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Cytoplasmic superoxide radical
a possible contributing factor to intracellular Aβ oligomerization in Alzheimer disease

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Soluble amyloid β (Aβ) oligomers cause memory loss and synaptic dysfunction in Alzheimer disease (AD). Despite intensive studies on Aβ assembly in vitro and in vivo, the localization and cellular mechanism of Aβ oligomerization are not fully understood. Previously, we demonstrated that cytoplasmic superoxide radicals contribute to drusen deposition, a hallmark of age-related macular degeneration as well as other geriatric diseases (fatty liver, skin thinning, and osteoporosis). Using a transgenic mouse model of AD, we recently clarified the role of cytoplasmic oxidative stress in cognitive impairment and oligomer formation. Moreover, we also found that these phenomena were associated with neuroinflammation, tau phosphorylation, and synaptic loss. Notably, studies using human brains support the involvement of cytoplasmic superoxide radicals in AD pathology. In this addendum to Murakami et al. (JBC 2011), we discuss and comment on intracellular Aβ oligomer formation and the possible therapeutic effects of intracellular redox state modulators.

Alzheimer disease (AD) is characterized by the presence of senile plaques that mainly contain 40- and 42-mer amyloid β-proteins (Aβ40, Aβ42).[1,2] These proteins are generated from amyloid β-protein precursor by β-site hAPP-cleaving enzyme 1 (BACE1), followed by γ-secretase processing (the amyloidogenic pathway). Many studies have demonstrated that soluble Aβ oligomers (50~60 kDa: Aβ-derived diffusible ligands, Aβ*56, and globulomers; > 100 kDa: amylospheroid, protofibrils, and annuli) rather than insoluble fibrils cause memory loss and synaptic dysfunction.[3] Oxidative stress contributes to the pathogenesis of several neurodegenerative diseases, such as AD, Parkinson disease, and amyotrophic lateral sclerosis.[4,5] In addition, a study that detected the in vitro radicalization of Aβ suggested that the Aβ-induced neurotoxicity observed in AD is associated with oxidative damage.[6]

Superoxide dismutase (SOD) is one of the major antioxidant metalloenzymes that converts toxic superoxide radicals (O_2^-) to H_2O_2, followed by Fenton reaction.[7] SOD consists of three isozymes: CuZn-SOD (SOD1), which is found in the cytosol, nucleus, and intermembrane space of mitochondria; Mn-SOD (SOD2), which is localized in the mitochondrial matrix; and extracellular SOD (SOD3), which is also a complex of Cu and Zn and is found in the blood. We previously reported that *Sod1*-deficient (*Sod1*$^{-/-}$) mice displayed drusen deposition, which is a typical characteristic of age-related macular degeneration,[8] fatty liver,[9] skin thinning,[10] and osteoporosis.[11] Some of these phenotypes can be alleviated by vitamin C.[10,11] Although mitochondrial oxidative stress has hitherto been believed to be a potent cause of neuronal disorders,[12] whether the damage induced by mitochondrial radicals is the main contributor to age-related diseases like AD is questionable because a systemic deficiency in SOD2 causes neonatal lethality in mice.[13,14] Our previous study, which involved the use of a human amyloid precursor protein (hAPP) transgenic AD mouse model lacking *Sod1* (*hAPP*/*Sod1*$^{-/-}$), shed new light on the importance of cytoplasmic superoxide radicals in the pathogenesis of AD, as the *hAPP*/*Sod1*$^{-/-}$ mice accelerated Aβ oligomerization, memory loss, neuroinflammation, tau phosphorylation, and synaptic loss compared with the control AD mouse.[15] As shown in **Figure 1**, western blotting of anti-Aβ1-16 antibody (6E10) revealed that the formation of Aβ hexamer was significantly increased in *hAPP*/*Sod1*$^{-/-}$ rather than *hAPP*/*Sod1*$^{+/+}$. These results are in good agreement with the previous study using anti-N-terminal Aβ antibody (82E1).[15] Intriguingly, the levels of SOD1 were significantly lower in human AD patients compared with non-AD individuals, but SOD2 and SOD3 were not.[15] Higher levels of BACE1 were also detected in the *hAPP*/*Sod1*$^{-/-}$ mice, indicating that the amyloidogenic pathway had been stimulated by the presence of cytoplasmic superoxide radicals.[16]

Intracellular Aβ oligomers have recently become the focus of much AD research. It is indispensible to elucidate where Aβ oligomerizes; i.e., whether Aβ oligomerizes in the cytosol after extracellular secretion and reuptakes into cells, or whether Aβ

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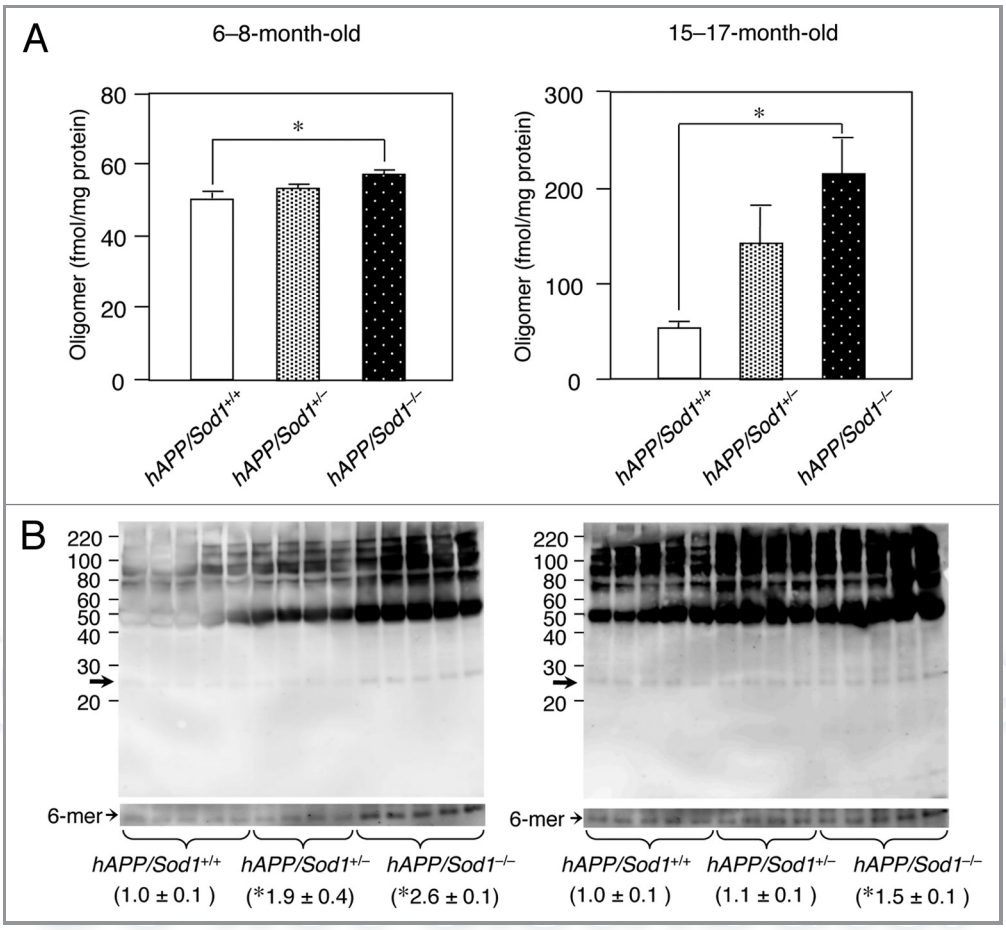

Figure 1. An increase of Aβ oligomer by *Sod1* deletion in Alzheimer mice. (A) ELISA analysis of 82E1-specific oligomers using the TBS-soluble fraction of brains of mice (n = 5~7 per genotype) of the indicated genotypes and age. In ELISA for Aβ oligomers (Immuno Biochemical Laboratories: IBL, Gunma, Japan), the same N-terminal Aβ antibody (82E1) is used both for antigen capture and detection. In the younger ages (6−8-mo-old), the level of only Aβ oligomers was significantly increased in *hAPP/Sod1*$^{-/-}$ as compared with the *hAPP/Sod1*$^{+/+}$ mice, but not Aβ42 or Aβ40 (ref. 15). On the other hand, older (15−17-mo-old) *hAPP/Sod1*$^{-/-}$ showed a significant elevation of Aβ oligomers as well as Aβ42 and Aβ40 (ref. 15) as compared with the *hAPP/Sod1*$^{+/+}$ mice. These data are rearrangement of the previous work (ref. 15). (B) Distribution of Aβ aggregates by western blotting of mice (n = 4~5 per genotype) of the indicated genotypes and age. The detailed procedure was described previously (ref. 15). In brief, Tris buffered saline (TBS)-soluble fractions (2 μg/μL) were subjected to western blotting using 10–20% Tricine gel (Invitrogen) and transferred to a PVDF membrane (0.2 μm pore size, Bio-rad). Anti-Aβ antibody (6E10, 1:1,000, Signet) was used for Aβ detection. Overexposed bands corresponding to the hexamer (arrows: ~30 kDa) were used for relative quantification. *left*: 6−8-mo-old, *right*: 15−17-mo-old. *p $<$ 0.05 vs. *hAPP/Sod1*$^{+/+}$, mean ± s.e.m.

oligomerizes in the extracellular space and reuptakes into cells. Alternatively, misfolding of Aβ in endoplasmic reticulum-Golgi may lead to oligomerization in the cytosol. Excessive free radicals in the cytosol could directly alter the conformation of intracellular Aβ; e.g., a toxic turn formation of intracellular Aβ42 were proposed by our groups.[6,17] Ohyagi and coworkers revealed that intraneuronal Aβ accumulation and memory disturbance occur before extracellular Aβ deposition in mice.[18] In the human brain, it was also reported that intracellular Aβ accumulation frequently precedes senile plaque formation.[19] Mori, Tomiyama, and coworkers suggested that intraneuronal Aβ oligomers induce neuronal death by activating endoplasmic reticulum stress, endosomal/lysosomal leakage, and mitochondrial dysfunction.[20] On the other hand, there have been several reports on the relationship between SOD1 protein and intracellular Aβ. As a result, it has been found that the interaction of intracellular Aβ with SOD1 decreases the activity of SOD1,[21] and the deletion of the copper chaperone for SOD1 enhances the cytoplasmic concentration of Aβ.[22] These findings strongly suggest the importance of cytoplasmic superoxide radicals in the intracellular Aβ-mediated AD pathologies and support our conclusion that it is an alternative therapeutic target for AD treatments. Further investigation of molecular mechanism will be required because cytoplasmic radicals may contribute to the pathogenesis of other various neurodegenerative diseases.

Regarding therapies based on SOD, several AD phenotypes in mice can be improved by the direct administration of SOD,[23] and the dietary intake of Cu stabilizes SOD1 activity.[24] However, it should be taken into account that excessive changes in the levels of redox-active metal ions could have adverse effects due to hydroxyl radical generation. On the other hand, a number of synthetic compounds that decrease the concentrations of cellular

radicals have been developed. Among them, EUK-8, a salen-manganese complex with SOD and catalase activities,[25] and porphyrin-like compound [Mn(III) tetrakis(4-benzoic acid) porphyrin chloride, denoted as Mn-TBAP[26]] are promising agents. Recently, some orally available compounds that protect against oxidative damage (mito-Q[27,28] and metalloporphyrin AEOL11207[29]) were found to rescue the phenotypes of mouse models of AD and Parkinson disease, respectively. On the other hand, unexpectedly, an increase of SOD1 activity may produce H_2O_2, resulting in generation of hydroxyl radicals. With regard to this context, decreasing H_2O_2 levels by catalase or glutathione peroxidase can also be valuable to attenuate oxidative stress. Recently, apomorphine[30] was reported to reduce the amounts of H_2O_2, leading to inhibition of intracellular hydroxyl radicals in AD. The combination therapy using various types of anti-oxidative stress drugs can be a promising way to delay AD. In conclusion, our research will thus not only help to advance our understanding of the molecular basis of intracellular Aβ oligomerization, but may eventually provide a starting point for the development of novel redox status modulators.

Disclosure of Potential Conflicts of Interest

No Potential conflicts of interest were disclosed.

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