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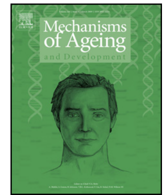

Original article

Molecular pathophysiology of impaired glucose metabolism, mitochondrial dysfunction, and oxidative DNA damage in Alzheimer's disease brain

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ABSTRACT

In normal brain, neurons in the cortex and hippocampus produce insulin, which modulates glucose metabolism and cognitive functions. It has been shown that insulin resistance impairs glucose metabolism and mitochondrial function, thus increasing production of reactive oxygen species. Recent progress in Alzheimer's disease (AD) research revealed that insulin production and signaling are severely impaired in AD brain, thereby resulting in mitochondrial dysfunction and increased oxidative stress. Among possible oxidative DNA lesions, 8-oxoguanine (8-oxoG) is highly accumulated in the brain of AD patients. Previously we have shown that incorporating 8-oxoG in nuclear and mitochondrial DNA promotes MUTYH (adenine DNA glycosylase) dependent neurodegeneration. Moreover, cortical neurons prepared from MTH1 (8-oxo-dGTPase)/OGG1 (8-oxoG DNA glycosylase)-double deficient adult mouse brains is shown to exhibit significantly poor neuritogenesis *in vitro* with increased 8-oxoG accumulation in mitochondrial DNA in the absence of antioxidants. Therefore, 8-oxoG can be considered involved in the neurodegenerative process in AD brain. In mild cognitive impairment, mitochondrial dysfunction and oxidative damage may induce synaptic dysfunction due to energy failures in neurons thus resulting in impaired cognitive function. If such abnormality lasts long, it can lead to vicious cycles of oxidative damage, which may then trigger the neurodegenerative process seen in Alzheimer type dementia.

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1. Introduction

About 47.7 million people worldwide suffer from dementia, with 7.7 million new cases every year (World Health Organisation, 2015). Sporadic AD (also known as late-onset AD) is the most common dementia subtype, accounting for 60–80% of all dementia cases (Prince et al., 2013; Sosa-Ortiz et al., 2012). AD is characterized by the accumulation in the brain of both senile plaques containing aggregated amyloid β (Aβ) and neurofibrillary tangles (NFTs) consisting of aggregated highly phosphorylated TAU protein, and by neuronal loss mainly in the cortex and hippocampus (Gotz et al., 2012; Querfurth and LaFerla, 2010). About 1% of AD cases develop as a result of mutations to any of three specific genes for the amyloid precursor protein (APP), the presenilin 1 protein and the presenilin 2 protein, with the latter two regulating APP processing through their effects on γ secretase (an enzyme that cleaves APP). Individuals with mutations in any of these three dominantly inherited genes, tend to develop AD symptoms before the age of 65, sometimes as early as age 30, and it has been shown that Aβ plaques can be present for more than 20 years before the onset of dementia in patients with such inherited mutations (Bateman et al., 2012; Haass and Steiner, 2002; Kang et al., 1987; Sinha et al., 1999; Takasugi et al., 2003). The vast majority of individuals with sporadic AD have late onset disease, occurring at age 65 or later, and similar to other chronic diseases, sporadic AD develops as a result of multiple factors rather than just from a single cause (Alzheimer's Association, 2015)

It has been shown by epidemiologic studies that insulin resistance and diabetes mellitus (DM) are risk factors for pathogenesis of dementia including AD (Bedse et al., 2015; de la Monte, 2014; Hao et al., 2015; Matsuzaki et al., 2010; Ohara et al., 2011; Sekita et al., 2010). Moreover, it was demonstrated through

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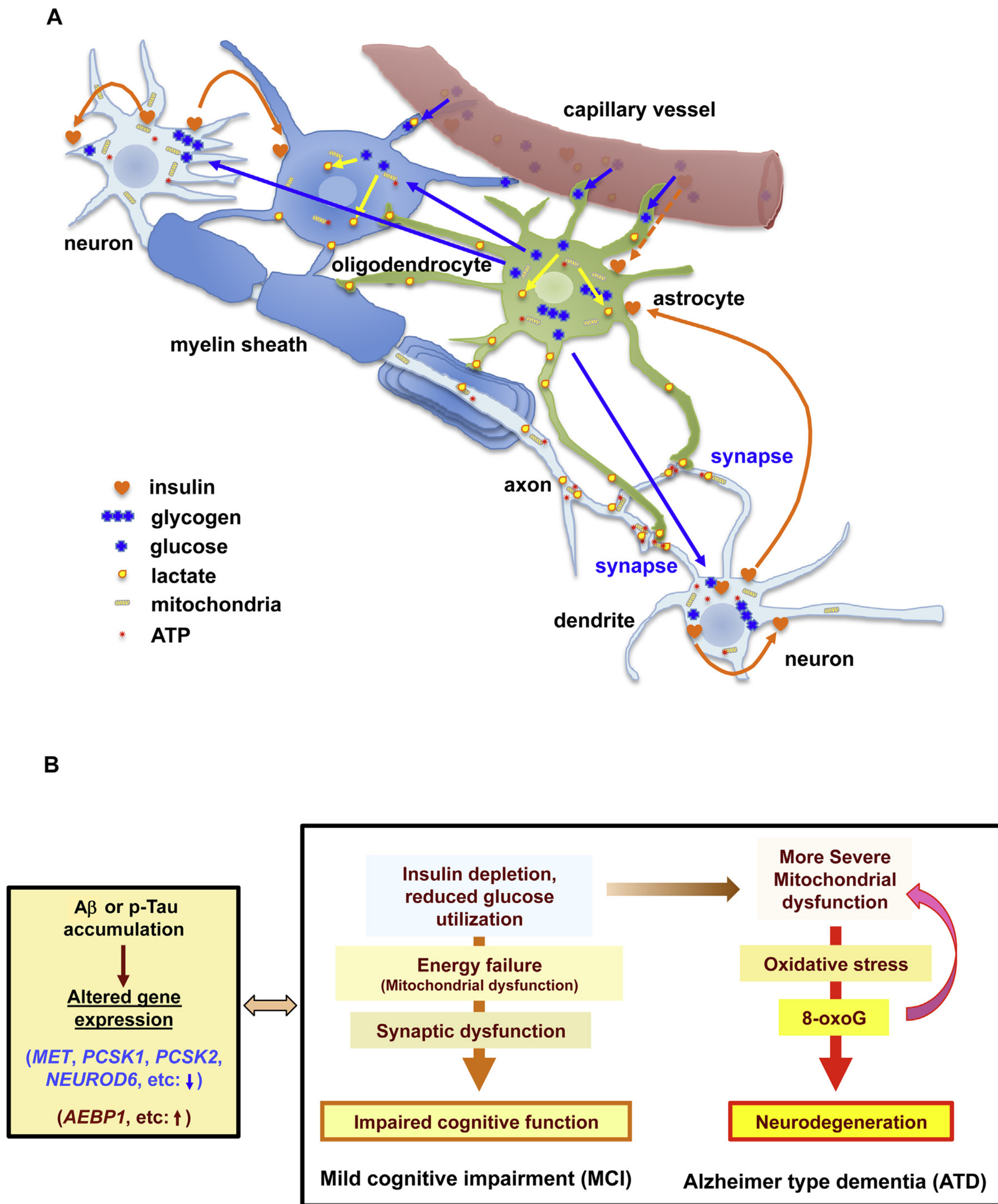

Fig. 1. Insulin production and glucose metabolism in normal brain and their impairments in AD brain. (A) Insulin production and glucose metabolism in normal brain. In normal brain, neurons in cortex and hippocampus produce insulin, which may regulate glucose utilization, glycogen storage or their metabolism in brain, probably mainly in astrocytes. (B) Impaired insulin production and glucose metabolism in AD brain may cause mitochondrial dysfunction and oxidative stress, which enhances the neurodegenerative process. In AD brain, up-regulation of *AEBP1* may cause a down-regulation of both *PCSK1* and *PCSK2*, which process proinsulin to insulin, resulting in a decreased insulin production in neurons, downergulation of *MET* further causes insulin resistance, and down-regulation of *NEUROD6* may cause mitochondrial dysfunction. In patients with mild cognitive impairment (MCI), such abnormality may induce synaptic dysfunction due to energy failure in neurons, thus resulting in impaired cognitive function. If such abnormality lasts long, mitochondrial dysfunction and oxidative stress might increase, resulting in a vicious cycle of oxidative damage and mitochondrial dysfunction, which may then trigger neurodegenerative processs similar to those in Alzheimer type dementia (ATD).

brain imaging by positron-emission tomography (PET) with use of ^{18}F-fluorodeoxyglucose (FDG) and Pittsburgh compound B (PIB) (FDG-PET and PIB-PET, respectively) that a significant decrease in cerebral glucose use in the precuneus region (known to be an area of early deposition of Aβ in both sporadic AD and inherited AD cases) was detected in mutation carriers 10 years before the onset of the expected symptom (Bateman et al., 2012). Though these data suggest that insulin resistance and DM may lead to the disturbance of glucose metabolism in the brain, the exact mechanisms on how insulin resistance and DM acts as risk factors for AD remain unclear.

The brain or central nervous system (CNS) utilizes a vast amount of energy to sustain its basic functions, such as maintaining or re-establishing of membrane potentials, signaling, and other essential cellular activities. While an adult human brain typically weighs

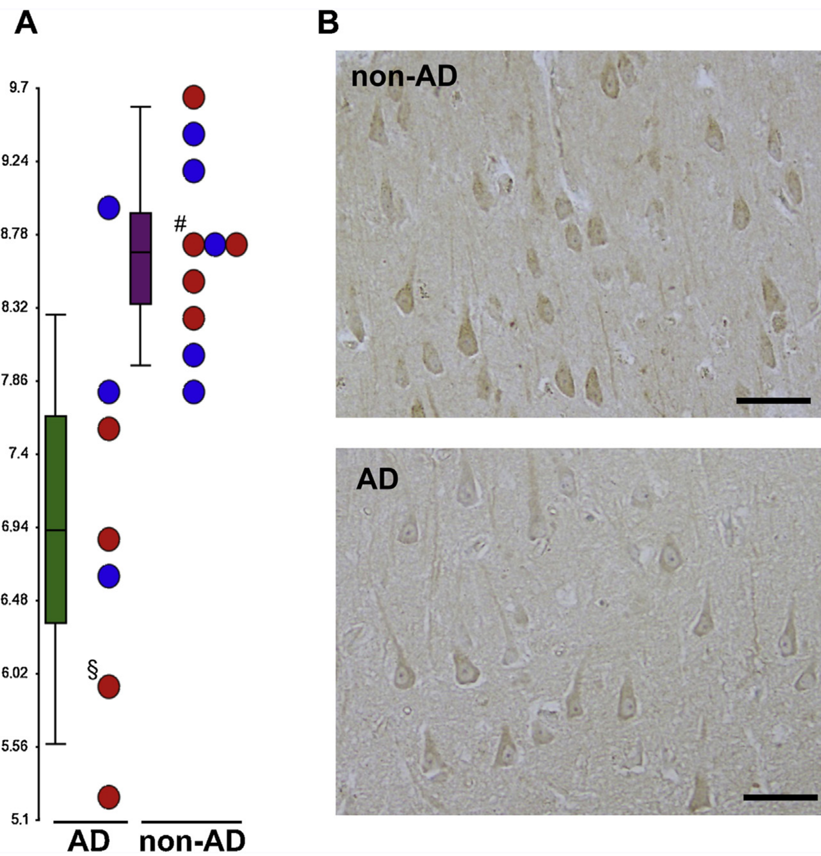

Fig. 2. Decreased expression of *PCSK1* mRNA and PCSK1 protein in post-mortem AD brains. (A) Raw expression levels ($\log_2$ transformed means) of *PCSK1* mRNA in individual subjects are plotted. In the whisker-box plots, the boxes indicate medians as well as the 25th and 75th percentiles, and the whiskers indicate the 10th and 90th percentiles (Hokama et al., 2014). Green box, AD; Purple box, non-AD. Blue circles, men; Red circles, women. (B) Immunohistochemistry with anti-PCSK1 antibody (Santa Cruz Biotechnology, sc-100578) using the same method as previously applied (Hamasaki et al., 2014), revealed decreased PCSK1 levels in the affected pyramidal neurons in CA1 region of the AD case (corresponding to individual shown with § in (A)), in contrast to those in the non-AD case (corresponding to individual shown with # in (A)). Scale bars = 50 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

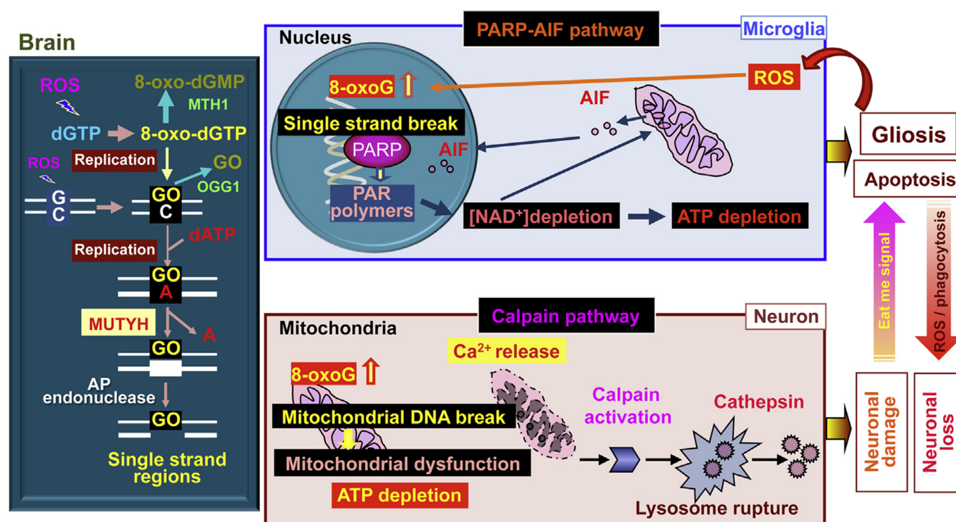

Fig. 3. 8-Oxoguanine accumulated in neurons or microglia is differentially involved in neurodegenerative processes. In the absence of MTH1, which hydrolyses 8-oxo–dGTP in the nucleotide pool to prevent its harmful incorporation into DNA, and/or OGG1, which excises a major oxidative base lesion 8-oxoG (GO) opposite cytosine from DNA, causes significant accumulation of 8-oxoG in the brain. At an early stage, buildup of 8-oxoG in the mitochondrial DNA of neurons causes calpain-dependent neuronal damage, such as nerve terminal or synaptic degeneration triggered by mitochondrial MUTYH-initiated BER of adenines opposite 8-oxoG. In the later stages, damaged neurons release eat-me signals that then activates the microglia. Ros produced by microglia causes a buildup of 8-oxoG in the nuclear DNA of microglia, causing PARP-dependent nuclear translocation of mitochondrial AIF triggered by nuclear MUTYH-initiated BER, thus aggravating microgliosis leading to neuronal loss.

only about 2% of the body weight, a resting brain consumes more than 20% of all the oxygen, thus indicating a 10-fold greater energy requirement than other tissues. This high demand for energy in the brain is mainly achieved by ATP production during oxidation of glu-

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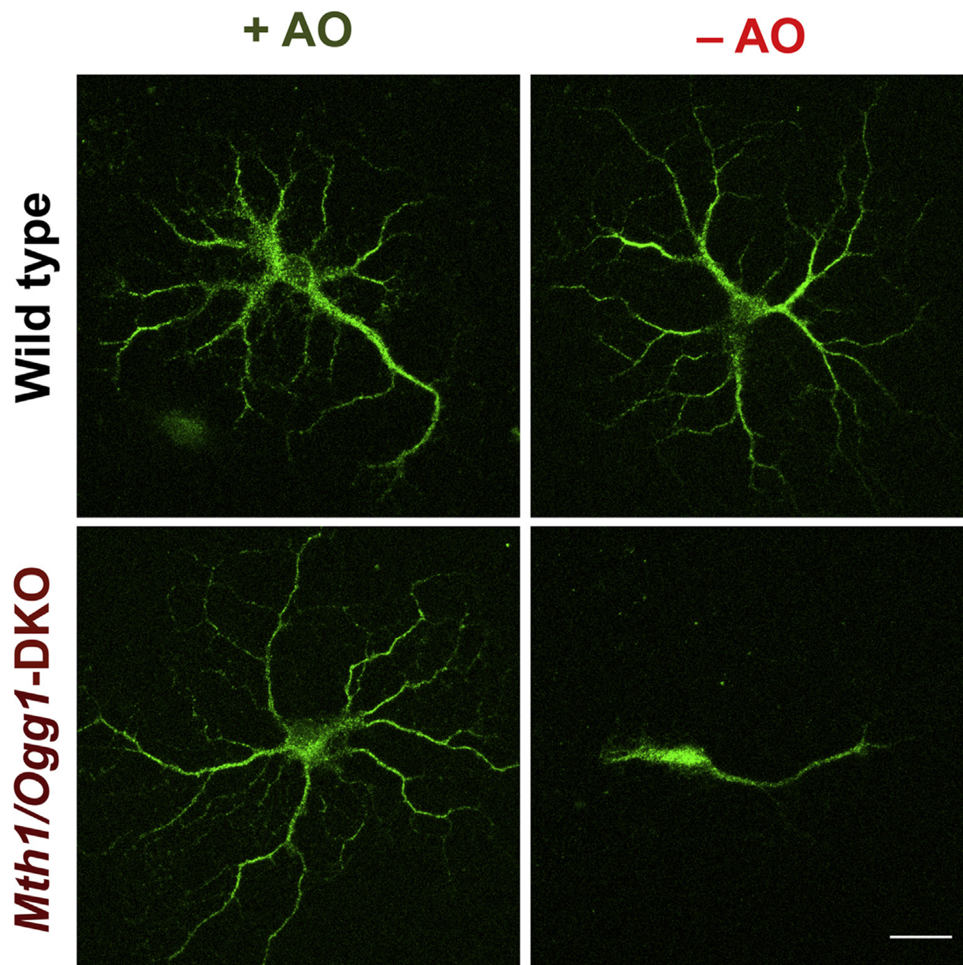

Fig. 4. Neuritogenesis of cortical neurons isolated from adult *Mth1/Ogg1*-double-knockout (DKO) was significantly impaired in the absence of antioxidants in contrast to those from wild-type mice. Adult cortical neurons isolated from *Mth1/Ogg1*-DKO and wild-type mice (15-week-old) were cultured for 5 days in the absence (−AO) or presence (+AO) of antioxidants and were subjected to MAP2-immunofluorescence microscopy (Green). Scale bar = 20 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cose or oxidative phosphorylation in the mitochondria (Chen and Zhong, 2013; MacKenna et al., 2012). It is well documented that impaired glucose metabolism or mitochondrial dysfunction is one of the major pathological changes observed in various neurodegenerative diseases (Bhat et al., 2015; Bullon et al., 2014) including AD, Parkinson's disease (PD) or Huntington's disease (HD), thus suggesting that regulation of glucose metabolism and maintenance of mitochondrial homeostasis are critical for brain function (Fig. 1A).

In the brain, astrocytes are the main energy reservoirs, accumulate glycogen, and help to sustain high-energy demands associated with neuronal activity. It has further been shown that insulin and insulin-like growth factors can modulate glucose metabolism, especially in the glycogen storage in astrocytes (Heni et al., 2011; Muhic et al., 2015) (Fig. 1A), thus suggesting insulin-resistance or DM may cause or worsen impaired glucose metabolism in AD brain. On the other hand, mitochondrial dysfunction is known to be mainly associated with synaptic damage due to increased oxidative damage in an AD brain (Bhat et al., 2015), suggesting that regulation of glucose metabolism and maintenance of mitochondrial homeostasis are concomitantly impaired in astrocytes and neurons, respectively, in an AD brain.

As markers for oxidative damage in AD brain, various oxidized molecules in lipids, proteins, and nucleic acids have been detected mostly in the neurons (Cobb and Cole, 2015; de la Monte et al., 2000; Lovell et al., 2011; Nunomura et al., 2001; Wang et al., 2006), and some of them may contribute to the AD pathology, though the details are still unclear. In this review, we will discuss how impaired glucose metabolism and mitochondrial dysfunction are involved in AD pathology and the possible involvement of oxidative DNA damage in these cases.

2. Brain insulin signaling and glucose metabolism modulate long-term memory formation

It has been shown that insulin controls not only whole-body energy and glucose homeostasis in the periphery of the human body but also exerts specific effects in the brain (Ghasemi et al., 2013; Gray et al., 2014; Kleinridders et al., 2014). The brain is known to contain a high concentration of insulin, which appears to be 10–100 times higher than in plasma, subject to change during brain development (Havrankova et al., 1979; Schechter et al., 1992). Moreover, brain insulin appears to be regulated completely independently from insulin in the periphery. This suggests that brain insulin is synthesized by the neural elements, and plays a role in the central nervous system which is unrelated to peripheral glucose metabolism (Havrankova et al., 1979). There are many literatures reporting that brain insulin is partly the result of an uptake from the peripheral blood through the blood brain barrier (BBB), via a specific transporter system coupled to insulin receptor (INSR) present in the brain microvessels (Cunnane et al., 2011; Pifferi et al., 2007). More-

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over insulin is also locally produced in the brain (Ghasemi et al., 2013). Recently, three groups independently reported that insulin is produced within various areas of the brain (Kuwabara et al., 2011; Mehran et al., 2012; Molnar et al., 2014). In hippocampus, granule cells in dentate gyrus (DG), pyramidal neurons in CA1 and CA3, and neuronal progenitors in subgranular zone of DG appear to produce insulin (Kuwabara et al., 2011), while in the cerebral cortex, GABAergic neurogliaform cells express insulin (Molnar et al., 2014) (Fig. 1A).

INSR and the closely related insulin-like growth factor (IGF)-1 receptor (IGF-1R), as well as their downstream targets such as INSR substrate-1 (IRS-1) and IRS-2, are distributed throughout the brain including olfactory bulb, cortex, hippocampus, hypothalamus and cerebellum (Kleinridders et al., 2014). Through these receptors and signaling pathways in the brain, insulin affects feeding behavior and how the body stores energy, the metabolism of glucose and fats in the liver and adipose, as well as various aspects of memory and cognition (Gray et al., 2014). Furthermore, insulin signaling also modulates neurotransmitter channel activity, brain cholesterol synthesis, and mitochondrial function (Kleinridders et al., 2014).

The cognitive enhancing effects of insulin are proposed to be mediated through the activation of INSR in the hippocampus, an important integration center for learning and memory in the mammalian brain (Benedict et al., 2007; Craft and Stennis Watson, 2004; Stockhorst et al., 2004). Although the brain express mainly insulin-independent glucose transporters, GLUT1 (endothelial cells of BBB, astrocytes), GLUT2 (hypothalamic and hippocampal neurons, astrocytes), GLUT3 (endothelial cells of BBB, astrocytes), GLUT5 (microglia), and GLUT6 (neurons), there are also some expression of insulin-dependent transporters, GLUT4 and GLUT8 (Duelli and Kuschinsky, 2001; Grillo et al., 2009; Wood and Trayhurn, 2003). GLUT4 and GLUT8 are localized in neuronal cell bodies in the cortex and cerebellum, but mainly in the hippocampus and amygdala, where they maintain hippocampus-dependent cognitive functions. Insulin translocates GLUT4 from cytosol to plasma membrane to transport glucose into cells, and GLUT8 from cytosol to rough endoplasmic reticulum to recover redundant glucose to cytosol after protein glycosylation (Jurcovicova, 2014).

Recently, it has been revealed that astrocytes play an essential role in long-term memory formation by converting glycogen into lactate and transporting it to the neurons (Newman et al., 2011; Suzuki et al., 2011) (Fig. 1A). Astrocytes express an insulin-independent glucose transporter, GLUT1, thus take up glucose through BBB in an insulin-independent manner, and convert the intracellular glucose to glucose-6-phosphate and then store as glycogen (Jurcovicova, 2014). Upon greater energy demand during synaptic transmission, glycogenolysis is triggered to produce lactate. Lactate is then transported to the extracellular space by monocarboxylate transporters (MCT1, MCT4), and is taken up by neurons via MCT2, and contributes to memory consolidation processes (Belanger et al., 2011; Bezzi and Volterra, 2011; Stobart and Anderson, 2013). While these steps are likely to be insulin independent, it has been reported that astrocytes express INSR and respond to insulin or IGF-1 (Garwood et al., 2015). Since insulin or IGF-1 promotes glycogen storage and cell proliferation in astrocytes (Heni et al., 2011; Muhic et al., 2015), the increase in glycogen storage in the astrocytes have a contributory effect of an insulin-dependent increase in glucose utilization during increases in neuronal activity associated with hippocampal-dependent learning, as well as GLUT4-dependent glucose uptake by neurons.

3. Impaired insulin production and signaling in AD brain

Genome-wide gene expression profiling of postmortem brains from sporadic AD patients have revealed altered expressions of neurological and immunological genes, genes encoding inflammatory molecules and metabolic enzymes (Bossers et al., 2010; Brooks et al., 2007; Colangelo et al., 2002; Hokama et al., 2014; Parachikova et al., 2007; Tan et al., 2010). Colangelo et al. (2002) reported altered gene expression profile supporting the hypothesis of widespread transcriptional alterations, misregulation of RNAs involved in metal ion homeostasis, transcription factor signaling deficits, decreases in neurotrophic support and activated apoptotic and neuroinflammatory signaling in moderately affected AD hippocampal CA1. Brooks et al. (2007) reported that 15 out of 51 members are statistically significantly down-regulated in hippocampus in AD brain of the glycolytic, tricarboxylic acid cycle, oxidative phosphorylation, and associated pathways, which suggests altered glucose metabolism in AD brain. Moreover, Tan et al. (2010) reported a significantly altered AD transcriptome (5485 genes) in the neocortex, characterized by synaptic dysfunction, perturbed neurotransmission and activation of neuroinflammation.

We have examined gene expression profiles in postmortem human brains donated for the Hisayama study (Hokama et al., 2014). The hippocampi of AD brains showed the most significant alteration in gene expression profile. In AD brains, 143 from the top 200 transcript clusters were markedly downregulated in the hippocampus beyond the expected level based on the cell population changes. Among the top 200 transcript clusters, 145 genes were eligible for generating functional gene networks. The most relevant network involved in insulin production and signaling. The second-most relevant network consisted of the genes encoding GABA receptors, synaptotagmin members, syntaxin, potassium channels, and regulators of G protein signaling. Expression of all of these genes was markedly decreased in the AD hippocampus, reflecting the neuronal dysfunction in AD brain. The third-most relevant network consisted of genes regulated by insulin signaling pathways. The alterations in the expression levels of the genes constituting these 3 networks were well preserved in the temporal cortex and to a lesser extent in the frontal cortex of AD brains. Comparative analyses of expression changes in the brains of AD patients and a mouse model of AD (3xTg-AD) were also performed, and genes involved in noninsulin-dependent DM and obesity were commonly altered in both AD brains and the AD mouse model, as were genes related to psychiatric disorders and AD. Interestingly, we found that the alterations in the expression profiles of DM-related genes in AD brains are independent of peripheral DM-related abnormalities, indicating that the altered expression of genes related to DM in AD brains resulted from AD pathology, which may thereby be exacerbated by peripheral insulin resistance or DM.

We discovered that the gene list reported by Tan et al., (2010) contained most of the genes from the three networks we found, confirming that there are common alterations of gene expression in AD brains from two independent studies (the Oxford Project to Investigate Memory and Ageing, and the Hisayama study) (Hokama et al., 2014). Our study (Hokama et al., 2014) and that of Bossers et al., (2010) and Tan et al. (2010), all showed that expression of the *PCSK1* (proprotein convertase subtilisin/kexin type 1) gene is reproducibly and most significantly down-regulated in the late stages of disease in AD brains (Fig. 2A). Moreover, our data showed that the extent of *PCSK1* down-regulation was most significant in the hippocampi of AD brains, with down-regulation occurring to a lesser extent in the temporal cortex and to an even lesser extent in the frontal cortex, in terms of the pathological severity. PCSK1 is essential, together with PCSK2, for proinsulin processing (Schechter et al., 1992; Seidah et al., 1999) in the pancreas. As shown in Fig. 2B, PCSK1 protein is highly expressed in hippocampal neurons in human brain, and its expression is significantly decreased in AD brain (Fig. 2B). Moreover, *PCSK2* expression level was also decreased in AD brains. These results indicated for the first time that neurons are equipped with enzymes for proteolytic maturation of insulin

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precursor expressed in neurons, and thus decreased expression of PCSK1 and PCSK2 in AD brain are expected to cause insulin depletion (Hokama et al., 2014).

We found that expression of *MET*, encoding a receptor for hepatocyte growth factor (HGF), was significantly decreased in AD patients. Expression of *MET* has been shown to be up-regulated by VEGF (vascular endothelial growth factor) and HGF (Gerritsen et al., 2003), and we also found that the expression level of the former is significantly decreased in AD brains, thus suggesting that the down-regulation of *MET* gene in AD brains is likely to reflect reduced expression of VEGF, whose expression is known to be up-regulated by insulin (Miele et al., 2000). Importantly, Fafalios et al. (2011) reported that MET is essential for an optimal hepatic insulin response by directly engaging INSR to form a MET-INSR hybrid complex, culminating in a robust signal output. They also found that the HGF-MET system restores insulin responsiveness in a mouse model of insulin refractoriness. In human brain, MET is mainly expressed in neurons in cortex and hippocampus as well as in astrocytes, and its expression in neurons is significantly decreased in AD brain, suggesting impaired insulin/HGF signaling in these neurons or astrocytes (Hamasaki et al., 2014).

The gene expression profiles in AD brains also revealed that *NEUROD6* encoding the neurogenic basic helix-loop-helix transcription factor, which has been shown to confer tolerance to oxidative stress by triggering an antioxidant response and sustaining mitochondrial biomass (Uittenbogaard et al., 2010), is significantly down-regulated in AD brains (Fowler et al., 2015; Hokama et al., 2014). Interestingly, expression of *NEUROD6*, whose variants were also found to be associated with AD, can be upregulated by 2-deoxyglucose (Fowler et al., 2015), suggesting a causative connection among decreased glucose uptake, mitochondrial dysfunction, and increased oxidative stress in AD brain (Fig. 1B).

Astrocytes express INSR and respond to insulin or IGF-1 (Garwood et al., 2015). Astrocytes contain glycogen, an energy buffer, which can bridge the local short-term energy requirements of the brain. Glycogen is the largest energy reserve of the brain, and has been found to be almost exclusively localized in astrocytes in the adult brain (Belanger et al., 2011). Glycogen levels reflect a dynamic equilibrium between glycogen synthesis and glycogenolysis (Belanger et al., 2011). In astrocytes cultured *in vitro*, stimulation with insulin or IGF-1 promotes glycogen storage, but not glucose uptake through glucose transporters (Heni et al., 2011; Muhic et al., 2015), thus suggesting that insulin produced by neurons might play an important role to maintain glycogen storage in astrocytes.

We suggest that in AD brain, proinsulin is not efficiently processed to the mature form of insulin due to the down-regulation of necessary proprotein convertases, PCSK1 and PCSK2, thus causing insulin depletion. Moreover, down-regulation of MET is likely one of the causes for decreased insulin responsiveness in AD brain.

4. Impaired glucose metabolism and mitochondrial dysfunction in AD brain

Sleigh et al. (2011) reported that phosphocreatine recovery after exercise, a measure of skeletal muscle mitochondrial function *in vivo*, was significantly slowed in patients with *INSR* mutations compared with that in healthy age-, fitness-, and BMI-matched controls suggesting that defective insulin signaling may cause mitochondrial dysfunction. Recently, Kleinridders et al. (2015) have shown that mice with a brain-specific knockout of *Insr* (NIRKO mice) develop age-related anxiety and depressive-like behaviors, accompanied by brain mitochondrial dysfunction with reduced mitochondrial oxidative activity, increased levels of reactive oxygen species (ROS), and increased levels of lipid and protein oxidation in the striatum and nucleus accumbens. It is strongly suggested that impaired insulin production and signaling in AD brain thus cause brain mitochondrial dysfunction due to severe impairment of glucose or glycogen metabolism (Fig. 1B). Indeed, brain imaging with FDG-PET tracer revealed that brain glucose uptake is significantly decreased in temporal-parietal cortex in AD brain, and its occurrence precedes cognitive dysfunction and pathological alterations decades earlier (Bateman et al., 2012; Cerami et al., 2015; Chen and Zhong, 2013; Cunnane et al., 2011; Dukart et al., 2013; Mosconi et al., 2014).

Current researches indicate that mitochondria are the primary metabolic platform, which can malfunction during insulin resistance (Bullon et al., 2014; Cheng et al., 2010; Kleinridders et al., 2014). One of the best-studied and most important signaling cascades activated by insulin is IRS-PI3K-AKT signaling cascade. AKT phosphorylates the FOXO transcription factors – FOXO1, FOXO3, FOXO4 – which control expression of genes including those that mediate gluconeogenesis, lipid metabolism and stress resistance (Cheng et al., 2009; Dong et al., 2008; Gross et al., 2008; van der Horst and Burgering, 2007). It has been demonstrated that both IRS1 and IRS2 are strong inhibitors of FOXO1, through AKT-mediated phosphorylation in liver (Dong et al., 2008; Guo et al., 2009). Insulin resistance, thereby, results in hyperactivation of FOXO1 that in turn induces HMOX1, the enzyme that consumes heme and disrupts the integrity of the mitochondrial electron transport chain, thus leading to increased production of ROS (Cheng et al., 2009). However, the exact molecular link between insulin resistance and mitochondrial dysfunction in AD brain still remains undefined. As described in the previous section 3, it is likely that down-regulation of *NEUROD6* in sporadic AD brain is also one of the causes for mitochondrial dysfunction and increased ROS production (Uittenbogaard et al., 2010).

In AD brains, two essential glucose metabolic pathways in mitochondria: Krebs cycle and oxidative phosphorylation are known to be distressed. Abnormal Krebs cycle or/and oxidative phosphorylation cause(s) not only glucose hypometabolism but also the increased generation of ROS, oxidative damage, and programmed cell death such as apoptosis. Because mitochondria are also the main location that suffers from ROS, oxidative stress further exacerbates mitochondrial dysfunction and this vicious cycle is more prone to occur and have been demonstrated to be an event occurring before the appearance of senile plaques and the onset of clinical manifestations (Chen and Zhong, 2013; de la Monte et al., 2000; Eckert et al., 2011; Maruszak and Zekanowski, 2011).

5. Oxidative DNA damage accumulated in AD brain

Reflecting the increased oxidative stress in AD brain, various oxidized bases in DNA have also been detected (Bradley-Whitman et al., 2014; Gabbita et al., 1998; Lyras et al., 1997; Mecocci et al., 1994; Wang et al., 2006; Wang et al., 2005). Among the various oxidized bases detected in either nuclear or mitochondrial DNA prepared from postmortem AD brains, the 8-oxoG accumulates in both, and is recognized as the most pronounced marker in AD brain (Fig. 1B). Immunohistochemical examination of postmortem AD brains revealed that cytoplasmic accumulation of 8-oxoG is evident in hippocampal CA1 and CA3 pyramidal neurons (Song et al., 2011), and in neurons of the temporal cortex (de la Monte et al., 2000), where Aβ is also highly accumulated. Accumulation of 8-oxoG in the AD brain is an early event, occurring before the onset of dementia (Coppede and Migliore, 2015; Lovell and Markesbery, 2007).

Many mouse models for familial AD have been established (Puzzo et al., 2015), and increased cytoplasmic immunoreactivity for 8-oxoG has been observed in the brains of some models (Aliev et al., 2003; Duffy and Holscher, 2013; Song et al., 2011; Xiong et al.,

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2011). 8-OxoG is most likely to be detected in mitochondrial DNA or cytoplasmic RNA. These observations support that oxidative stress is increased in mouse models of AD, similar to those observed in postmortem AD patient brains.

Among the various types of oxidative lesions found in nucleic acids, 8-oxoG is one of the major sources of spontaneous mutagenesis (Nakabeppu et al., 2007a; Ohno et al., 2014). The buildup of 8-oxoG in DNA is caused by direct oxidation of guanine in DNA itself or through the incorporation of 8-oxoG from nucleotide pools in which 8-oxo-2′-deoxyguanosine triphosphate (8-oxo-dGTP) is generated under oxidative condition. 8-oxo-dGTP can be utilized by DNA polymerases as a precursor for DNA synthesis, consequently, 8-oxoG is incorporated into the nascent strand opposite adenine and cytosine in the template with almost equal efficiency, resulting in an A:T to C:G transversion mutation. In contrast, G:C to T:A transversion arises from replication of 8-oxoG-containing template DNA (Nakabeppu et al., 2007a).

Three enzymes, MTH1, OGG1 and MUTYH, have been shown to play important roles in counteracting the buildup of 8-oxoG in DNA of human and rodent cells (Nakabeppu, 2014). MTH1 (also known as NUDT1), an oxidized purine nucleoside triphosphatase, efficiently hydrolyzes 8-oxo-dGTP in nucleotide pools, thereby avoiding incorporation of 8-oxoG into DNA (Nakabeppu, 2001a, 2014). On the other hand, OGG1 with 8-oxoG DNA glycosylase activity excises 8-oxoG opposite cytosine in DNA, thereby preventing the accumulation of 8-oxoG in DNA (Nishioka et al., 1999; Boiteux and Radicella, 2000). Adenine inserted opposite 8-oxoG in template DNA are excised by MUTYH with adenine DNA glycosylase (Ohtsubo et al., 2000; Oka and Nakabeppu, 2011). All three enzymes are known to function both in nuclei and mitochondria (Nakabeppu, 2001b).

In non-diseased postmortem human brains, MTH1 protein is most highly expressed in the stratum lucidum of the CA3 hippocampal subfield corresponding to mossy fiber synapses, followed by perikarya of the DG granular neurons and pyramidal neurons of the entorhinal cortex (Furuta et al., 2001), and weakly expressed in the cytoplasm of CA1 and CA3 pyramidal neurons (Song et al., 2011). In AD brains, MTH1 synaptic expression in CA3 as well as cytoplasmic expression in CA1 and CA3 neurons were significantly decreased, whereas increased expression was observed in the entorhinal cortex (Furuta et al., 2001; Song et al., 2011). It is noteworthy that decreases in the MTH1 levels in CA1 and CA3 neurons correlate with an increased 8-oxoG levels in these neurons (Song et al., 2011).

In non-diseased postmortem human brains, substantial levels of both nuclear and mitochondrial forms of OGG1 are expressed in frontal, temporal and parietal lobes and cerebellum. In contrast, the protein levels of nuclear OGG1 in the frontal lobes from patients with late-stage AD were significantly decreased, and nuclear OGG1 in temporal lobe and cerebellum from patients with mild cognitive impairment (MCI) were significantly increased. There was no significant difference in mitochondrial OGG1 levels among control, MCI and late-stage AD cases (Shao et al., 2008). Irrespective of the alteration in OGG1 protein levels in MCI or late-stage AD brains, 8-oxoG DNA glycosylase activity was significantly decreased in nuclear fractions and to a lesser extent in mitochondrial fractions from MCI and late-stage AD brains (Shao et al., 2008). Shao et al. (2008) also found that both nuclear and mitochondrial forms of OGG1 are modified by 4-hydroxynonenal, a neurotoxic by-product of lipid peroxidation in aged brains. This modification of mitochondrial OGG1 is likely to be elevated in MCI, perhaps underlying the decreased mitochondrial OGG1 activity in MCI.

In non-diseased postmortem human brains, the mitochondrial form of OGG1 (OGG1-2a) is strongly expressed in the superior occipital gyrus, orbitofrontal gyrus and entorhinal cortex, is expressed at much lower levels in CA1, CA3 and CA4, and is absent from the DG. In late-stage AD brains, OGG1-2a was detected as associated with NFTs, dystrophic neurites and reactive astrocytes, suggesting highly increased oxidative stress in mitochondria (Iida et al., 2002).

Mutations in *OGG1* (C796 deletion, Ala53Thr, Ala288Val) specific to AD patients have previously been reported (Mao et al., 2007). Mutant OGG1-1a protein with the C796 deletion has an altered carboxy terminal sequence (267aa to 345aa), resulting in the complete loss of 8-oxoG DNA glycosylase activity. The two other missense mutations (Ala53Thr and Ala288Val), which are likely to be rare polymorphic variants, conferred significantly reduced repair capacity to OGG1-1a, as well as reduced binding capacity to its partner proteins, poly(ADP-ribose) polymerase 1 (PARP-1) and X-ray repair cross-complementing protein 1 (XRCC1) (Jacob et al., 2013). All three mutations also alter the amino acid sequence of OGG1-2a (C796 deletion alters 267aa to 424aa). This suggests that both the nuclear form (OGG1-1a) and the mitochondrial form (OGG1-2a) lose repair capacity.

To date, no association of *MUTYH* polymorphism or its altered expression in AD brain has been reported.

In the transgenic (Tg)-$APP^{Arc/Swe}$ mouse model, there is a transient increase of at least 4-folds *Ogg1* mRNA levels in the hippocampus, frontal cortex, cerebellum and other regions in 4-month-old mice compared with the levels found in 6-week-old Tg-$APP^{Arc/Swe}$ and wild-type mice, and the levels are 2–3-fold higher than those found in 4-month-old wild-type mice. The *Ogg1* mRNA levels in 12-month-old Tg-$APP^{Arc/Swe}$ mouse brains were significantly decreased in all the brain regions examined and are equivalent to the levels in 6-week-old mouse brains (Lillenes et al., 2013). The Tg-$APP^{Arc/Swe}$ model has early onset senile plaque formation (4–6 months) and increased intraneuronal Aβ aggregation (1 month) prior to extracellular Aβ deposition, suggesting that the increased expression of *Ogg1* is likely to be a protective response to oxidative damage caused by the accumulation of intraneuronal Aβ aggregation, as seen in preclinical AD brains. Such a protective response is likely to be diminished in late stages of AD pathology, as found in late-stage AD patient brains.

It has been shown that exercise induces neuroprotection of the hippocampus in *APP/PS1* transgenic mice, and that the protection is associated with increased levels and repair activity of mitochondrial OGG1 (Bo et al., 2014), suggesting a protective role of OGG1 in AD pathology. This possibility should be examined using AD mouse models with OGG1-deficiency.

While MTH1 and MUTYH have not yet been investigated in any AD mouse model, it would be interesting to examine their expression levels and to determine whether the progression of AD pathology in the AD mouse can be altered with MTH1 or MUTYH deficiency.

6. 8-Oxoguanine accumulated in DNA may be involved in AD pathology

Observations in neurodegenerated postmortem brains (Bradley-Whitman et al., 2014; Coppede and Migliore, 2015; Lovell and Markesbery, 2007; Lovell et al., 2011; Nakabeppu et al., 2007b) and studies using animal models for various neurodegenerative diseases have shown that 8-oxoG accumulation in nuclear or mitochondrial DNA in neurons under oxidative conditions somehow results in neurodegeneration, and that MTH1 or OGG1 protects neurons by preventing 8-oxoG accumulation (Cardozo-Pelaez et al., 2012; De Luca et al., 2008; Liu et al., 2011; Miller-Pinsler et al., 2015; Sheng et al., 2012; Ventura et al., 2013; Yamaguchi et al., 2006).

Oka et al. (2008) demonstrated that accumulation of 8-oxoG in nuclear and mitochondrial DNA triggers two distinct cell death

pathways that are independent of each other. Both pathways are initiated by the accumulation of MUTYH-generated single-strand breaks (SSBs) in nuclear or mitochondrial DNA. When 8-oxoG accumulates to high levels in nuclear DNA, poly(ADP-ribose) polymerase (PARP) binds to the SSBs generated by MUTYH-initiated base excision repair (BER). This increases poly(ADP-ribose) polymer (PAR) resulting in nicotinamide adenine dinucleotide (NAD^+) and ATP depletion followed by nuclear translocation of apoptosis-inducing factor (AIF). AIF then executes apoptotic cell death. Thus, 8-oxoG accumulated to high levels in mitochondrial DNA causes degradation of mitochondrial DNA through MUTYH-initiated BER, resulting in mitochondrial dysfunction and activation of calpains, which in turn cause lysosomal rupture and cell death (Oka and Nakabeppu, 2011).

In the early phase of 3-nitropropionic acid (3-NP)-induced striatal degeneration, MTH1 and/or OGG1-deficient medium spiny neurons accumulate high levels of 8-oxoG and SSBs in mitochondrial DNA in an MUTYH-dependent manner, resulting in calpain activation and neuronal damage. In the later phase, dead neurons or damaged neurons activate microglia, which produce ROS, and activated microglia accumulate high levels of 8-oxoG and SSBs in nuclear DNA. In activated microglia, SSBs accumulated in nuclear DNA cause activation of the PARP-AIF pathway in a MUTYH-dependent manner, thus exacerbating microgliosis and neurodegeneration (Fig. 3) (Sheng et al., 2012).

Under oxidative conditions, 8-oxoG is highly accumulated in mitochondrial DNA but not in the nuclear DNA of neurons, and this accumulation in mitochondrial DNA is efficiently suppressed by the increased expression of MTH1 (De Luca et al., 2008). These observations indicate that the 8-oxoG accumulated in mitochondrial DNA is derived from the 8-oxo-dGTP accumulated in the nucleotide pool under oxidative conditions (not from direct oxidation of guanine in DNA), because only mitochondrial DNA and not nuclear DNA is replicating in post-mitotic neurons. On the other hand, microglial proliferation can be induced under inflammatory responses in the brain with an increased production of ROS; therefore, microglia accumulate 8-oxoG in nuclear DNA (Fig. 3) (Sheng et al., 2012).

Administration of a calpain or PARP inhibitor significantly ameliorated 3-NP-induced striatal degeneration and decreased microgliosis in MTH1/OGG-deficient mice, indicating that calpain-dependent neuronal damage causes microgliosis, and that microgliosis indeed exacerbates neurodegeneration (Sheng et al., 2012). It is noteworthy that activation of calpain and PARP is a hallmark of neurodegeneration under oxidative conditions, in both animal models and in AD brains (Kauppinen and Swanson, 2007; Martire et al., 2015; Saito et al., 1993; Yamashima, 2013). Calpain and PARP are therefore considered to be general therapeutic targets for various neurodegenerative diseases. Accumulation of 8-oxoG in neurons and microglia in the brain should be considered activators of calpain and PARP in neurodegenerative disorders; therefore, molecules involved in BER and related DNA metabolism should be considered novel therapeutic targets.

7. Future perspective

Recently, we evaluated the contribution of MTH1 and OGG1 in the prevention of the mitochondrial dysfunction during neuritogenesis *in vitro* (Fig. 4) (Leon et al., 2016). We isolated cortical neurons from adult wild-type and MTH1/OGG1-deficient mice and maintained them with and without antioxidants for 2–5 days, then examined the neuritogenesis. In the presence of antioxidants, both MTH1/OGG1-deficient and wild-type neurons exhibited efficient neurite extension and arborisation. However, in the absence of antioxidants, the accumulation of 8-oxoG in mitochondrial DNA of MTH1/OGG1-deficient neurons was increased and neurons exhibited significantly poor neurite outgrowth with decreased complexity of neuritic arborisation, indicating that MTH1 and OGG1 are essential for neuritogenesis or protection of nerve fibers under oxidative conditions. These observations indicate that mitochondrial dysfunction caused by oxidative damage in neurons results in degeneration of axons or dendrites, as well as neuronal death, which may represent a part of early pathological features of AD brain. Such degenerating neurons may trigger microglial activation, resulting in neuronal loss by phagocytosis (Fig. 3).

Now that many different mouse models of AD are available, whether the introduction of MTH1 or OGG1 deficiency exacerbate AD pathology, or the overexpression of MTH1 or OGG1 as transgene supress the progression of AD pathology, should be addressed using different AD model mice. Such approach will shed light on the development of new therapeutic approaches for AD.

Competing financial interests

The authors declare no competing financial interests.

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