科技资料

Molecular Biology and Genetics of Alzheimer's Disease

MOLECULAR BIOLOGY AND GENETICS OF ALZHEIMER'S DISEASE

Proceedings of the International Symposium on Dementia: Molecular Biology and Genetics of Alzheimer's Disease, Niigata, Japan, 11–14 November 1989

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PREFACE

Research on Alzheimer's disease is rapidly progressing in the various fields, especially in molecular biology and genetics. This volume contains the proceedings of the 'International Symposium on Dementia: Molecular Biology and Genetics of Alzheimer's Disease' held in Niigata, Japan, on November 11–14, 1989.

This symposium was organized in five sections. The first deals with PHF (paired helical filaments) and related subjects, in which ultrastructure and chemical constituents of PHF and ubiquitin dependent proteolysis of tau protein are mainly discussed. The second section presents the studies on analytical, functional and genetic aspects of β-amyloid protein and its precursor. In this section there were a lot of enthusiastic discussions concerning the source of amyloid in the brains of Alzheimer's disease and Down's syndrome and the processing and biological function of precursor protein.

Section III deals with trophic activity in Alzheimer brain. These activities are important for the understanding of the mechanisms involved in processes of neuronal degeneration.

Section IV provides the chromosome restriction maps of human chromosome 21 and the results of linkage studies of familial Alzheimer's disease (FAD). The genetic heterogeneity in FAD is suggested by these studies and it is crucial to collect more pedigrees by world-wide collaboration with the accurate clinical and pathological diagnosis for making the search for the FAD gene successful.

Section V covers the molecular and cell biology of prion in which the significance of the amino acid sequence of prion protein was demonstrated.

The organizers wish to thank all of the speakers for the excellent presentations that made the meeting a success.

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INTRODUCTION

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The purpose of this Symposium is to gain insight into the pathogenesis of SDAT from a molecular and genetic point of view. Accordingly, most of the presentations in this Symposium focus on genetics, amyloid and tangle. However, to begin with I should like to refer to the etiology of SDAT in a more general way. There are possibly many more pathogenetic factors involved in SDAT, but for simplicity, I have chosen only five of these: genetic, immunological, toxic, homestatic and metabolic factors. Below a brief explanation is given for each of these factors.

I. Genetic factors

There are several findings which support the presence of genetic factors in SDAT.

Population studies have suggested an increase in the risk of contracting the disease among relatives of patients with SDAT. A higher risk was found among the siblings of patients who have had an early onset of the disease(1).

Twin studies have also indicated a higher concordance between identical twins but not between non-identical twins(2).

Pedigree studies further support the view that genetic factors are of importance in the etiology of SDAT. These investigations meet the criteria for autosomal dominant inheritance. With regard to genetic linkage, it was shown that there is an increased risk of developing SDAT if a C4B2 allele is present at the locus, namely the locus of immunological complement(3).

There is evidence of an association between familial AD and one of two alleles localized on chromosome 21, but it has also been reported once on chromosome 19(4).

From the similarity between Down's syndrome and SDAT it has long been suggested that chromosome 21 is involved in the pathogenesis of SDAT. A recent finding that A4 or beta-protein is due to chromosome 21 seems to support this idea strongly. However, it is now known that the locus is found at some distance away from that of Down's syndrome. I sincerely hope that exciting reports on this topic will be made at this Symposium.

Although the gene for SDAT has not been localized, it is hoped that a new breakthrough will be made in the near future such as the finding of dystrophin in the study of muscular dystrophy.

II. Immunological factors

Amyloid, which exists at the center of the senile plaque, was reported to be due to

dysfunction of the immune system. Thus disturbances of immunoglobulin have been considered as etiological or pathogenetic factors in SDAT. Certainly there are several reports indicating the presence of immunoglobulin in amyloid of senile plaque as well as in the walls of small vessels(5). The detection of brain autoantibody in cerebrospinal fluid in SDAT patients has suggested that immunoglobulin aberrations may be the main factors involved in this disease. This cerebrospinal immunocytochemical reaction was blocked by pre-incubation of brain sections with rabbit anti-acetylcholine antibody. Antibody specifically binding to cholinergic neurons has been identified in sera from SDAT patients(6,7). In this context it is tempting to assume that plaque formation as well as a decrease in presynaptic cholinergic markers in SDAT may be due to an abnormality of the immune system.

III. Toxic factors

Aluminium has long been suggested as a pathogenetic factor. It was reported that aluminium selectively accumulated on nuclear chromatin of brain cell nuclei from patients with SDAT. It has also been shown that in senile plaque focal deposition of aluminium silicate takes place at the center core. Moreover, aluminium is reported to be elevated in neurofibrillary tangle-bearing neurons in SDAT(8).

Slow virus was for some time thought to be a possible pathogenetic factor. For example, Creutzfeld-Jacob disease (CJD), which is known to cause dementia, can be transplanted to the brain of chimpanzees, indicating that this is a so-called 'transmissible dementia'. It was therefore suggested that familial senile dementia might be due to infection from this virus(9). On the other hand, in the process of purifying the pathogen of Scrapie disease, which is also transmissible, the infectious protein, called prion, was identified. However, the possibility that prion is a pathogenetic factor in SDAT is uncertain since it was reported that anti-prion antibody did not cross-react with amyloid of senile plaque. Nevertheless, the story of slow virus has remained obscure.

IV. Homeostatic factors

In patients with SDAT many neurohormonal and neurochemical changes are reported which are considered to be of pathogenetic importance in the disorder. A typical example is regression of two enzymes, choline acetyltransferase and choline esterase, which will result in severe disturbance of the acetylcholine system as reported in the brain of patients with SDAT(10,11).

Although disturbance of the acetylcholine system will explain the reduced memory function, it cannot explaine the extrapyramidal symptoms, emotional impairment, or depressed mood, some of which may be due to the reduction of the dopamine, noradrenaline or 5-hydroxytryptamine system. In all, damage to one of these cannot be pathognomonic enough. However, it is conceivable that the reduction in the neurohormonal level in the brain of SDAT patients will result in an inability to downregulate the stress response, which may ultimately lead to a degeneration of the hippocampus.

Moreover, it is worth noting that the activity as well as the amount of monoamine

oxidase is increased in the SDAT brain, especially in the cortex(12). It is tempting to assume that degenerative processes as a result of SDAT will induce the growth of extraneuronal cells which contain higher amounts of monoamine oxidase B. It would be of particular interest to correlate such cell growth with trophic factors. Neurite growth to counterattack cell death may be related to the phosphorylation of tau protein which is discussed below and is one of the major topics of this session.

V. Metabolic factors

Many abnormalities in metabolic rate, metabolic pathway and metabolic regulation have been reported in SDAT patients. For example, the most prominent abnormality in SDAT patients is a 44% reduction in the cerebral metabolic rate of glucose(13).

Vitamin B_{12} deficiency is also known to be associated with SDAT(14). Since cobalamin is actively transported across membranes, the reduced concentration of vitamin B_{12} in the patients with late-onset dementia could be considered as a marker for reduced membrane transport capacity. It can be assumed that other essential nutrients are also insufficiently transported across membranes thus giving rise to brain dysfunction.

Protein catabolism seems to be seriously impaired in the brain of SDAT patients. Ubiquitination is an essential step in protein breakdown in the cell. However, in the brain of SDAT patients it does not work normally, but results in the accumulation of insoluble ubiquitinated proteins. Amyloid deposition in senile plaque may also be due to dysfunction of protein catabolism. As discussed in this symposium, the protease inhibitor domain in beta-amyloid protein precursor protein (abbreviated as APP, BPP or BAPP) may be involved in dysfunction of protein breakdown. However, some reservations are necessary before drawing any conclusions about the structure of precursor protein since there is no guarantee that the inhibitor domain itself, for example, has the same inhibitory ability as the free Kunitz-type inhibitor molecule.

Another abnormality in protein metabolism can be seen in its phosphorylation. It is well known that tau protein in neurofibrillary tangles (NFT) or paired helical filaments (PHF) is highly phosphorylated while this protein in normal brain is not. This may suggest that some kinase which specifically phosphorylates tau protein may be over-expressed in the brain of SDAT patients. We have therefore started to identify and characterize this kinase from the brain of rats and cows(15). We have named this enzyme 'T-kinase' or 'protein kinase T' (PKT) for the following reasons.

As shown in Fig. 1, this enzyme was activated by tubulin. However, tubulin does not directly interact with the kinase but through tau protein. Probably the conformational change in tau protein induced by tubulin will make its epitope accessible to the kinase. Moreover, since the addition of colchicine abolished this stimulatory effect, the effector must be tubulin in the polymerizing state but not tubulin monomer or microtubule. In other words, this kinase could be closely related to the polymerization process of tubulin, which is necessary for neurite extension. Addition of so-called second messengers such as cAMP, cGMP, Ca or the Ca-Calmodulin system had no effect or was even inhibitory in some cases. Since A-kinase and C-kinase are named after their effector we have called this enzyme 'T-kinase'.

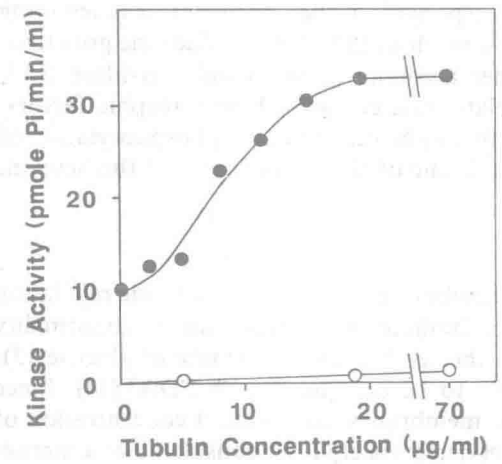

FIG. 1 *Stimulation of the incorporation of phosphate into tau by tubulin. Tau (0.2 mg/ml) was phosphorylated for 1 h in the presence (closed circle) or absence (open circle) of the kinase.*

Some other properties of this enzyme are as follows.

1. The molecular weight has been estimated at 50K, as assessed by gel filtration.
2. This enzyme is serine (threonine) kinase and it does not phosphorylate tyrosine residues.
3. The substrate specificity of this enzyme is very restricted because among the brain proteins, only tau protein and MAP2 are phosphorylated with this kinase. Besides, histone H1 is phosphorylated but no kination takes place of casein or other proteins. The T of T-kinase also indicates this substrate specificity.

Tau protein which has been phosphorylated with this kinase shows very similar properties to those involved in PHF.

1. It is less soluble than untreated tau protein
2. It gives less mobility on SDS PAGE than does untreated tau protein
3. Anti-PHF antibody cross-reacts with treated tau protein but not with untreated tau protein as shown in Fig. 2. This shows immunoblotting with anti-PHF antibody. Only phosphorylated tau protein produced the bands.

These results strongly suggest that this PKT is operating in the SDAT brain to phosphorylate tau protein.

Next we raised antibody against the whole PKT molecule as well as partial sequences of it. This research and also cloning of the gene of this enzym are now in progress in our laboratory and the results will be published elsewhere.

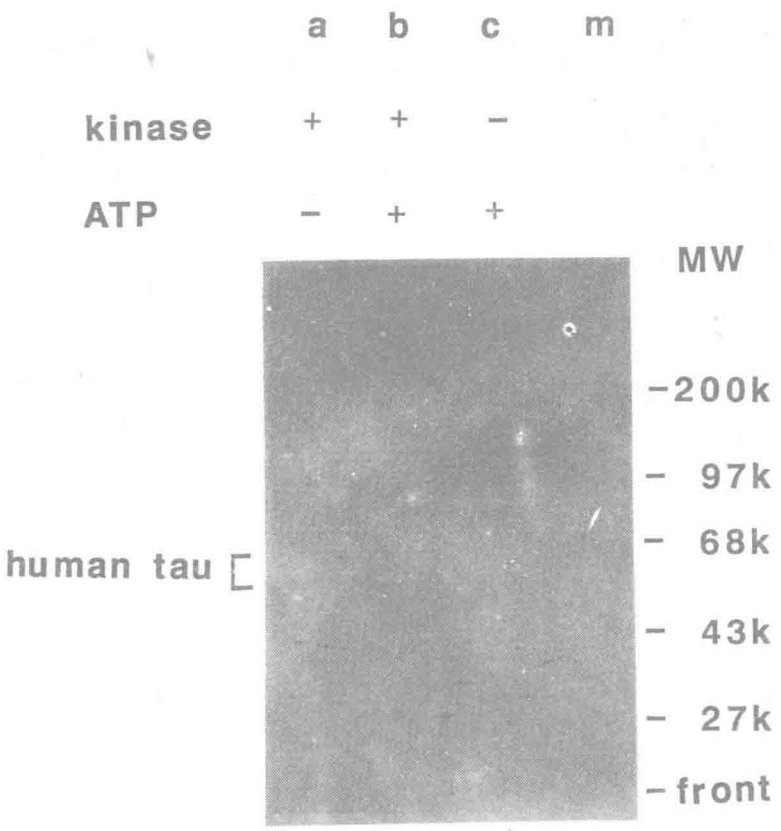

FIG. 2 *Western blot analysis using anti-PHF antibody. Human dephosphorylated tau (0.24 mg/ml) was mixed with tubulin (0.8 mg/ml), the kinase (300 units/ml) and ATP (0.1 mM), as indicated by the plus signs at the top of the figure. The mixtures were then incubated at 37°C for 1 h. The mixtures (2.3 μ g) were electrophoresed on SDS-polyacrylamide gel and then transferred to a nitrocellulose membrane. Western blot analysis was performed using anti-PHF antiserum (×100 dilution). Lane m shows electrophoretic pattern of prestained proteins used for molecular weight standards.*

CONTENTS

PHF/TAU AND RELATED SUBJECTS

Molecular biology and genetics of Alzheimer's disease.
T. Miyatake, D.J. Selkoe and Y. Ihara, editors

ULTRASTRUCTURE OF NEUROFIBRILLARY TANGLES IN THE BRAIN WITH ALZHEIMER'S DISEASE

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INTRODUCTION

Histopathologically, both senile plaques (SP) and neurofibrillary tangles (NFT) are numerously observed in the brain with Alzheimer's disease. For this reason, SP and NFT are very important findings in Alzheimer's disease. Since Kidd (1963)[1] reported the ultrastructure of NFT, the NFT has been called PHFs (paired helical filaments) which is made up of two helically wound filaments. Since then, many researchers have examined PHFs in biochemical, immunological and histopathological fields. In the present study, a more detailed ultrastructure examination of NFT than that of already reported studies was carried out.

The original report of this study was published in Virchows Arch B Cell Pathol (1989)[2].

MATERIALS AND METHODS

The material used in the present study consisted of eight cases of Alzheimer's disease. Parts of the cerebral cortex from the temporal lobes and hippocampal formation were removed, cut into small pieces and immersed in 3% glutaraldehyde in phosphate buffer (pH 7.4) for 2h. They were immersed in 2.5% osmium tetroxide in phosphate buffer (pH 7.4) for 2h. The tissues were dehydrated in alcohol and embedded in epon. Thick sections were obtained with toluidine blue for light microscopy. Ultra-thin sections 200-300 Å were stained with uranyl acetate, lead acetate or alkaline-bismuth, and examined with 150 KV accelerated voltage in a JEOL 2000 EX electron microscope.

RESULTS

Numerous fibrils of neurofibrillary tangles (NFT) running in several directions were observed in the cytoplasm of nerve cells.

Observing the fibrils in longitudinal sections by high magnification, they seemed to consist of paired helical filaments twisting together and containing many globular subunits. Some filaments seemed to wind in a left-handed manner, while others appeared to be wound in a right-handed manner (Fig. 1). However, observing the whole structure longitudinally, the fibrils consisted of about eight protofilaments made up of globular subunits which wound helically in a longitudinal direction : the diameter of each globular subunit was about 35 Å.

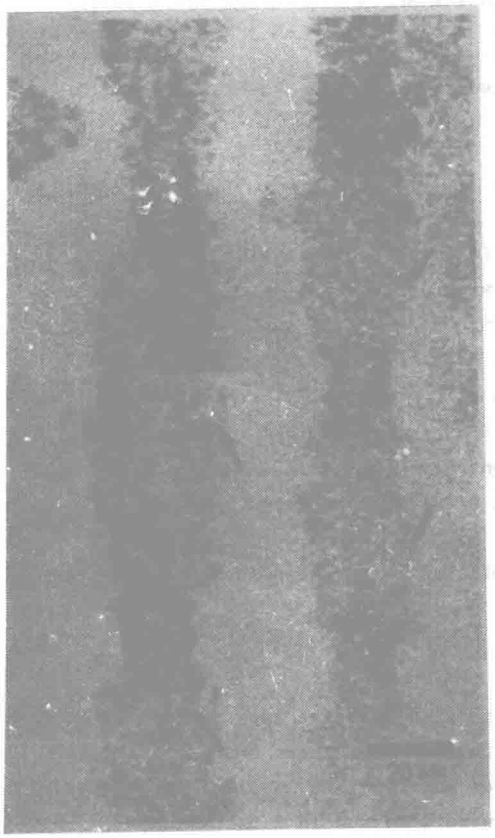

Fig. 1. The fibril on the left seems to wind in a left-handed manner (arrows) and consists of many globular subunits. However, the fibril on the right seems to be wound in a right-handed manner (arrows). The length of the segment between two constrictions of the left fibril is 110 nm.

Fig. 2. A fibril consists of about eight protofilaments made up of globular subunits, which are arranged in a longitudinal direction (arrows). In the narrow parts of the fibril (arrow heads), the width of the fibril is about 18 nm and globular subunits of the protofilaments are tightly connected, but they are more loosely connected in wider parts of the fibril. The width of the widest parts is about 34 nm. The length between two constructions of a fibril in a longitudinal direction is about 74-81 nm. The average diameter of globular subunits is about 35 Å.

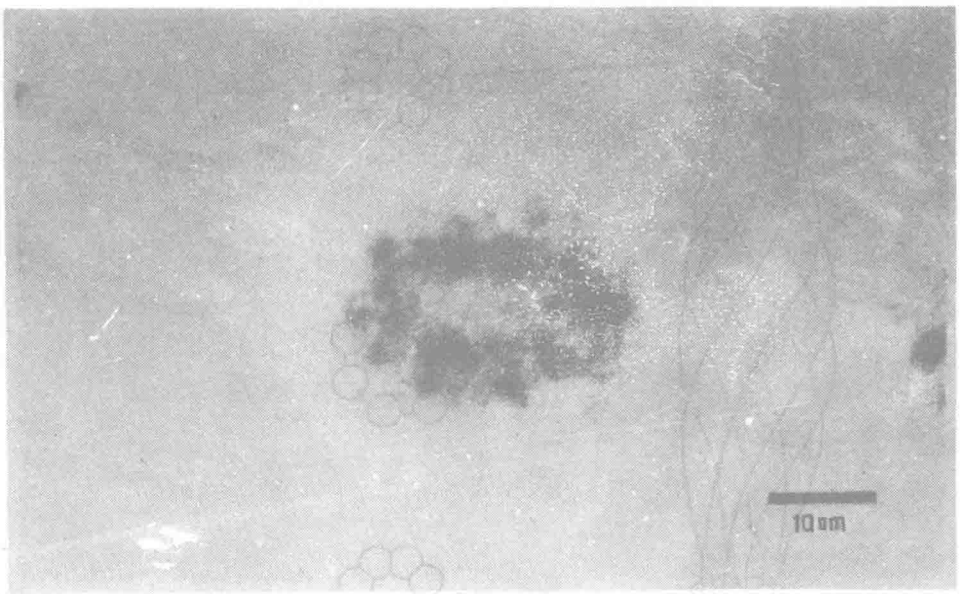

Fig. 3. The transverse section of a fibril showing the widest part. The fibril shows hollow structure and seems to consist of about eight globular subunits.

In narrow parts of the fibril, globular subunits of the protofilaments were tightly connected but in wider parts of the fibril, the subunits were more loosely connected. The length between two constrictions of a fibril in a longitudinal direction was 70 to 85 nm. The width of a fibril was about 10-20 nm at the narrowest and 30-35 nm at the wider parts (Fig. 2). In a tilting experiment the narrowest part did not move along an axial direction.

On the other hand, in transverse sections, almost all fibrils showed a hollow structure. The number of globular subunits in transverse sections seemed to be about eight (Fig. 3).

From the above findings the fibril of NFT seems to be a twisted tubule having periodical constrictions and is made up of eight helically wound protofilaments consisting of globular subunits (Fig. 4).

DISCUSSION

In 1963, Terry[3] reported the ultrastructure of neurofibrillary tangles (NFT) in Alzheimer's disease. He suggested that the fibrils of NFT show twisted tubules which have regular constrictions in every 800 Å length in a longitudinal direction. On the other hand, Kidd (1963)[1] reported that the filaments composing the bundles of NFT are seen to be double helics. Wisniewski et al. (1976)[4] reported that Alzheimer's neurofibrillary tangles consist of paired