: Transgenic mouse, Cathepsin D, Mitochondrial bioenergetics, Apoptosis, Cathepsin D knockout mice, Dopamine, Autophagy, Lysosome, Life expectancy, Behavior, Neuronal, ceroid, lipofuscinosis.

Introduction: Preface

Previous studies using a CTSD gene knockout mouse model (CtsdKO or CDKO) have confirmed this association. CDKO mice showed intracellular accumulation of protein aggregates, decreased proteasome activity (another critical protein degradation system), and, importantly, early postnatal lethality at around day 26 of life. These findings highlight the critical role of CTSD in maintaining cellular health and preventing the accumulation of potentially toxic proteins.

To study the protective properties of CTSD, the researchers developed a new transgenic mouse strain that overexpressed human CTSD specifically in cells of the nervous system. This was achieved by using the Cre-loxP recombination system, which allowed CTSD gene expression to be activated only in cells expressing the Nestin protein, a marker of neural stem cells and neurons. Thus, CTSD was overexpressed selectively in neural tissue, eliminating the potential side effects of systemic overproduction of the enzyme. These mice, designated CDtg, demonstrated normal behavior and sensitivity to neurotoxic agents such as MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine), indicating that CTSD overexpression does not have significant negative consequences.

On the experiment that plays a decisive role in the study: The key experiment involved crossing CDtg mice with CDKO mice. The results were dramatic: overexpression of CTSD in the nervous system of CDKO mice significantly extended their lifespan, partially restored the reduced proteasome activity, and, most importantly, reduced the accumulation of Aβ42, an amyloid peptide that plays a key role in the development of Alzheimer's disease. Thus, the experimental data confirm the protective role of CTSD against proteotoxicity and the accumulation of toxic protein aggregates. Importantly, overexpression of other cathepsins (B and L) did not show a similar effect, confirming the specificity of the role of CTSD.

In conclusion, the study demonstrated the potential of CTSD enhancement strategy as a therapeutic approach to combat neurodegenerative diseases. The developed transgenic mouse line represents a valuable model for further research in this area, allowing to study the mechanisms of CTSD-mediated neuroprotection and to develop new treatment strategies. Moreover, the results of the study confirm the importance of maintaining lysosomal homeostasis to prevent neurodegenerative processes and open new avenues for the development of therapeutic strategies aimed at enhancing lysosomal protein degradation in the nervous system. This is of great importance for the search for effective drugs to combat Alzheimer's disease and other neurodegenerative diseases for which there is currently no effective treatment.

Introduction

Lysosomes are intracellular organelles that perform a vital function of recycling cellular components. They act as recycling centers, breaking down damaged proteins, organelles, and other cellular waste through a process called autophagy. This process is critical for maintaining the health and normal functioning of cells. Dysfunction of the autophagic system is closely associated with the development of neurodegenerative diseases such as Parkinson's disease (PD) and Alzheimer's disease (AD). Many genes that are mutated in PD are involved in or affect the normal functioning of the autophago-lysosomal pathway. Insufficient efficiency of this pathway contributes to the accumulation of amyloid plaques and tau proteins, the hallmarks of AD. Cathepsin D (CTSD), an aspartate protease activated in the acidic environment of lysosomes, plays a central role in lysosomal degradation. Its key function is supported by the fact that genetic deficiency of CTSD causes neuronal ceroid lipofuscinosis (NCL), a severe neurodegenerative disease, in humans. Patients with NCL, which is caused by a mutation in the CTSD gene, exhibit profound motor impairment. Even decreased CTSD levels in neurons of the substantia nigra, a brain region critical for motor function, are observed in Parkinson’s disease, implicating it in the pathogenesis of this disease. The association between Parkinson’s disease and the accumulation of α-synuclein, a protein that forms aggregates in neurons in this disease, is closely linked to lysosomal dysfunction. Lysosome dysfunction, specifically decreased CTSD activity, prevents the efficient degradation of α-synuclein, leading to its accumulation and, ultimately, neuronal death. This underscores the critical role of lysosomes and CTSD in maintaining the health of the nervous system.

METHODS

Mice: To study the role of CTSD in neurodegenerative processes, genetically modified Ctsd knockout (CDKO) mice are used. These mice, lacking a functional CTSD gene, display severe pathological changes. They exhibit neuronal, inflammatory and systemic abnormalities and, most importantly, have a significantly reduced lifespan, dying at approximately 26 days of age due to intestinal necrosis. This confirms the vital role of CTSD in maintaining the body's homeostasis. A more detailed study of CDKO mice showed the accumulation of autophagosomes , structures involved in autophagy, as well as α-synuclein. This clearly demonstrates that the absence of CTSD disrupts the autophago-lysosomal pathway, leading to the accumulation of proteins that would normally be disposed of. In addition, CDKO mice were found to accumulate insoluble Aβ42, a peptide that is a key component of amyloid plaques in Alzheimer's disease. This suggests that CTSD dysfunction may be involved in the pathogenesis of various neurodegenerative diseases. Thus, the study of transgenic mice with cathepsin D gene knockout provides convincing evidence of the important role of this lysosomal enzyme in maintaining cellular homeostasis and preventing the development of neurodegenerative diseases. Disruption of its function leads to impaired autophagy, accumulation of toxic proteins and, as a consequence, severe pathological changes, which makes CTSD a promising target for the development of new therapeutic strategies in the fight against Parkinson's disease, Alzheimer's disease and other neurodegenerative diseases. Further research in this direction can lead to the creation of new methods of treatment and prevention of these severe diseases, significantly improving the quality of life of patients. In particular, the study of mitochondrial bioenergetics in the context of CTSD deficiency may provide additional clues to understanding the mechanisms of neurodegenerative processes and developing targeted therapeutic approaches aimed at improving cellular metabolism and preventing neuronal apoptosis. Deep understanding of the relationship between CTSD function, autophagy, accumulation of toxic proteins and changes in mitochondria represents a huge potential for developing effective treatment strategies for neurodegenerative diseases. It is important to take into account the complex nature of these diseases and develop therapeutic approaches aimed at correcting several interrelated pathological mechanisms.

Chemicals: Sigma–Aldrich supplied chloroquine (CQ) (C-6628-25G), MPTP (M0896-10 MG), 1-methyl-4-

phenylpyridinium (MPP+) (D048-100 MG), and staurosporine (S4400-0.5 MG). Soligomycin, FCCP, and antimycin were purchased from Agilent /Seahorse Bioscience.

Neuronal cultures: Primary neuronal culture Neurons of the cerebral cortex were isolated from mouse embryos at the age of 24, 25 and 26 days. For this purpose, mouse brain was dissected in ice-cold Hanks' solutions lacking Ca2+ and Mg2+. For this purpose, mouse brain was dissected in ice-cold Hanks' solutions lacking Ca2+. The cerebral cortex was incubated for 15 min at 37 °C with the addition of papain (Worthington). After tissue mincing, the cells were isolated by centrifugation at 25 °C for 5 min at 1000 × g. The cells were then resuspended in Neurobasal medium containing 2% B27 supplement (Invitrogen 17504-044), 1% Pen-Strep (at a concentration of 10,000 units/mL) and placed in 24- or 6-well plates pre-coated with poly-L-lysine (concentration of 0.1 mg/mL). plate. The plates with cultures were kept in a humidified incubator at 37° C. For Immunocytochemical analysis of these cultures used NeuN and GFAP antibodies and found that cultures aged DIV7-14 contained more than 80% neurons.

Measuring mitochondrial function: Mitochondrial function in mouse primary cortical neurons was measured using a Seahorse Bioscience XF24 Extracellular Flux Analyzer24,27,28,29,30,31,32,33. Cells were seeded at 80,000 cells per well, and the concentrations of oligomycin , FCCP, and antimycin A were used at 1 µg/mL, 1 µmol/L, and 10 µmol/L, respectively. Following measurements, total protein in each well was determined using a DC protein assay (Bio- Rad), and the oxygen consumption rate (OCR pmol / min) was normalized to µg protein in each well.

Proteasome activity assays: We used 40 µg of cortical extracts (in triplicate and with n = 3 mice each) and 50 µmol/L substrate in an assay buffer consisting of 50 mmol/L Tris (pH 7.5), 2.5 mmol/L EGTA, 20% glycerol, 1 mmol/L DTT, 0.05% NP -40. Fluorescence was measured at an excitation wavelength of 380 nm and an emission wavelength of 460 nmol/L every 5 min for 2 h. 2.12. For the open-label test, the fluorescence was measured at an excitation wavelength of 380 nm and an emission wavelength of 460 nmol/L every 5 min for 2 h. fields 23,24 used Ethovision software to conduct this test for 5 minutes for each mouse individually.

Behavioural tests: The mouse movements were tracked, recording the total distance traveled (cm), position within the field (time in the center compared to the wall in seconds), and average speed (cm/s). For Zero Maze (diameter 70 cm), which consists of two parts with sides 15 cm high and two parts with a wall 0.5 cm, which were placed in the arena and tracked by an Ethovision camera for 4 minutes. Each mouse was placed in the arena and tracked by an Ethovision camera for 4 minutes. Recordings were animal position in the arena at 5 frames per second and time spent in the open or closed arm. SDI grip force system (San Diego Instruments) was used to measure hindlimb grip strength and limb strength in mice using the Meyer method36. For the Tail test Flick used the overhead halogen light source, providing thermal stimulation of the 4 mm x 6 mm area for the tail. For the experiment with the rotating rod 23,24 the paradigm included three days of testing mice on a rotating rod (San Diego Instruments), which gradually accelerated from 4 to 40 revolutions per a minute, and displays the reaction time in 0.01 s increments. For the rotating test, rod 23,24 the paradigm included 3 days. For every mouse every day registered delay falls. For the Morris water maze test23,24,37,38 a 120 cm diameter pool and a 10 cm diameter platform positioned 0.5 cm below the water surface were used. Four trials were conducted per day on days 1–5, so that all starting positions were used equally (in random order). Mice give 60 s to find the platform and 10 s to stay on the platform. The running wheel analysis was used with with help cells with a running wheel (Lafayette Instrument). Mice were housed individually in cages with a running wheel, where they were provided with food and water ad libitum .C using the Activity system software Wheel Monitoring (AWM) were measured distances during the day and nights.

Analysis of striatal monoamines by HPLC: WITH using a dedicated HPLC analysis with an Antec electrochemical detector Decade II (oxidation 0.4) are extracted and determined using a dedicated HPLC analysis using an Antec electrochemical detector Decade II (oxidation 0.4). The copy number of mitochondrial DNA was determined by real-time PCR using the forward primer 5′-ccccagccataacacagtatcaaac -3′ and the reverse primer 5′- gcccaaagaatcagaacagatgc -3′ in ABI 7500 (Applied Biosystems)25,39, 40, 41, 42. Real-time PCR conditions were next 94° C for 2 min, then 40 cycles of denaturation at 94° C for 15 s, annealing and extension at 60° C for 1 min.

Quantification of mtDNA: Mitochondrial DNA copy number was normalized by real-time PCR of the 18 S nuclear sequence using forward primer 5′-aaacggctaccacatccaag -3′ and reverse primer 5′-caattacagggcctcgaaag -3′.

TUNEL – Coloring: CDKO, CDtg and CDtg mice at p23–25 was used for TUNEL staining (InvitrogenTM Click - iTTM Plus Assay Kits TUNEL for detection of apoptosis in situ, catalog C 10617), DNase-treated sections were used as positive controls, TdT treatment was not was used.

RESULTS

Investigation of the effects of CTSD overexpression on cellular bioenergetics and mitochondrial function

Generation of transgenic mice with CTSD of the nervous system (CDtg):CTSD transgenic mice (CTSDfloxedstop) have been created that are capable of overexpressing CTSD in Cre -expressing tissues . When crossed with Nestin - cre transgenic animals, increased expression of CTSD mRNA is observed in the double transgenic mice. animals. Shown wild-type genomic structure, pCAG - loxp - STOP - loxp - hCTSD insertion into the Hprt site, and genomic structure in Nestin - cre expressing cells after crossing with Nestin - cre mice . There are genotyping results of 4 offspring of CTSDfloxedstop and Nestin - cre mice using PCR # 1 in the diagram, indicating that hCTSD is inserted into the Hprt allele . PCR products #1 indicate that the mice carry a CTSDfloxedstop knockout . PCR products using primers corresponding to the cDNA Cre recombinases indicate that the mouse carries the Nestin - cre transgene . Therefore, lane 1 represents a transgenic animal with CTSDfloxedstop and Nestin - cre . After PCR with primers PCR#1 and cre, DNA is extracted from 6 offspring with cre + and either + PCR#1 results.

This work is devoted to the study of the effect of increased CTSDa expression on cellular bioenergetics and mitochondrial function. The authors used a mouse model with transgenic overexpression of CTSD and conducted a series of experiments to assess the impact of this change on various aspects of cellular function.

*CTSDa overexpression had no significant effect on mitochondrial function measured by oxygen consumption and ATP production.

* No changes in the expression of genes related to autophagy, lysosomal pathways, or mitochondrial biogenesis were detected, indicating that CTSDa does not directly influence these processes.

* There were also no significant differences in mitochondrial DNA levels between CTSDa-overexpressing mice and controls.

The authors concluded that although CTSDa is a lysosomal enzyme, its overexpression does not result in significant changes in mitochondrial function or biogenesis.

Additional notes: The work contains a detailed description of the methods used and statistical analysis of the data.

discuss the limitations of their study and suggest directions for future work.

This work represents a well-considered study that contributes to our understanding of the role of CTSDa in cellular processes.

Sources:Xiaosen Ouyang , Willayat I. Wani, Gloria A. Benavides , Matthew J. Redman , and Hy Vo performed the experiments. Thomas van Groen . Victor Darley-Usmar and Jianhua Zhang . Xiaosen Ouyang And Jianhua Zhang Acrobat PDF (2 MB) - Cathepsin D overexpression in the nervous system rescues lethality and A β 42 accumulation of cathepsin D systemic knockout in vivo- Acta Pharmaceutica Sinica B Volume 13, Issue 10, October 2023, Pages 4172-4184

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