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Review Article

Neurons in the Klotho Mutant Mouse Show Biochemical and Morphological Characteristics Resembling Age-Related Disorders

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Abstract

The klotho gene is considered to regulate the lifespan of animals including humans. The klotho mutant mouse, which has a defective klotho gene, shows many age-related disorders and has a short lifespan, around 2 months old; the overexpression of this gene in the normal wild-type mouse extends lifespan significantly. In klotho mutant mice, various organs/tissues have been analyzed extensively, especially with regard to the problem in ion homeostasis. However, the central nervous system (CNS) of this mutant mouse has not attracted as much attention, probably due to its protection from peripheral circumstances. Although it is suggested that some neurons in the CNS of klotho mice are degenerative, no distinct evidence has yet emerged. There are alterations in neurofilaments and microtubules with their constituent or associated proteins, and significant differences in the distribution and expression of these structures and proteins between wild-type and mutant CNS. The expression of antiapoptotic and proapoptotic proteins were significantly changed in klotho mutant mice compared with wild-type mice. Lysosomes, lysosome-like profiles and synapse-related structures with their respective associated proteins were also altered in the mutant mice. Neuronal degeneration was evident in some restricted regions of the CNS, such as in the hippocampus. Gliofilaments in astrocytes and GFAP were drastically altered in density and expression, respectively. These alterations in klotho mutant mouse CNS are similar to those in aged animals or humans, despite some differences between them, probably because of very early death of the mutant mice, which indicate that the mutant mice would be a valuable genetic model for multiple analyses on the aging of the CNS including the autonomic nervous system. Prevention of neurodegeneration in the mutant mice with nutritional manipulation using antioxidants such as polyphenols is also discussed. [*Tzu Chi Med J* 2008;20(3):155–160]

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1. Introduction

The klotho gene, a senescence-related gene, was originally identified by insertional mutagenesis in a mouse strain and encodes a putative type I transmembrane protein, the klotho protein, composed of 1014 amino acids in both mice and rats [1,2] and 1012 amino acids in humans [1]. It has been considered a suppressor of phenotype for aging, because when the expression of this gene is suppressed in mice, these gene-suppressed mice, called klotho mutant mice, characteristically exhibit age-related syndromes/deteriorations, such as arteriosclerosis, osteoporosis, skin atrophy, infertility, thymic atrophy, pulmonary emphysema and growth arrest, even when they are still premature in stage around the age of 4 weeks [1]. The lifespan of klotho mutant mice is extremely short; they generally die around 8 weeks of age. However, since klotho mutant mice do not show some phenotypes frequently seen in aged humans, such as cancer, cataract and brain atrophy including the deposition of amyloid or senile plaque [1,3,4], it remains unclear if these mutant mice are suitable as an animal model for human aging, especially for brain aging.

Although neuronal cell degeneration or death was suggested to occur in klotho mutant mice, especially in the hippocampus due to cognition impairment [3] or cerebellar Purkinje cells [1], no morphological sign for such deterioration has been demonstrated thus far. Klotho mutant mice show obviously lower motility with gait disturbance, as already indicated by Kuro-o et al [1], and when they do not move, when they are at rest, it is clear that their limbs, especially the posterior ones, are flattened (Fig. 1). Such disturbance in the motor function of skeletal muscles strongly suggests that motor neurons, either in the pyramidal or extrapyramidal system, are impaired. Therefore, in addition to the hippocampal neurons, it is of interest to analyze the morphology of Purkinje cells and spinal cord anterior horn cells. These two kinds of neurons are motor in nature; Purkinje cells belong to the

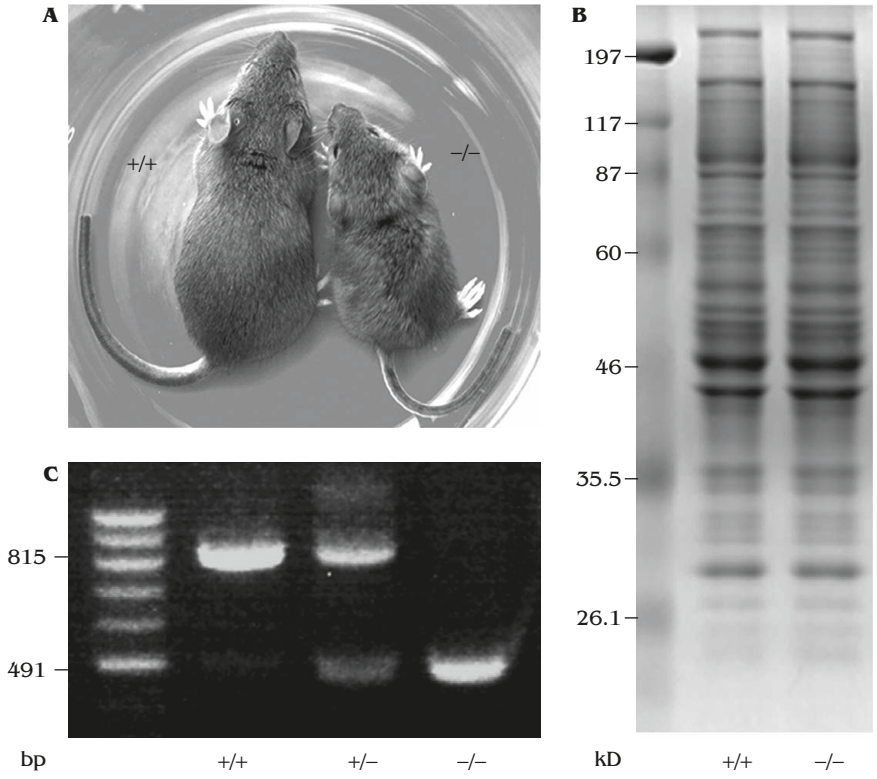

Fig. 1 — (A) Differences in appearance between wild-type (+/+, left) and homozygous mutant (–/–, right) mice at the age of 6 weeks. These littermate mice are 1 week younger than those used for our present study. Compared with the wild-type mouse, the mutant mouse is already much smaller in size and has a less fine coat of hair. Their tail tips were cut for genotype analysis, as shown in B. (B) Mouse genomic DNA screened by PCR analysis with specific primers from wild-type (+/+), heterozygous (+/–) and homozygous mutant (–/–) mice. Amplified DNA from wild-type mice shows a single band at 815 bp and that from homozygous mutant mice at 491 bp, while that from heterozygous mice are at both bands. (C) Homogenized postnuclear supernatants (PNS), from wild-type (+/+) and mutant (–/–) CNS tissues, transferred onto gels, were stained with Coomassie brilliant blue. Protein concentrations from both PNS were identical in total, although some bands were different in density from each other. These samples were used for immunoblotting to compare the expression levels of the various proteins described in this review.

extrapyramidal system while anterior horn cells are pyramidal, and they are both easy to identify and be compared among respective animals. Since the mutant mice tend to be smaller as well as thinner after 3 weeks of age, and such differences were significantly distinct by the age of 6 weeks (Fig. 1A), many internal organs, such as the digestive system, must be disordered due partially to impairment of the autonomic nervous system. However, it is complicated to analyze the relationship between the internal organs and autonomic nervous system because of the involvement of the endocrine system, and so these points will not be discussed here.

2. Neurodegeneration with aging

The mechanism for neurodegeneration in the central nervous system (CNS) of klotho mutant mice has not extensively been analyzed, probably due to less morphological as well as physiological alterations in the CNS when compared to other organs, such as kidney, artery, bone, lung, skin and genital organs [1], with particular attention being paid to ion homeostasis related to kidney or bone [5–7] or vascular/endothelial dysfunction [8,9]. This is despite the fact that the klotho gene is more highly expressed in the brain than in other organs, with the exception of the kidney which has the highest expression of this gene [1,10]. Age-associated degeneration of neurons is detectable in aged animals and also in many age-related neurodegenerative diseases, such as in Alzheimer's and Parkinson's diseases. Although the neurofibrillary tangles composed of a kind of microtubule-associated protein (MAP), tau, and senile plaques/amyloid deposits, both of which are hallmarks for Alzheimer disease [11,12], are not visible in klotho mutant mice, the absence of these pathological alterations do not support the idea that the CNS of klotho mutant mice is not associated with aging, because the normal aged animals do not show such degenerative changes either. Our detailed analysis of the whole CNS of klotho mutant mice in comparison with their wild-type littermates by immunoblotting, immunohistochemical and electron microscopic techniques (shown in Figs. 1 and 2) have convincingly demonstrated that the CNS neurons in these mutant mice are not simply immature or undeveloped but aging in nature after having almost developed, showing distinctive signs of neuronal degeneration or cell death even in their premature condition at less than 2 months of age [13]. Neuronal and glial intracellular structures and the expression patterns of many proteins associated with cell degeneration/death [14,15] in these cells in the klotho mutant CNS are very similar to those seen in aged animals and also in some neurodegenerative diseases.

3. Relationship between aging and cytoskeletons in the CNS

From our morphological viewpoint, the most powerful evidence to support the idea that neurons in klotho mutant mice are aging but not immature in nature was due to the increase in neurofilaments (NFs) in the cell bodies of both Purkinje cells and spinal cord anterior horn cells (Fig. 2). If the animals are immature and not fully developed, few NFs would be visible in neuronal cell bodies, especially in Purkinje cells in which NFs are much less numerous than in the axonal compartment [16]. Among the normal neuronal cytoskeletons, NFs are the most susceptible cytoskeleton associated with aging or neurodegenerative diseases; they are increased in density in most axonal compartments and sometimes also in other neuronal compartments, dendrites and cell bodies [15,17,18]. In addition to the increase in the distribution density of NFs in neuronal cell bodies, NFs were more numerous in the axonal compartment in klotho mutant mice than in wild-type mice, as is also demonstrated in aged rats [19,20]. In general, the phosphorylation level of NF subunit proteins, especially of NF-H, becomes higher with age and in neurodegenerative conditions [15,18]. The relative reduction in the amount of NF-M was reported in aged rats [19,20] and also in klotho mutant mice [21]. Thus, changes in NF structural profiles and in the nature of their subunit proteins are critical for identifying neuronal aging or degeneration. Thus, these morphological and biochemical alterations in NFs and their subunit proteins, respectively, observed in klotho mutant mice strongly suggest that the CNS tissue in these mice is aging or degenerating.

In addition to NFs, another neuronal cytoskeletons, i.e., microtubules and its constituent protein, tubulin, do not show any significant difference in distribution density in neurons including their cell bodies (Fig. 2) and in expression level between wild-type and klotho mutant mice. However, in Purkinje cell dendrites, which are occupied predominantly with microtubules with few NFs even in normal adult animals [16], there was a detectable morphological change in the organization of this cytoskeleton; microtubules were aligned significantly closer to each other in mutant than in wild-type mice. Although there is no report on the expression of a disease-related MAP, tau, in klotho mutant mice, we found that another MAP, i.e., MAP2, was reduced in expression and also in histological labeling density, especially in the cell bodies. Since the reduced expression of MAP2 has been indicated in SAMP10 mice, another animal model of accelerated brain aging [22], our result also supports that the CNS neurons in klotho mutant mice are aging in nature.

Moreover, gliofilaments in astrocytes and the expression of their constituent protein, GFAP, were

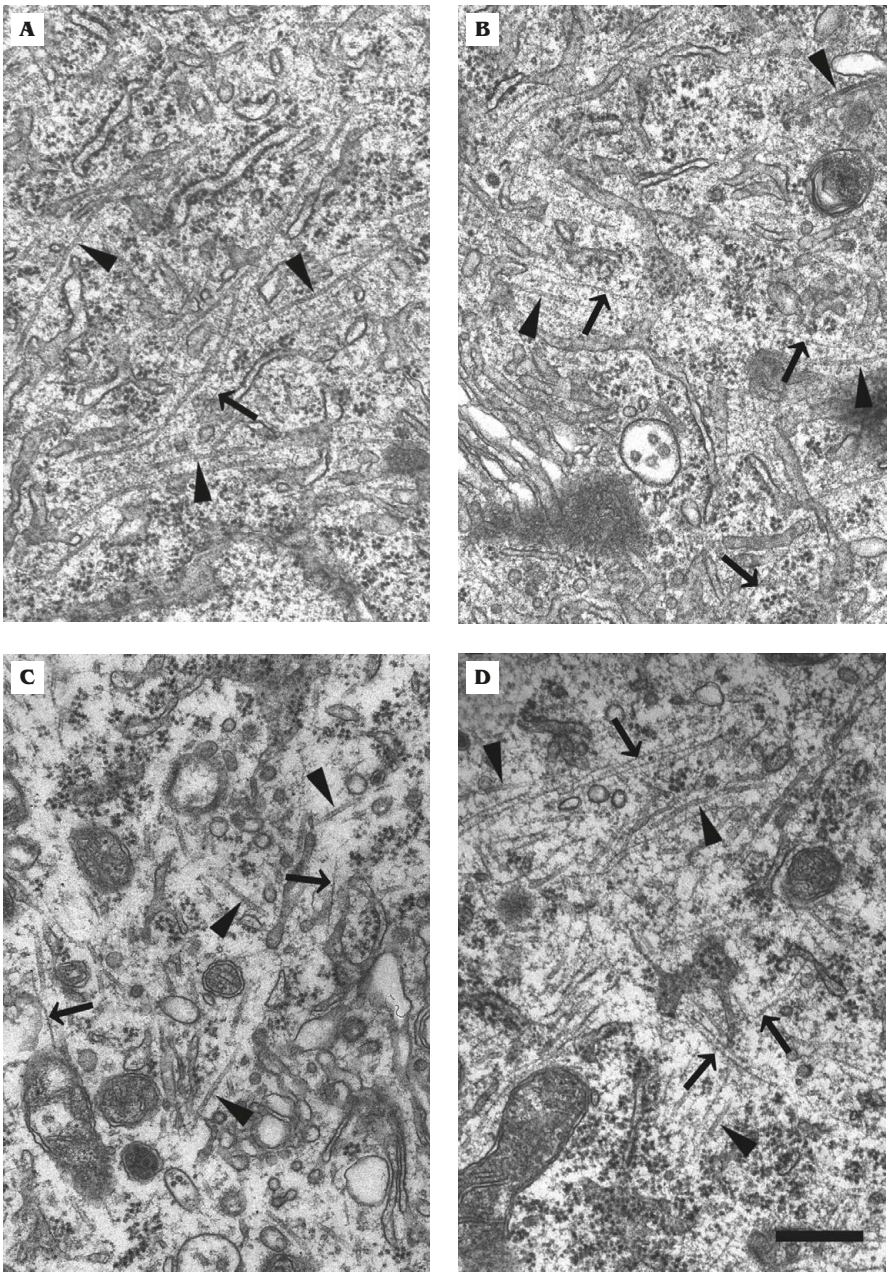

Fig. 2 — Electron micrographs of cerebellar Purkinje cells (A and B) and spinal cord anterior horn cells (C and D) from wild-type (A and C) and mutant (B and D) mice. Cytoskeletons, neurofilaments (NFs) and microtubules, in the cell bodies from these neurons were compared between wild-type and mutant mice. NFs (arrows) were few in number in wild-type mice (A and C), there were hardly any in the Purkinje cell (A), but were obvious in mutant mice (B and D), showing significant increase in the anterior horn cell (D). On the other hand, microtubules (arrowheads), which are much thicker (25nm) than NFs (10nm), were always conspicuous in both neurons from the respective mice. The increase in the distribution density of NFs in klotho mice indicates that CNS neurons in the mutant mice tend to be more mature than the wild-type mice or aged after maturation, indicating clearly that the klotho mutant CNS neurons are not less mature in nature than wild-type mice. Scale, 0.5μm.

drastically increased in distribution density and expression level, respectively, in klotho mutant mice. Such changes in glial structures and protein expression (15) also strongly suggest that the CNS of klotho mutant mice is either degenerating or aging, but not immature in nature.

Therefore, the klotho mutant mouse is a good model for brain aging and more suitable for analyzing neurodegenerative alterations dependent on aging processes than other proposed animal models. Klotho mutant mice have very short longevity, meaning that the aging process progresses rapidly,

making it convenient to analyze or modify the lifespan of these animals in the lab with short experimental periods (23).

4. Possible rescue of neuronal degeneration and extension of lifespan with nutritional manipulation

The short-lived klotho mutant mice are defective in the klotho gene, resulting in the absence of the expression of the protein, and introduction of exogenous klotho gene into these mutant mice improve or rescue their disordered phenotypes (1). More importantly, overexpression of the klotho gene significantly extends the lifespan of wild-type normal mice (24). Moreover, the klotho protein appears to be associated with removal of reactive oxygen species (25). Therefore, in addition to gene manipulation, nutritional/pharmacological manipulations of klotho mutant mice with some powerful antioxidants are expected to improve or rescue, at least to some extent, age-related deteriorations, especially CNS neuronal disorders, in these animals. In klotho mutant mice, treatment with antioxidant vitamin, α-tocopherol, was reported to ameliorate cognition impairment, and prevent lipid peroxidation and apoptosis in the hippocampus, although there was no effect on their lifespan (3).

However, other antioxidants such as polyphenols, widely present in fruits/vegetables, had some neuroprotective effects (26). Among the polyphenols, resveratrol, rich in grapes and red wine, is known to increase longevity in short-lived animals, whose experimental data are much easier to interpret than in animals with relatively longer lifespans such as rodents (23). Resveratrol and also other polyphenols, such as green tea polyphenols, may reduce the degree of impairment in neurodegenerative conditions such as Alzheimer's and Parkinson's diseases (27,28). Interestingly, resveratrol is considered to be associated with caloric restriction, affecting neuronal energy homeostasis by the activation of AMP-activated kinase, a sensor of cellular energy levels (29). In addition to the antioxidant function of both resveratrol (26) and klotho protein (25), the presumed energy-related functions of resveratrol seem to be associated with reduction of insulin/insulin-like growth factor I signaling, which is also brought about by klotho protein, providing the possible common mechanism by which both chemicals delay aging by energy or caloric balance/regulation (24,29–31). Taken together, nutritional manipulation with various polyphenols (including resveratrol and red wine extracts), which is much safer and more applicable to humans than genetic manipulation, could be expected to improve disordered CNS morphology and function of klotho mutant mice and also aged animals, including humans, resulting in lifespan extension without impairment of neuronal cognitive function.

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