学 位 論 文 の 要 旨

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学 位 論 文 名　Analysis of the Light-Sensitivity of the Photoreceptor Cells of the Ataxia and Male Sterility (AMS) Mouse, an *Nna1* Mutant

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論文内容の要旨

INTRODUCTION

The Ataxia and Male Sterility (AMS) mouse is a mutant that arose spontaneously in a colony of autoimmune-prone MRL/*lpr* mice in our laboratory, the major symptoms of which derive from a near-complete loss of Purkinje cells in the cerebellum and azoospermia due to abnormal cellular death in the course of spermatic differentiation in the seminiferous tubules of the testes. The autosomal recessive disease-phenotype of the this mouse was found to be allelic to that of the previously known Purkinje Cell Degeneration (PCD) mouse, and mutations that cause a loss-of-function of the *Nna1* gene were found to be responsible for the symptoms in these mice. The *Nna1* gene is phylogenetically well preserved and it encodes an ATP/GTP binding protein (Nna1) with a putative zinc carboxypeptidase domain that is essential for Purkinje cell survival in mice. However, the level of dependency of the cell survival on the function of the *Nna1* gene differed depending on the cell types in these mice. In this report, degeneration of the retina, caused by the photoreceptor cell death, was shown to be one of the manifestations of the pleiotropic effect of the *Nna1* mutation on various cell lineages in the AMS mouse, and examined how genetic damage caused by the *Nna1* mutation affects the photosensitivity of the AMS mouse photoreceptor cells.

MATERIALS AND METHODS

AMS and control MRL mice housed with a under the 12-hour light-dark cycle or in a dark environment from birth until the time of examination. The intentional light exposure was

performed by 1 or 5 klux of diffuse, cool, white fluorescent light for 6 or 24 hours (hr) in wire-topped cages, the outside of which was covered with alminium foil. The mice were euthanized and eyes were enucleated, and were processed for paraffin sections, frozen sections and transmission electron microscopical examination. The thickness of the outer nuclear layer (ONL) of the retina was measured at the positions 500 μm superior and inferior to the optic nerve head (ONH) of hematoxylin and eosin (HE)-stained paraffin sections using light microscopic 40x images. For electron microscopy, mice retinas were observed the morphological changes after irradiation. The number of apoptotic cells (TUNEL positive cells), present in a 100 μm^2 area of the ONL 500 μm superior and inferior to the ONH of ten μm thick frozen sections, was counted, and was calculated to be the mean of figures obtained from the two areas mentioned above by the count obtained from three independent observers without knowledge of the objects.

RESULTS AND DISCUSSION

The adult AMS showed age-dependent gradual degeneration of the retina. The ONL thickness of the AMS mice at 12 weeks of age was only 46% of that of the age-matched control MRL mice, or 66% of that of the 4-week-old AMS mice. However, the basic 10-layer histological structure of the retina was well-preserved.

The examination of the dark-reared mouse retinas revealed a complete effect of light protection against the photoreceptor cell loss only in the 4-week-old AMS, whereas in adult AMS mice older than 6 weeks of age, there was as much retinal degeneration as that observed in the AMS mice housed under the regular light environment. This indicates that under the genetic defect of *Nna1* in the AMS mouse, another event is necessary to trigger death mechanisms in photoreceptor cells before 4 weeks of age, but in the adult mice, the retinal degeneration progresses autonomously, independent of external light stimulation. This might reflect the difference in the biochemical activities, including that of the Nna1, in the photoreceptor cells between the developing and the adult retinas, because major cellular events, including cell proliferation or cell death, related to the postnatal retinal development of the mouse end by the 3rd postnatal week.

Effects of the intentional light irradiation on the AMS retina were as follows. The retinal degeneration induced by the 1 klux-24 hr irradiation, a relatively weak light stimulation, was exacerbated in the AMS mouse, which suggested that the susceptibility to light was enhanced in the photoreceptor cells already stressed by a defect in the *Nna1* gene. On the other hand the short- and long-term effects of 5 klux intensive light stimulation on the ONL thickness were different from these of the weak light stimulation. The decrease of the ONL thickness in the first

24 hr after the 5 klux-24 hr light irradiation appeared only in the AMS retina, as if it indicated increased susceptibility of this mutant to intensive light exposure. However, the ONL atrophy observed 1 week after the irradiation was similar in the following 4 groups of mice, irrespective of whether they were mutant or wild-type, and regardless of whether they were irradiated for 6 or 24 hr. Two important points that arose from these results were that the illumination with 5 klux light for 6 hr was sufficient to trigger the death of all light-sensitive cells and prolongation of the irradiation for a further 18 hr did not increase the number of dying cells, and that the number of survived cells, all of which had been non-susceptible to intense light during the indicated period of irradiation, did not differ between the mutant AMS mice and the control MRL mice. The presence of non-susceptible cells to the condition of the illumination may be relevant to a state of photoreceptor-specific metabolism. Furthermore, *Nna1* seemed to be silent in these non-susceptible cells, otherwise the intracellular stress due to the Nna1 defect would have caused exacerbation of the retinal degeneration in the AMS mice.

The morphological changes appeared in the first 24 hr post-5 klux-irradiation were examined. Severe damage of the photoreceptor cells, such as degeneration and cell death in the ONL and vacuolation and disarrangement of the layer of rods and cones, were shown by light microscopic observations in the retina of both mice. Apoptosis and cell death-induced changes were observed further in detail by electron microscopy. These changes gradually weakened and subsided in the MRL retina by around 24 hr post-irradiation, but remained to a greater extent in the AMS retina at 24 hr post-irradiation. The apoptotic signals by TUNEL staining in the fluorescent micrographs were most apparent at 2 hr post-irradiation, and seemed to be stronger in the AMS mice than in the MRL mice. However, semi-quantitation of the apoptotic cells in the ONL showed that there was no difference in the TUNEL positive cell number per unit area at any of the time points examined after the irradiation between the AMS and MRL mice. At 1 week after the intensive irradiation, the acute morphological changes described above disappeared, and just simple atrophy of the ONL was observed in all four groups. However, further ONL atrophy on and after 2 weeks post-irradiation was observed only in the AMS mice, and not in the MRL mice. This suggests that the function of Nna1 becomes turned on in some of the remaining photoreceptor cells.

CONCLUSION

The AMS mouse, an *Nna1* mutant, is a mouse model of the hereditary retinal degeneration. The present experiments revealed that the functional loss of Nna1 and the light stimulation were closely related, but mutually independent, death triggers of the photoreceptor cells.