Autologous fluorescence exclusion method of lipofuscin in a brain tissue section

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Abstract

Lipofuscin has autologous fluorescence. When an immunofluorescent stain is applied to an autopsy brain tissue specimen, it is necessary to erase the autologous fluorescence of the lipofuscin. However, in the conventional report, it was difficult to remove lipofuscin enough. We examined the autologous fluorescence removal of lipofuscin in brain tissue using various methods. The autologous fluorescence could be completely removed by using Autofluorescence Eliminator Reagent R for 45 minutes.

Keywords: lipofuscin, Parkinson’s disease, mitochondria

Introduction

It is one of the representative neurodegenerative diseases, and most are isolated, and Parkinson's disease occurs. The selective degeneration of nigral dopamine-producing cells is found in the same disease. The contribution of a mitochondrial functional disorder is assumed by the condition of the patient. However, the cause and the mechanism of the mitochondrial disorder are still unknown. On the other hand, pathologic elucidation of isolated Parkinson's disease has been tried through analysis of the gene responsible for hereditary Parkinson's disease. The parkin was discovered to be the gene responsible for familial Parkinson's disease (PARK 2), which occurs most frequently [1]. The parkin protein has ubiquitin ligase (E3) activity. It seems to be associated with the selective degeneration of nigral dopamine-producing cells [2,3]. It is very important to this study that an autopsy brain tissue specimen is analyzed. It is an essential technique to stain the brain tissue of the patients by conducting autologous fluorescence removal of the lipofuscin.

Methods

Immunological analysis

We used mouse antibody against parkin; PRK8 (Sigma-Aldrich Co., MO, USA). Polyclonal anti-Tom 20 antibody was purchased from Santa Cruz Biotechnology Inc. (Heidelberg, Germany). For immunocytochemical analysis, cells were fixed with 4% PFA in phosphate-buffered saline (PBS) for 12 min and treated with 0.5% Triton X-100 in PBS for 5 min. For immunohistochemical analysis, 10-μm thick cryosections were made from an autopsied brain that was stored in liquid nitrogen. Conventional pathological analysis confirmed no abnormality in the brain sections. After blocking with 5% bovine serum albumin (BSA) in PBS for 20 min, cells were incubated with primary antibodies. Control experiments were performed using normal rabbit or mouse serum instead of primary antibodies. Secondary antibodies included fluorescein

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isothiocyanate-conjugated anti-mouse IgG and tetramethylrhodamine isothiocyanate-conjugated anti-rabbit IgG. To reduce autofluorescence, sections were treated with Potassium metabisulfite-oxalic acid or Autofluorescence Eliminator Reagent (Millipore Corporation, MA, USA) according to the manufacture's protocol and our lab protocol.

Results

As shown in Figure1, the classic methods with the conventional report were tried, but the autologous fluorescence could not be removed. Therefore, different methods using Autofluorescence Eliminator Reagent[R] (Life Technologies Corp., CA, USA) were tried. It was added for five minutes according to the manual, but was not able to remove the autologous fluorescence very much. Therefore, the latent time was extended, and it was observed that the autologous fluorescence could be completely erased within 45 minutes (Figure2). The immunostaining of the cerebrum normal graft. As shown in Figure2, fluorescent antibody stained cerebrum normal parkin and mitochondria. Red mitochondria completely accorded with green parkin.

Discussion

Lipofuscin is inclusion bodies accumulating with aging. Lipofuscin is the name given to finely granular yellow-brown pigment granules composed of lipid-containing residues of lysosomal digestion. It is considered one of the aging pigments, found in almost the whole body including the liver, kidney, heart muscle, adrenals, nerve cells, and ganglion cells. It is specifically arranged around the nucleus, and is a type of lipochrome. In particular, self-possession of brown lipofuscin is found in cytoplasm of nerve cells which have aged. A large quantity of lipofuscin is present in a nerve cell of the autopsy brain tissue [4].
The presence of lipofuscin can complicate the use of fluorescence microscopy in CNS (e.g., fluorescent retrograde tract tracing and immunocytochemistry) because of its broad excitation and emission spectra [5-7]. The spectra of lipofuscin overlap those of all commonly used fluorophores, making distinctions between specific labeling and nonspecific autofluorescence difficult or impossible [8-12]. We performed an immunofluorescent stain of autopsy brain tissue. This autologous fluorescence could not be erased easily by conventional methods . Therefore, we examined an original condition and were able to achieve our purpose.

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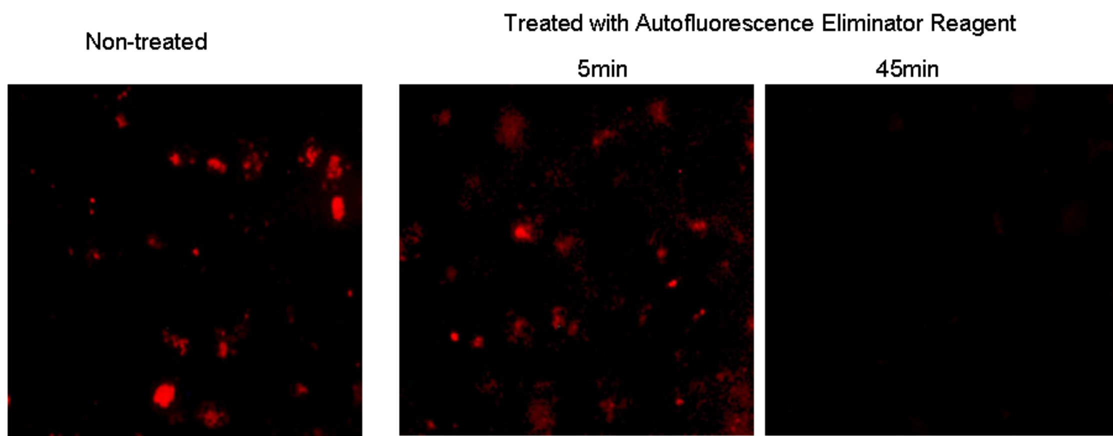

Figure 1. Autofluorescence and treatment of Autofluorescence Eliminator

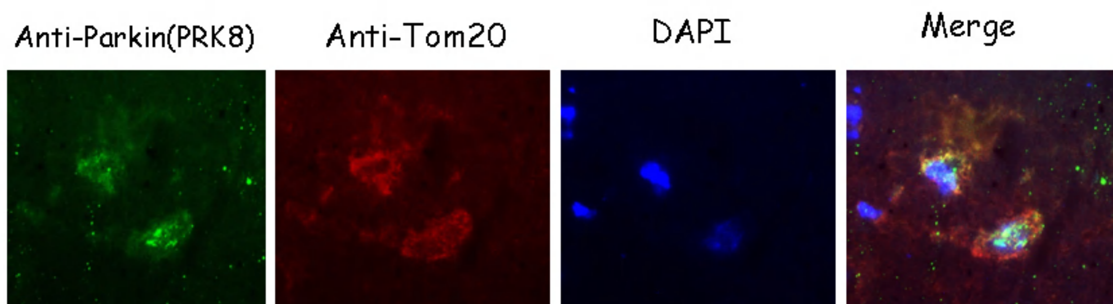

Figure 2. Immunofluorescent images from autopsied cerebral cortex with treatment of Autofluorescence Eliminator