

Department of Pathology Division of Neuropathology

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General Summary

Our research projects have concerned neurodegenerative disorders caused by the 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

Accumulation of subunit c of mitochondria ATP synthase in the central nervous system in lysosomal diseases

Objective: This study investigated the accumulation of subunit c of mitochondria ATP synthase (SCMAS) in the central nervous system in lysosomal disorders.

Material and methods: We analyzed the central nervous system of mouse models of prosaposin deficiency and mucopolysaccharidosis type II (MPS II) with biochemical methods, the amino-cupric-silver method, and immunohistochemical methods with antibodies against accumulating materials, such as SCMAS.

Results: In the central nervous system of mouse models of prosaposin deficiency and MPS II, the numbers of SCMAS-immunoreactive neurons increased in proportion to the amino-cupric-silver-impregnated neurons.

Discussion: SCMAS is a candidate for amino-cupric-silver-impregnated material in the central nervous system of mouse models of lysosomal disorders.

Detection of point mutations in the isocitrate dehydrogenase gene

Objective: A novel mutation of isocitrate dehydrogenase (IDH) was found in gliomas and in hematopoietic and chondroid neoplasms. Different methods, such as direct polymerase chain reaction (PCR) sequencing, post-PCR fluorescence melting curve analysis, and real-time PCR assays for single nucleotide polymorphisms (SNaPshot, Life Technologies, Carlsbad, CA, USA), and immunohistochemical studies for the IDH1 R132H mutation are available to determine the IDH mutation status. This study assessed the Cycleave PCR method (Takara Bio Inc., Otsu, Shiga, Japan) using chimeric probes containing RNA and DNA for investigating the status of IDH mutations.

Material and methods: We designed chimeric probes and PCR primers for detecting mutations of IDH1 (132R and R132H, R132S, R132C, R132G, R132L, R132V, and R132P) and IDH2 (172R and R172G, R172K, and R172K). For positive and negative controls, we also made recombinant plasmid vectors containing the wild-type IDH gene segments or each mutation. The Cycleave PCRs were evaluated in the various ratios of wild-type

and mutated recombinant plasmid vectors. The assumed tumor condition of 2 IDH mutations was also investigated.

Results: The specificity of Cycleave PCR method for IDH mutations was 100%. The IDH2 R172M probe, containing a palindromic sequence, was unavailable for Cycleave PCR. We could detect more than 0.2% of a single mutation in the wild-type vector. When a tumor contained 2 IDH mutations, not less than 2% of a single mutation was detectable.

Discussion: The Cycleave PCR method can be used for IDH genotyping.

Nuclear functional domain in retinal cells of spinocerebellar ataxia 7

Spinocerebellar ataxia 7 (SCA7) is an autosomal dominant neurodegenerative disorder characterized by cerebellar ataxia and retinal degeneration. SCA7 is caused by a polyglutamine expansion in ataxin 7. The pathologic hallmark of SCA7 is the formation of neuronal intranuclear inclusions (NIIs) through the accumulation of mutated ataxin 7. Nuclear functional domains related to the formation of NIIs, especially promyelocytic leukemia nuclear bodies, could be accumulation sites of the pathological ataxin 7 with expanded polyglutamine. Spliceosomes and Cajal bodies could be related to the formation of NIIs in SCA7. In this study, we examined the relation of spliceosomes and NIIs in the retina of SCA7 knock-in mice. Ataxin 7-immunoreactive NIIs were present in all cell layers of degenerated SCA7 knock-in mice retina. In the retina of wild-type mice, NIIs immunoreactive for coilin (a molecular marker of Cajal bodies) and sm (a marker of spliceosome) were observed in the ganglion and internal granular cell layers. In SCA7 knock-in mice, sm-immunoreactive structures were found in the external granular cell layer of the retina, and coilin-immunoreactive structures were unchanged. These findings indicate that alteration of the nuclear spliceosome functional domain is related to RNA metabolism in retinal cells in SCA7.

Publications

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Reviews and Books

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