学 位 論 文 の 要 旨

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学 位 論 文 名　Different types of neural cell death in the cerebellum of the Ataxia and Male Sterility (AMS) mutant mouse

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論 文 内 容 の 要 旨

Introduction

Animal models of human diseases, even if they are not identical to human diseases, provide us with opportunities to analyze their pathogenic processes. Our mutant mouse, named as AMS after its clinical symptoms, manifests ataxia and male sterility due to the presence of a disease-causing *ams* mutation which shows an autosomal recessive inheritance pattern. The disease-responsible lesion for ataxia results in a near-complete loss of Purkinje cells by 6 weeks of age. In the present study we focused on the presence of dying cells in the AMS cerebellum to explain its cellular basis of acute cerebellar atrophy, and found that different types of neural death indicative of primary and secondary cell death due to the above mutation contributed to the morphological changes observed in the cerebellum.

Materials and Methods

Mice: *ams* heterozygous partners were mated to obtain ataxic *ams* homozyogous mice (AMS mice) and control *ams* heterozygous or wild type mice.

Morphometry: The area of the cerebellum in the mid-sagittal plane was measured. Images of the cerebellum on a monitor were manually copied on paper and then were cut apart according the

nueroanatomical units. The weight of the cut paper was then converted to the actual area by the value obtained from the known constant area of a hemocytometer gauge.

Evaluation of apoptosis: Morphological observations by light and electron microscopy, the terminal deoxynucleotidyl transerase-mediated dUTP nick-end-labeling (TUNEL) method and immunohistochemical method were carried out to evaluate apoptosis.

Results and Discussion

Cerebellar atrophy in AMS mice: The size of the cerebellum of the AMS mouse was smaller than that of age-matched control ones, but the normal development of the folia and formation of cortical structures except acute loss of Purkinje cells by 6 weeks of age were apparent. The area of the cerebellum of the control mice increased gradually from 3 to 6 weeks of age and then remained relatively constant to 12 weeks of age. In contrast, the increase in the area of AMS cerebellum between 3 and 5 weeks of age was minimal and thereafter the area gradually decreased. The ratio of the area of the AMS to that of the control was 85% at 3 weeks of age and decreased to 44% at 12 weeks of age. These results show the cerebellar atrophy to be quite acute and the results of the individual measurement of the area of the molecular and granular layers and comparison between them show shrinkage of the molecular layer was somewhat more rapid than that of the granular layer. Atrophy of the molecular layer seemed to be mainly ascribed to a complete loss of the dendrites of the Purkinje cells.

Morphology of neural cell death in the AMS cerebellum. The light microscopic morphological change of the dying Purkinje cells was conspicuous, i.e. shrinkage of the cytoplasm with eosinophilic stainability accompanied by condensation and fragmentation of the nucleus and an irregular swelling of the dendrite. A complete disappearance accompanied by these changes made us quite naturally consider the mode of Purkinje cell death to be apoptosis. However, electron microscopic observations did not reveal any typical nuclear morphology in the dying Purkinje cells. In contrast, electron microscopic observations of the granule cell revealed a substantial number of classical apoptotic bodies throughout the granular layer.

Quantification of apoptosis in the AMS cerebellum: TUNEL positive granule cells gradually increased beginning at 3 weeks of age and the maximal number showed a plateau level at around 6, 7 weeks of age and thereafter decreased. On the other hand very few TUNEL positive Purkinje cells were found irrespective to the presence of many dying Purkinje cells at 3 weeks old. Immunohistochemistry for cleaved caspase-3 also showed many positive cells parallel to the number of TUNEL positive cells in the granular layer. Double fluorescent staining for TUNEL and cleaved caspase-3 revealed the presence of a substantial number of cells that were either double positive or single positive for each marker. Fluorescent double positive nuclei clearly showed the transfer of the enzyme to the nucleus, thus indicating the activation of the apoptotic process in the granule cells.

These results indicate that the death of Purkinje cells was autonomic due to the direct effect of the *ams* mutation. In addition, the death of a substantial number of granule cells might be secondary to the loss of post-synaptic Purkinje cells, because the time-dependent numerical change of the granule cell death followed that of the Purkinje cells.

We tried to morphologically reveal the location and time of appearance of the molecules controlling the apoptotic process and the cascade of the activation of caspases, but failed to show them clearly. Regarding immunohistochemistry, only the anti-cleaved caspase-3 antibody used in this study provided stable and reliable staining.

Conclusion

The cerebellum of the ataxic AMS mutant mouse was morphologically examined. Regarding the mode of cell death, Purkinje cell death was autonomic but it did not show any characteristics of classical apoptosis. In contrast, the death of granule cells was secondary to Purkinje cell death, thus showing the characteristics of classical apoptosis. As a result, different types of neural death were thus considered to contribute to the acute atrophy of the cerebellum that was observed at a young age in the AMS mouse.