

# Roles of Microtubules in Maintenance of Nerve Cell Networks

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## 1. Introduction

### Recent topics of neural networking studies

Various higher brain functions such as reflex, memory, emotion, imagination and so on, are supported by complicated neuronal networks. To keep the precise connections of the wires is very important for the central nerve functions. The discovery of neural stem cell provided us many clues to understand the mechanism of neural networking. Now, we know that the networking neurons and the supportive neuroglia cells are yielded from the neural stem cells by regulation of several specific bHLH transcription factors (Sakamoto, M., et al., 2003, Liu, Y. et al., 2004, Parras, C.M. et al., 2002). In these processes, the networking cells project axons to connect the dendrite of counterpart cells precisely. Since the connections between differentiated nerve cells must be kept for the functions, the morphological disruptions lead to some neural disorders. Recent brilliant studies about the microtubule dynamics enhance our understandings of the mechanism of neural network maintenance and the disorders.

### 1.1 Neural networking and neural stem cell during neural development

During early neural development, neural stem cells transform from neuroepithelial cells into radial glial cells (Hatakeyama, J. et al., 2004). The radial glial cell in ventricular zone, projected a long radial glial process to cerebral membrane, self-renews and produces an immature neuron (Miyata T., et al., 2004). The immature neuron transforms into multipolar cell with many process containing actin fibers. Cyclin-dependent kinase 5 (Cdk5) regulates the formation of these process and the transform of multipolar cell into bipolar locomotion cell having a leading process (Kawauchi, T., et al., 2006). The bipolar locomotion cells move to the precise layer along the radial glial fiber, and differentiate into mature networking neurons. To construct an ordered six layer structure of mammalian cerebral cortex, the locomotion of these neural stem cell lineage cells is strictly regulated by Reelin signal pathway affecting microtubule dynamics (Liu, J.S., 2011). So, some disorders of the microtubule regulation can cause structural errors of neural network development.

## 1.2 Maintenance of neural network in adult hippocampus

In adult hippocampus, it was shown that neurogenesis also occurs constantly (Eriksson, P.S. et al. 1998). Like early neural development, these new neurons are produced from radial glia cells (Fukuda, S., et al., 2003). Since the neurogenesis and activity-dependent synaptic plasticity are accelerated by long term learned behavior (Bruehl-Jungernab, E., et al., 2006), it can participate in functional remodeling of neural networks during the formation of memories. A recent interesting study indicates microtubule transport systems in the dendrites play important roles in maintenance of the synaptic plasticity (Okada, D., et al., 2009). It suggests that the healthy microtubule kinetics is needed to maintenance the neural networking during the formation of memories. What microtubule is all about?

## 2. The kinetics of microtubules and cell functions

### 2.1 Function of microtubules

The cytoskeleton is the essential infrastructure of all cells; it consists of microtubules, actin microfilament, and intermediate filaments. Microtubules are a major component of the cytoskeleton and form a highly organized network of intermingled filaments in eukaryotic cells. Microtubules are important components of several subcellular structures, including the mitotic apparatus, cilia, flagella, and neurons. Microtubules are fundamentally composed of a protein called tubulin. Tubulin is made of  $\alpha$ - and  $\beta$ -tubulin. The molecular weight of each is about 50 kDa. There are many microtubule-associated proteins (MAPs) (Wade, R.H., 2009) in addition to the tau protein, which contributes to the formation of microtubules. The tau protein is enriched in axons. Two types of high-molecular-weight MAPs (200-300 kDa) and the lower-molecular-weight ones (~55 kDa) have been isolated from the brain. For example, MAP2 is found in the cell body and dendrites. In addition, microtubules interact with many proteins, including motor proteins, such as kinesin and dynein.

Microtubules play many roles in cellular processes, such as cell division, cell motility, and morphogenesis, and they are required for brain function. Purich and Kristofferson (1984) have reviewed microtubule assembly. Wade has described the function of the cell division of microtubules in detail (Wade, R.H., 2009). The motor proteins kinesin and dynein use microtubules as pathways for transport and are also involved in cell division. Microtubules organize the spatial distribution of organelles. Actin and microtubule cytoskeletons determine cell shape and polarity during morphogenesis and promote stable cell-cell and cell-matrix adhesions through their interactions with cadherins and integrins, respectively (Hall, A., 2009).

### 2.2 Polymerization of tubulin: Microtubule assembly

Tubulin is widely distributed in eukaryotic cells, and the specific self-assembly of tubulin results in microtubule formation. Microtubules are hollow tubes approximately 25nm in diameter. Tubulin is composed of two subunits of  $\alpha$ - and  $\beta$ -tubulins that bind one mole of guanosine triphosphate (GTP) each. GTP binding to  $\alpha$ -tubulin is present at the non-exchangeable site in  $\alpha$ -tubulin, and that binding to  $\beta$ -tubulin is at an exchangeable site in  $\beta$ -tubulin. Some reports have focused on microtubule assembly kinetics (Detrich, et al., 1985; Barton, J.S., et al., 1987; Caplow, M., and Shanks, J., 1990). The polymerization mechanism of tubulin is fundamentally due to the polymer self-assembly theory of Oosawa and Kasai (1962). Magnesium is required for tubulin polymerization (Weisenberg, R.C., 1972; Olmsted

J.B., and Borisy, G.G., 1975), and calcium inhibits microtubule assembly. The assembly kinetics of the microtubule protein is altered by the ionic strength, temperature, and magnesium ion but not by the pH (Barton, J.S., et al., 1987). Timasheff and Grisham have reviewed in detail an *in vitro* assembly process from tubulin and the mechanism of microtubule assembly (Timasheff, S.N., and Grisham, L.M., 1980).

On the growth of microtubules, Mitchison and Kirshner (1984) proposed a behavior called dynamic instability. Horio and Hotani (1986) confirmed alternate phases of growth and shrinkage of microtubule assembly using a light optical technique.

Post-translational modifications of tubulin building generate functional diversity of microtubules. Hammond et al. (2008) have reported that tubulin modifications influence microtubule-associated proteins, such as severing proteins, plus-end tracking proteins, and molecular motors. In this way, tubulin modifications play an important role in regulating microtubule properties, such as stability and structure, as well as microtubule-based functions, such as ciliary beating, cell division, and intracellular tracking (Hammond, J.H., et al., 2008).

### **2.3 Relationship between microtubule assembly and GTP hydrolysis**

Tubulin used in our experiments was prepared from bovine brain by the modified procedure of Lee et al. (Weisenberg, R.C., and Timasheff, S.N., 1970; Lee, J.C., et al., 1973; Na, G.C. and Timasheff, S.N., 1981). Microtubule assembly was monitored by turbidity at 350 nm using a spectrophotometer with a recorder. GTP hydrolysis accompanies microtubule formation. GTP bound at an exchangeable site is hydrolyzed. GTPase activity was evaluated by the measurement of GDP produced using HPLC with an ODS column (Seckler, R., et al., 1990). We examined the effects of the magnesium ion on microtubule assembly and the GTPase activity of tubulin. GTPase activity was clearly observed at a 2 mM magnesium ion concentration, while the formation of microtubules under the same conditions was not observed (Doi, H., et al., 1991). Microtubule assembly and GTPase activity were examined in the presence of 0.1 mM calcium ion as well. GTPase activity was apparently observed at 2min after heating at 37 °C, while there was no turbidity. The results described above indicate that the GTPase activity of tubulin occurs before microtubule assembly. The facts support the results of O'Brien et al. (O'Brien, E.T., et al., 1987) rather than those of Carlier (Carlier, M.-F., 1982).

## **3. Some evidence of nerve cell dysfunction caused by the microtubules disorder**

Here, we present some evidence of nerve cell dysfunction caused by the microtubules disorder. Our series of experiments using a neural cell line PC12 demonstrated that the oxidative damage of microtubules causes the morphological abnormality cell (Yamanaka, Y., et al., 2008).

### **3.1 Function of microtubules in neuronal cells**

In neurons, microtubules play a variety of roles in brain function. As in many other cells, microtubules form organized structures within a cell that can act as structural scaffolds. With respect to specific for neuron, microtubules have three functions. First, the stabilization of microtubules is sufficient to induce axon formation during neuronal development, and

they act as signal molecules for initial neuronal polarization (Witte H et al., 2008). Second, the development of dendritic spines that are major sites of excitatory synaptic input is regulated by microtubules (Gu, J., et al., 2008). Third, microtubules participate in the trafficking of synaptic cargo molecules that are essential for synapse formation, function, and plasticity. Cargos are transported between axons and dendrites mediated by motor proteins moving along microtubules to their plus or minus ends (Hirokawa N and Takemura, R., 2005). The motor proteins are the minus-end directed dynein and plus-end directed kinesins (Schliwa, M., 2003, Vale, R.D., 2003). On the other hand, several studies have shown the importance of the actin-based transport mechanism at excitatory synapses. Actin, which is abundant in highly dynamic structures, such as growth cones and dendritic spines, receives the cargo following passage of the microtubules. Neuronal transmission is achieved partly by collaboration of both microtubules and actins.

As reported above, it is clear that microtubules play an essential role in neuronal development, function, and transmission. Disruption of neuronal microtubules means functional failure of brain. Indeed, microtubule dysfunction and impairment of neurotransmission were observed in neurodegenerative diseases, such as Alzheimer's disease (refer to Chapter 4) and Parkinson's disease. The Alzheimer brain is characterized by the presence of aberrant amyloid plaques, neurofibrillary tangles, and alpha-synuclein. Neurofibrillary tangles are composed of paired helical filaments made from abnormally formed tau protein. In the normal brain, tau binds to microtubules and, thereby, stabilizes neuron structure and promotes tubulin assembly into microtubules. However, hyperphosphorylation of tau is assumed to be the cause of the formation of paired helical filaments; namely, it could result in the self-assembly of tangles of paired helical filaments and straight filaments.  $\alpha$ -Synuclein is a microtubule-associated protein (MAP) that is colocalized with tubulin in Lewy's bodies. The deposition of  $\alpha$ -synuclein as fibrillary aggregates in neurons or glial cells is observed in a Lewy variant of Alzheimer's disease (Spillantini, M.G., et al., 1997) and Parkinson's disease (Lücking, C.B. and Brice, A., 2000). It has been reported that  $\alpha$ -synuclein could promote tubulin polymerization in microtubules (Alim MA et al., 2004), whereas other studies have indicated that  $\alpha$ -synuclein inhibits tubulin polymerization (Chen L et al., 2007, Zhou RM et al., 2010).

Cumulative evidence suggests that neurodegenerative diseases are associated with neuronal cytoskeletal alterations. These findings suggest that elucidating the biology of the cytoskeleton could be a target for drug therapy.

### **3.2 The PC12 cells as a model for neurite outgrowth**

To study the behavior of the neuronal microtubules, PC12 would be an appropriate cultured cell line. It can enable us to conduct a visual assessment of neurite behavior from formation to disruption.

The adrenal pheochromocytoma (PC12) cell line has been well studied as a model for neurite outgrowth. It was originally isolated from a tumor in the adrenal medulla of a rat in 1976 (Greene, L. A., and Tischler, A. S., 1976). One of the main characteristics of PC12 cells is to differentiate into sympathetic neuron-like phenotypes in response to nerve growth factor (NGF) (Figure 1A, 1B). The mechanism of NGF-induced neuronal differentiation has not been fully elucidated; however, it has been reported that the regulator of G-protein signaling (RGS) proteins associates TrkA with activated signaling proteins of the Ras/pErk1/2 pathway (Willard, M.D., et al., 2007, Nusser, N., et al., 2002).

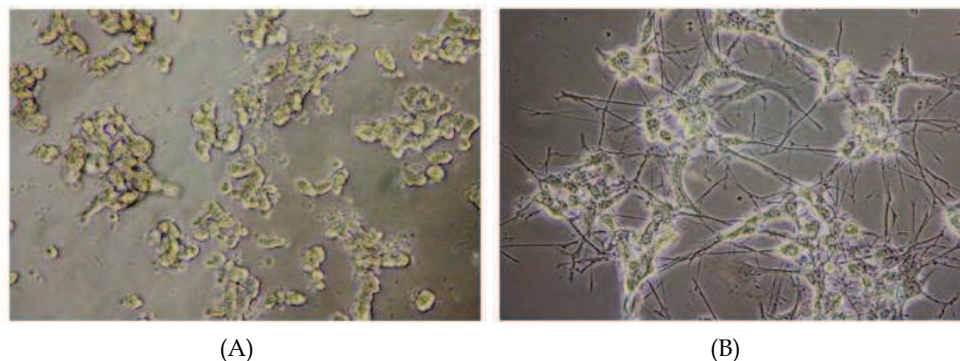


Fig. 1. Phase contrast microscopic observation of PC12 cells (A) before differentiation (B) after differentiation. For differentiation, the undifferentiated cells were treated with 100 ng/mL NGF. NGF induced the apparent morphological transformation of PC12 cells into neuronal-like cells within 3 days

### 3.3 The oxidative damage to PC12 cells

Many studies have demonstrated that lipid peroxidants are present in the AD brain (Keller, J. N., and Mattson, M. D., 1998; Markesbery, W. R., and Carney, J. M., 1999;). We tried to verify whether lipid peroxidation was induced in PC12 cells by exogenously added phosphatidylcholine hydroperoxides (PCOOH) which is a primary product of lipid peroxidation. Lipid peroxidation was measured according to the method of Hedley and Chow (1992), which utilizes time-resolved flow cytometry. Table 1 shows the fluorescence of undifferentiated and differentiated cells before and after exposure to PCOOH for 24 or 48 h. Compared with that of undifferentiated cells, the fluorescence of differentiated cells was significantly decreased in the presence of 100  $\mu$ M PCOOH for 48 h ( $P < 0.05$ ). The fluorescence of undifferentiated cells exposed to the same concentration of PCOOH was slightly but not significantly affected. These results suggest that PCOOH induces membrane lipid peroxidation in PC12 cells before and after differentiation. The levels of peroxidation were higher in the membranes of differentiated cells than in those of undifferentiated cells. It is likely that differentiated cells are more sensitive to oxidative stress. Considering that lipid peroxidation was certainly induced in differentiated PC12 cells, this experimental system may be useful as a model for AD brain cells.

Conc. of PCOOH ( $\mu$ M)	Before Differentiation (%)		After Differentiation (%)	
	24h	48h	24h	48h
0	100	100	100	100
100	88.8	79.2	84.9	63.8*

Table 1. Relative fluorescent intensity of *cis*-parinaric acid bound by the cell membrane

Neurites consist mainly of microtubules, whose function is significantly based on the ability of tubulin to polymerize and depolymerize. To examine the effect of PCOOH on microtubule formation from tubulin, we measured the GTPase activity of PC12 cells. GTPase activity is an indicator of microtubule formation and, therefore, provides the degree of microtubule assembly (O'Brien, E.T. et al., 1987; Seckler, R., et al., 1990; Doi, H., et al.,

1991). The specific activity of GTPase derived from differentiated cells was significantly decreased in the presence of 50  $\mu\text{M}$  PCOOH ( $P < 0.01$ ) (Figure 2). In the case of exposure to 100  $\mu\text{M}$  PCOOH, the value was decreased by one-tenth compared to that in the absence of PCOOH. In undifferentiated cells, the specific activity of GTPase decreased by half in the presence of 50  $\mu\text{M}$  PCOOH (Figure 3). The difference in sensitivity might be due to the presence or absence of neurites. Although GTP hydrolysis accompanies the polymerization reaction (Doi, H., et al., 1991), GTP resynthesis does not occur in the reverse reaction of depolymerization (David-Pfeuty, T., et al., 1977). Thus, PCOOH disrupts existing microtubules and inhibits new microtubule formation from tubulin.

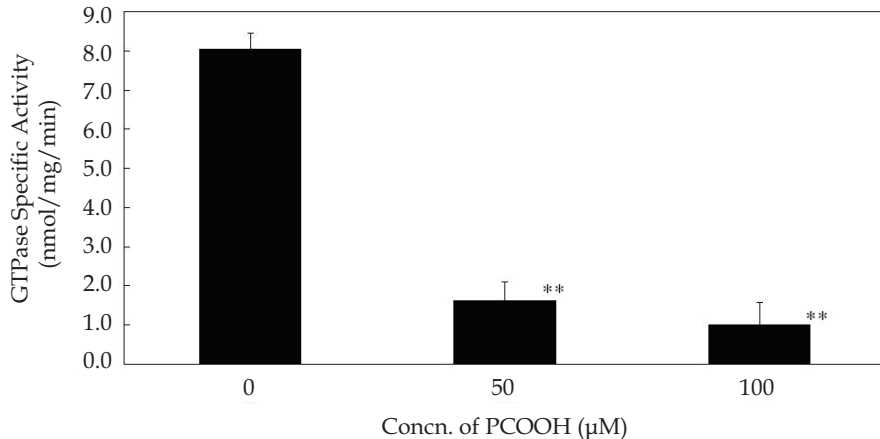


Fig. 2. GTPase-specific activity of the differentiated cells incubated with PCOOH at various concentrations for 24 h. The data represent means  $\pm$  SD, \*\* $P < 0.01$  compared with the control value

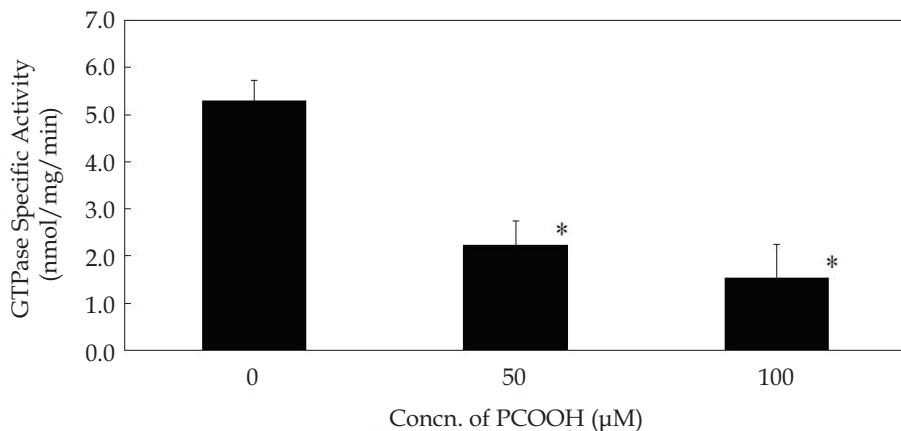


Fig. 3. GTPase-specific activity of the undifferentiated cells in the same condition as that described in Figure 2. The data represent means  $\pm$  SD, \* $P < 0.05$  compared with the control value

To visualize PCOOH-induced damage to the tubulin, we performed immunofluorescence microscopy using an antibody to monoclonal mouse anti- $\alpha$ -tubulin clone B-5-1-2. Undifferentiated or differentiated cells were individually cultured with 250  $\mu$ M of PCOOH for 6 h. After that, the cells were stained by the antibody to monoclonal mouse anti- $\alpha$ -tubulin clone B-5-1-2 and the antibody to Cy3-conjugated sheep anti-mouse IgG. As shown in the photographs in Figure 4A, the undifferentiated cells looked like grape clusters, and the cell shape was clear. However, after exposure to PCOOH, the cell shape was drastically changed (Figure 4B), becoming too vague to identify. The fluorescence emitted from cells was weakened. PCOOH may have induced cell shape alteration by the degradation of tubulin, which was more marked in differentiated cells than in undifferentiated cells. Although the extended neurites were observed clearly in the absence of PCOOH (Figure 4C), they disappeared when exposed in PCOOH for 6h (Figure 4D). The shape of the small cell was vague, as it was in undifferentiated cells, and the fluorescence emitted from cells became extremely weak. The fact that neurites composed of microtubules are easy to be injured may account for the higher vulnerability of differentiated cells.

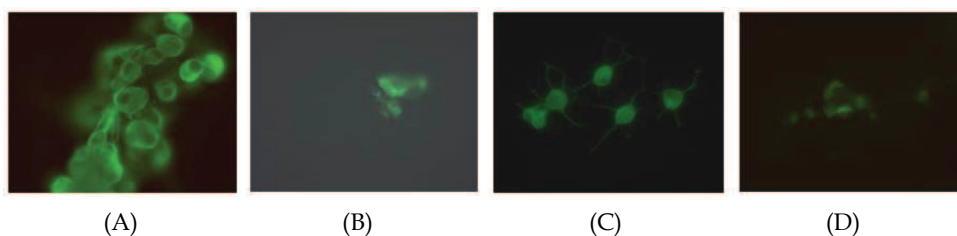


Fig. 4. Fluorescence microscopic observation of cells after tubulin antibody staining. Representative fields are shown: undifferentiated cells before (A) and after exposure to PCOOH for 6h (B), and differentiated cells before (C) and after exposure to PCOOH for 6h (D)

Furthermore, we tried to verify that the tubulin depolymerization induced by PCOOH could be attenuated by antioxidant. Differentiated cells were cultivated with 5  $\mu$ M retinol or ascorbic acid beforehand and then exposed to PCOOH. The GTPase activity of cell extracts derived from cells treated with retinol was three-fold higher than that of untreated control cells (Figure 5). Incorporation of antioxidants in cells before exposure to PCOOH protected tubulin depolymerization. This experimental data might lead to the development of an effective strategy to prevent some neurodegenerative diseases.

#### 4. Ageing of central nerve system and the microtubules disorder caused by neural malnutrition

As people get older, the brain functions decline in varying degree. Although the causes are still unknown, the neurogenesis in hippocampus is decreased dramatically with ageing (Cameron, HA., et al., 1999). The other hand, we can detect neurofibrillary tangles in aged entorhinal cortex or brain cortex of neurodegenerative disorder. These tangle formation are concerned with aggregation of tau, which is a microtubule binding protein. In this section, we will discuss the factors determining the ageing-related neural functional decline in Alzheimer's disease from the aspect of the axonal microtubules disorder caused by neural malnutrition.

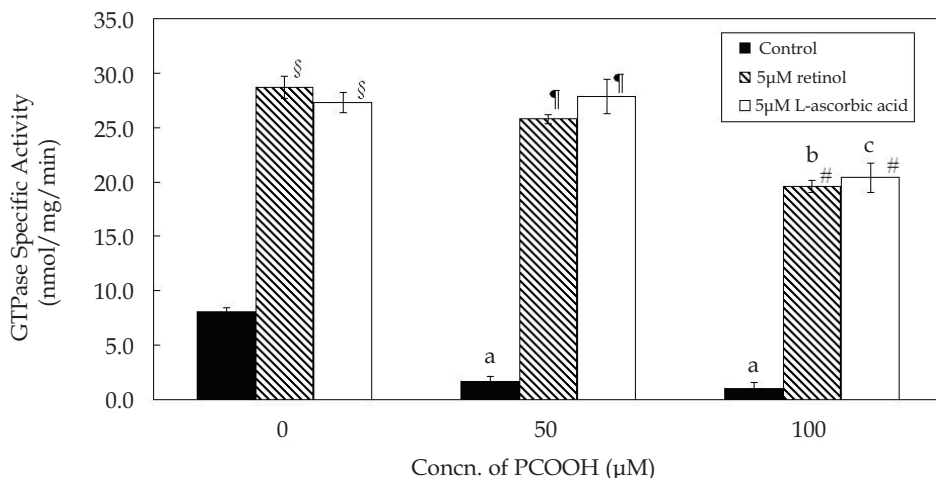


Fig. 5. GTPase-specific activity of the differentiated cells incorporated 5 µM retinol or 5µM ascorbic acid before exposure to PCOOH. The data represent means  $\pm$  SD, §, ¶, # $P$ <0.01 compared with the corresponding control value, a $P$ <0.01 compared with the data without PCOOH, b,c $P$ <0.05 compared with the data without PCOOH

#### 4.1 Microtubule degeneration and Alzheimer's disease

Alzheimer's disease (AD) is characterized by neuronal cell death and two kinds of deposits, neurofibrillary tangles (NFT) and senile plaques. Abnormal microtubule-binding tau proteins were isolated from AD by Liu et al. (1991). As is well known, in an AD brain, aberrant accumulation of amyloid- $\beta$ -protein ( $A\beta$ ) occurs ahead of the accumulation of paired helical filament in NFT. Imahori and Uchida (1997) observed extensive phosphorylation of tau and programmed cell death in a primary culture of embryonic rat hippocampus with  $A\beta$  (Imahori, K., and Uchida, T., 1997). There are several important reports on the phosphorylation of tau protein in AD by the group of Iqbal (Alonso, A.D.C., et al., 1994; Gong, C-X., et al., 1994; Iqbal, K., et al., 1994; Gong, C-X., et al., 1995). Glycogen synthase kinase -3 $\beta$ (GSK-3 $\beta$ ) is responsible for most of the abnormal hyperphosphorylation of tau observed in paired helical filaments, which are diagnostic for AD (Imahori, K. and Uchida, T., 1997). The tau protein is a microtubule-associated protein that contributes to the formation of microtubules. It is considered that hyperphosphorylated tau is free from microtubules and induces the destruction of the cytoskeleton.

It is possible that microtubules are related to many neurodegenerative diseases in addition to AD. In the brain with Alzheimer's disease, glycation end products are observed. Microtubule-associated protein T is glycated at the tubulin binding site (Ledesma, et al., 1995). The facts observed in microtubule-associated proteins of tau and T appear to indicate that they play a role in microtubule assembly. Furthermore, microtubule assembly is not likely to take place when tubulin has been modified.

#### 4.2 Lipid hydroperoxides in neurodegenerative disease

A part of the oxygen introduced in cell produces reactive oxygen species as a by-product in an electron transport system because of NADPH-dependent oxidase. Materials in cell are



exposed by oxidative stress, and then oxidative modifications of lipid in the cell membrane and DNA are introduced. The role of oxidative stress in Alzheimer's disease has been reported in several studies, some of which showed elevated markers of oxidative stress, including lipid oxidation products (Sultana, R., et al., 2006). Oxidized lipid hydroperoxides are a characteristic of neurodegenerative disease, and oxidized lipid by-products were enriched in the brain with Alzheimer's disease (Yoo, M-H., et al., 2010). Hydroperoxides of phospholipid were detected in brain samples from patients with Alzheimer's disease using oxidative lipidomics (Tyurin, V.A., et al., 2008).

#### **4.3 Inhibition of microtubule assembly by lipid hydroperoxides**

We have investigated the effect of lipid hydroperoxides on microtubule assembly (Kawakami, M., et al., 1993; Kawakami, M., et al., 1998). Lipid hydroperoxides were prepared from soybean phosphatidylcholine by photosensitized oxidation in methanol, with methylene blue being added to the phosphatidylcholine-methanol solution as a sensitizer (Kawakami, M., et al., 1998). Microtubule formation was inhibited dose-dependently by lipid peroxides. This result suggests the possibility that the interaction between tubulin and lipid peroxides may be the cause of some brain diseases. Matsuyama and Jarvik speculated that microtubule integration was a key to Alzheimer's disease (Matsuyama, S.S. and Jarvik, L.F., 1989).

Bizzozero et al. (2007) also indicated by *in vitro* experiments that lipid hydroperoxides were most likely responsible for protein oxidation. Lipid peroxidation scavengers, such as butylated hydroxytoluene, prevent the carbonylation of cytoskeletal brain protein-induced glutathione depletion (Bizzozero, O.A., et al., 2007).

#### **4.4 The mechanism of tubulin modification by phosphatidylcholine hydroperoxides**

We examined the concentration-dependent effects of phosphatidylcholine hydroperoxides on the ability of tubulin to polymerize into microtubules (Kawakami, M., et al., 2000). The results demonstrated that even very low concentrations of peroxides were sufficient to interfere with tubulin and, therefore, microtubule function. In the fluorescence spectra of tubulin before and after interaction with phosphatidylcholine hydroperoxides, a red shift in the emission maximum was observed. This fact indicates a conformational change upon the reaction, namely, that fluorescent aromatic amino acids become easier to dissolve on reaction with phosphatidylcholine hydroperoxides. The interaction mechanism may be a hydrophobic one because no effect on electric conductivity was observed, indicating that modulation of ionic binding was not involved.

#### **4.5 Possibility of recovery of tubulin function deteriorated by lipid hydroperoxides**

The effects of lipid hydroperoxides on microtubule assembly were studied in an *in vitro* assay system, as were the protective effects of vitamin A derivatives ( $\beta$ -carotene, retinal, and retinol). All vitamin A derivatives had the ability to protect against the inhibitory effects of lipid hydroperoxides, presumably owing to their antioxidant activities. This suggests a mechanism for the ability of vitamin A to inhibit cell ageing.

Glutathione and cysteine were used as water soluble reductants (Kawakami, M., et al., 1999). Tubulin GTPase activity deteriorated by lipid hydroperoxides was restored by the addition of water soluble reductants as well. These chemicals also have a protective effect on cellular ageing by the reduction of materials oxidized *in vivo*.

The detection of microtubule assembly-promoting material was tried using tubulin GTPase activity as the assay of microtubule assembly. Kawaguchi, M., et al. (2007) found a peptide with a molecular weight of 1340.8 from Japanese classified barley flour.

#### **4.6 Polymerization and calcium binding to tubulin-colchicine complex**

Calcium plays important roles as a messenger in a signal transaction by changing its concentration. The calcium concentration is continually changing, while the concentration is fundamentally very low in a cell. This means that the change affects the functions of many cell constituents.

Calpain is a neutral cysteine proteinase activated by calcium in cytosol, and it converts p35 to p25 (Lee, M-S., et al., 2000). In the brain of AD patients, p25 is stimulated. P25 induces the activation of cyclin-dependent kinase 5 (CDK5). CDK5 is also a factor for the hyperphosphorylation of tau. Indirubins, which are inhibitors of CDK5/p25, repress cell death (Leclerc, S., et al., 2001).

We are interested in the effect of calcium on tubulin polymerization because calcium is an inhibitor of microtubule assembly. Another reason may be the contribution of calpain, which is regulated by calcium, to AD. Instead of tubulin, the tubulin-colchicine complex was used (Doi, H., et al., 2003a). The high affinity sites of calcium took part in the polymerization of the complex in the GTP state, while the low ones participated in the depolymerization. The complex had 2 high-affinity sites with a dissociation constant of  $11.5 \times 10^{-6}$  M and 16 low-affinity sites with a dissociation constant of  $2.27 \times 10^{-4}$  M in the GTP state. In the case of the GDP state, the dissociation constant of the high-affinity site was  $7.2 \times 10^{-6}$  M, and that of the low affinity site was not observed. The ultracentrifugal experiment indicated a slightly more compact structure in the GTP state compared with the GDP state. The partial specific volumes of the tubulin-colchicine complex in the state of GTP were 0.739 and 0.744 ml/g in imidazole and BES buffers, respectively (Doi, H., et al., 2000b). The sedimentation coefficient  $S_{20,w}^0$  increased from 5.38 S with no calcium to 5.75 and 6.08 S with calcium concentrations of 0.1 and 0.5 mM, respectively, in the absence of the magnesium ion. In an imidazole buffer, the sedimentation coefficients  $S_{20,w}^0$  were 5.82 and 6.06 S in the presence of 0 and 2 mM  $MgCl_2$ , respectively. These results indicate that the tubulin-colchicine complex causes the calcium affinity to become low after polymerization with its conformational change. This means that the assembly induces the stability of microtubules from calcium.

### **5. The microtubules disruption and some neurodegenerative diseases**

Finally, we will discuss the association between the microtubules disorders and other some neurodegenerative diseases. Each neurodegenerative disease has specific aberrant intracellular structures like neurofibrillary tangles of AD (Chiti, F. and Dobson, C.M., 2006). Recently, TRA DNA-binding protein of 43kD (TDP-43) has been spotlighted as a common factor associated with the formation of these aberrant structure (Neumann, M. et al., 2006, Arai, T. et al., 2006, 2009, Fujishiro, H. et al. 2009, Schwab, C. et al., 2008). Although several diseases show only TDP-43 intracellular accumulation, TDP-43 is combined with other protein such as tau in many neurodegenerative diseases. It suggests that TDP-43 is a causal factor of microtubules disruption in these diseases. Although the intrinsic or extrinsic causes of many neurodegenerative diseases have been investigated aggressively, the breakdown of microtubules maintenance system by lack of brain blood flow has not been understood well.

Since neurons require sufficient energy supply for maintaining their high-performance, the lack of energy might damage the microtubule dynamics. As mentioned above, the microtubules disruption can be a trigger of neural degeneration. Further investigation for causes of microtubule disruption in neurons might contribute for our understanding neurodegenerative disease.

## 6. Acknowledgements

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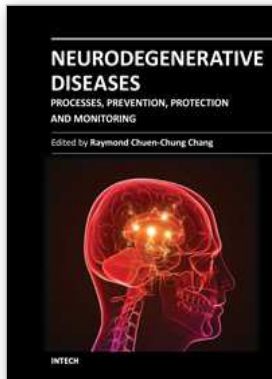
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Neurodegenerative Diseases - Processes, Prevention, Protection and Monitoring focuses on biological mechanisms, prevention, neuroprotection and even monitoring of disease progression. This book emphasizes the general biological processes of neurodegeneration in different neurodegenerative diseases. Although the primary etiology for different neurodegenerative diseases is different, there is a high level of similarity in the disease processes. The first three sections introduce how toxic proteins, intracellular calcium and oxidative stress affect different biological signaling pathways or molecular machineries to inform neurons to undergo degeneration. A section discusses how neighboring glial cells modulate or promote neurodegeneration. In the next section an evaluation is given of how hormonal and metabolic control modulate disease progression, which is followed by a section exploring some preventive methods using natural products and new pharmacological targets. We also explore how medical devices facilitate patient monitoring. This book is suitable for different readers: college students can use it as a textbook; researchers in academic institutions and pharmaceutical companies can take it as updated research information; health care professionals can take it as a reference book, even patients' families, relatives and friends can take it as a good basis to understand neurodegenerative diseases.

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