Department of Neuroscience
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General Summary

Our research projects have concerned neurodegenerative disorders caused by intracellular accumulation of abnormal proteins. We are also studying mouse models of neurodegenerative disorders and autopsy cases by means of standard morphologic analysis and molecular biological analysis.

Research Activities

Pathophysiological study of polyubiquitination in lysosomal diseases
Objective: This study investigated the pathophysiology of polyubiquitination in lysosomal diseases.
Material and methods: We analyzed skeletal muscle and central nervous system (CNS) neurons from model mice of Pompe disease, prosaposin deficiency, Niemann-Pick disease type C, Fabry disease, Hunter disease, and Sly disease and human patients with Pompe disease and Gaucher disease by means of immunohistochemical methods and antibodies against ubiquitin, K48 polyubiquitin, K63 polyubiquitin, p62, microtubule-associated protein light chain 3 (LC3), and lysosome-associated membrane protein 2 (LAMP2).
Results: Skeletal muscle from patients with Pompe disease contained aggregates immunoreactive for p62, ubiquitin, K48 polyubiquitin, K63 polyubiquitin, and LC3. Neurons from the CNS of prosaposin deficiency and Niemann-Pick disease type C (mice and humans) had small numbers of aggregates immunoreactive for p62, ubiquitin, K48 polyubiquitin, K63 polyubiquitin, and LC3. In mice models of Fabry disease, Hunter disease, and Sly disease and in human patients with Fabry disease and Gaucher disease, the CNS contained LAMP2-immunoreactive lysosomes with vacuolation. There were, however, few aggregates immunoreactive for p62, LC3, or ubiquitin.
Discussion: We confirmed that polyubiquitination involving the ubiquitin proteolysis system and the autophagy lysosome system would function in Pompe disease, prosaposin deficiency disease, and Niemann-Pick disease C.

Histopathological analysis of rat peripheral nerves with an in vivo cryotechnique
Experimental animals are used to examine the pathogenesis of various disorders of peripheral nerves. However, observing the fine structure of the peripheral nerves can be difficult with conventional histopathological analysis of paraformaldehyde-fixed, paraffin-embedded specimens. We attempted to examine the peripheral nerves of experimental rats with an *in vivo* cryotechnique. Sciatic nerves of rats were rapidly frozen by pouring isopentane-propane cryogen over them. The sciatic nerves were placed in a freeze-sub-

stitution solution (absolute acetone containing 2% paraformaldehyde), and the temperature of the sciatic nerves was gradually returned to room temperature. After being washed, the nerves were transferred to chloroform and embedded in paraffin. Sections of the paraffin-embedded samples were used for hematoxylin and eosin, Bodian, and Klüver-Barrera staining and immunohistochemical analysis. The axons were preserved from shrinkage, and the fine structure of the examined nerves could be observed. The structure of the nodes of Ranvier could be visualized with this preparation. Schmidt-Lanterman incisures were visible with immunostaining for E-cadherin or β-catenin. Preparation of specimens with our *in vivo* cryotechnique provided good resolution for microscopic examination of the peripheral nerves.

Publications

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