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NEURONAL INTERMEDIATE FILAMENT ACCUMULATION IN THE CENTRAL NERVOUS SYSTEM NEURONS OF NUDE RATS (F344/NJcl-*rnu/rnu*)

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Introduction

Neuronal intermediate filaments are cytoskeletal proteins including neurofilament (NF) triplets of light, medium and heavy subunits and α-internexin, which are classified into type IV intermediate filaments (IFs).[2-4] Accumulation of NF in the neurons has been reported in several neurodegenerative human diseases including neuronal intermediate filament inclusion disease (NIFID).[1-3] NIFID is one of the frontotemporal lobar degeneration-fused in sarcoma protein (FTLD-FUS),[1] and a rare neuropathological human disorder of early onset with variable clinical signs including frontotemporal dementia, pyramidal and extrapyramidal signs.[2] Grossly, there is focal atrophy of the frontal lobes, and to a less degree, temporal and parietal lobes, and the caudate is frequently affected.[2] Microscopically, all of the type IV neuronal IFs including α-internexin accumulate excessively as neuronal cytoplasmic inclusion, which is present throughout the brain including the spinal cord.[2] In addition, neuronal loss and gliosis are also common findings.[2] α-Internexin accumulation is the pathological hallmark of human NIFID, which is variably ubiquitinated, but do not contain tau.[1,2] A transgenic mouse model overexpressing rat α-internexin (Tg-mice with rat α-internexin) of human NIFID has been found to demonstrate abnormal neuronal IF accumulation and motor coordination deficits.[3] To our knowledge, this is the first description on spontaneous α-internexin accumulation in the CNS neurons in animals.

Materials and Methods

Nude rat (F344/NJcl-*rnu/rnu*) were purchased from CLEA Japan, Inc. (Tokyo, Japan). 23 animals aged eight to 18 weeks which showed no apparent clinical abnormalities and received no prior treatments were necropsied. Gross examination revealed no abnormalities in any organ from any animal. Immunohistochemistry (IHC) was performed using the following primary antibodies.

mouse monoclonal anti-rat α-internexin (GeneTex, Inc., Irvine, California, USA)
mouse monoclonal anti-human NFs (Dako, Burlingame, California, USA)
mouse monoclonal anti-bovine ubiquitin (LifeSpan Biosciences, Inc., Seattle, Washington, USA)
mouse monoclonal anti-human tau (AnaSpec, Inc., Fremont, California, USA)

Results

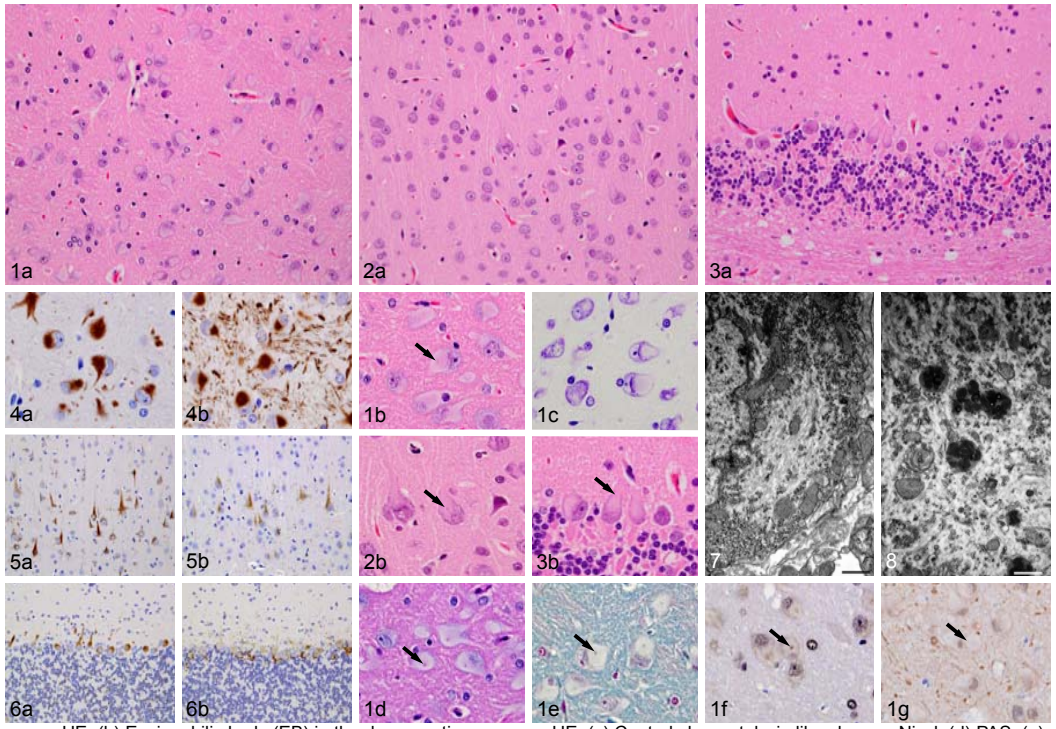

Figure 1. Thalamus. (a) Pale eosinophilic material accumulation in the neurons. HE. (b) Eosinophilic body (EB) in the degenerative neurons. HE. (c) Central chromatolysis-like change. Nissl. (d) PAS. (e) Schmorl. (f) IHC for ubiquitin. (g) IHC for tau. **Figure 2**. External pyramidal layer in the cerebral cortex. (a) Swollen dendrites. HE. (b). Higher magnification. **Figure 3**. Purkinje cell layer. (a) Swollen dendrites. HE. (b). Higher magnification. **Figure 4**. Thalamus. (a) IHC for α-internexin. (b) IHC for NFs. **Figure 5**. External pyramidal layer in the cerebral cortex. (a) IHC for α-internexin. (b) IHC for NFs. **Figure 6**. Purkinje cell layer. (a) IHC for α-internexin. (b) IHC for NFs. **Figure 7**. Purkinje cell. Filamentous structure in the degenerative neurons. TEM. **Figure 8**. Purkinje cell. Aggregates of secondary lysosomes in the neurites. TEM.

These results revealed that the pale eosinophilic materials in the degenerative neurons were accumulations of the neuronal IFs including α-internexin and NFs, and that EB was an aggregate of ubiquitinated materials with lipofuscin-like nature. There were no other histopathological abnormalities including neuronal necrosis or loss, gliosis or microglial proliferation in any area, or torpedo in Purkinje cell axon. Neuronal changes were observed in three animals out of 23 animals examined (13 %). These three animals were neither littermates nor from the same parents.

Discussion

α-Internexin is expressed earlier and more abundantly than NFs throughout the developing CNS neurons.[4] As development continues, the expression levels of α-internexin decrease, whereas those of NFs increase.[4] α-Internexin is expressed at relatively low levels in comparison to NFs, and selective expression is observed in the cerebellar granule cells and the cerebral layer II neurons in human adult brain.[2,4] IHC for α-internexin in the unaffected mature nude rats revealed selective expression only in the cerebellar granule cells. Therefore, these results indicated that neuronal α-internexin accumulation excluding the cerebellar granule cells in mature nude rats could have pathological significance in human NIFID as well.

In Tg-mice with rat α-internexin, Purkinje cells are most affected by α-internexin overexpression.[3] Fusiform swellings of the axons of Purkinje cells, called torpedoes, are observed in the granular layer in the cerebellar cortex.[3] In contrast, in nude rats, α-internexin accumulation in the dendrites of Purkinje cells and the pyramidal cells in the cerebral cortex were observed toward the molecular layer in the cerebellar cortex and the more superficial layer of the cerebral cortex, respectively. It is suggested that the pattern of α-internexin accumulation in the dendrites, not in the axons, could be an identifying feature in nude rats; however, the reason remains unclear.

A motor coordination test using a rotarod demonstrated that Tg-mice with rat α-internexin have a deficit in motor coordination as early as three month of age.[3] It remains unclear whether nude rats without apparent clinical abnormalities such as tremors or any other overt symptoms of disorders had presented poor motor coordination. In addition, there were no gross abnormalities including focal atrophy of the brain or histopathological abnormalities including neuronal loss or microglial proliferation in any area of the affected animals. Therefore, the nude rats of this study could not be diagnosed as rat NIFID. On the other hand, α-internexin is a major component of the pathological hallmark, and neuronal α-internexin accumulation demonstrates diagnostic specificity for human NIFID.[2] Therefore, it was suggested that nude rats (F344/NJcl-*rnu/rnu*) can be used as a spontaneous animal model for human NIFID.

References

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