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Original Contribution

Protective role of glutathione *S*-transferase A4 induced in copper/zinc-superoxide dismutase knockout mice

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ABSTRACT

Copper/zinc-superoxide dismutase (SOD1) plays a protective role in cells by catalyzing the conversion of the superoxide anion into molecular oxygen and hydrogen peroxide. Although SOD1 knockout (KO) mice exhibit a reduced life span and an elevated incidence of dysfunctions in old age, young SOD1 KO mice grow normally and exhibit no abnormalities. This fact leads to the hypothesis that other antioxidative proteins prevent oxidative stress, compensating for SOD1. Differently expressed genes in 3-week-old SOD1 KO and littermate wild-type mice were explored. A gene remarkably elevated in SOD1 KO mouse kidneys was identified as the glutathione *S*-transferase Alpha 4 gene (*Gsta4*), which encodes the GSTA4 subunit. The GSTA4 protein level and activity were also significantly increased in SOD1 KO mouse kidneys. The administration of an iron complex, a free radical generator, induced GSTA4 expression in wild-type mouse kidneys. Iron deposition detected in SOD1 KO mouse kidney is thought to be an inducer of GSTA4. In addition, overexpression of mouse GSTA4 cDNA in human embryonic kidney cells decreased cell death caused by both 4-hydroxynonenal and hydrogen peroxide. These findings suggest that compensatory induced GSTA4 plays a protective role against oxidative stress in young SOD1 KO mouse kidneys.

Oxidative stress caused by accumulated reactive oxygen species (ROS)[1] is closely involved in a variety of pathological processes, including inflammation, cancer, neurodegenerative disorders, and aging. Most cells have a lot of defense systems for avoiding the toxic effects of ROS. A variety of antioxidative proteins and low-molecular-weight antioxidants are highly regulated to maintain cellular homeostasis. Among them, superoxide dismutase (SOD) is thought to play a central role because of its ability to scavenge superoxide anions, the primary ROS generated from molecular oxygen in cells. Mammals have three isozymes, Cu/Zn-SOD (SOD1), Mn-SOD (SOD2), and extracellular SOD (EC-SOD, SOD3). SOD1 is mainly localized in the cytosol and SOD2 is located exclusively in mitochondria. SOD3 protein is secreted into the extracellular space, where the majority of it becomes anchored to sulfated glycosaminoglycans in the tissue interstitium. Mutation of SOD1 causes familial amyotrophic lateral sclerosis (FALS), which is a neurological disease characterized by selective motor neurons in the brain and spinal cord. Several lines of transgenic mice that express the mutant human SOD1 linked with FALS develop progressive neurodegeneration and a phenotype that clearly resembles human FALS [1,2]. In contrast, young SOD1 knockout (KO) mice grow normally and exhibit no clear abnormalities except for a small body size and anemia [3]. However, adult or old SOD1 KO mice exhibit elevated incidences of alcohol-induced liver injury [4], hepatocarcinogenesis [5], ischemia/reperfusion-induced renal failure [6], hearing loss [7], lipid accumulation in the liver [8], and female infertility [9]. These phenotypes are probably caused by accumulated toxic organic peroxides such as lipid peroxides and unsaturated aldehydes resulting from the constitutive SOD1 deficiency.

The glutathione *S*-transferases (GSTs; EC 2.5.1.18) constitute a major group of phase II detoxification proteins that protect against a variety of reactive chemicals, such as chemotherapeutic agents and chemical carcinogens, and secondary metabolites during oxidative stress, such as α,β-unsaturated aldehydes, quinones, and hydroperoxides [10]. Mammalian cytosolic GSTs are all dimeric with subunits of 199–244 amino acids. Based on amino acid sequence similarities, at least seven classes of cytosolic GSTs are recognized in mammals, which are designated as Alpha, Mu, Pi, Sigma, Theta, Omega, and Zeta [10]. Among them, GSTA4-4, which is a homodimer of the GSTA4 subunit, belongs to the GST Alpha family and exhibits uniquely high glutathione conjugation activity toward 4-hydroxynonenal (4-HNE), an end-product of lipid peroxidation [11,12].

Abbreviations: ROS, reactive oxygen species; SOD, superoxide dismutase; FALS, familial amyotrophic lateral sclerosis; GST, glutathione *S*-transferase; 4-HNE, 4-hydroxynonenal; CDNB, 1-chloro-2,4-dinitrobenzene; MDA, malondialdehyde; Fe–NTA, ferric nitrilotriacetate; HEK293, human embryonic kidney 293; ARE, antioxidant-responsive element; Nrf2, nuclear factor E2-related factor 2.

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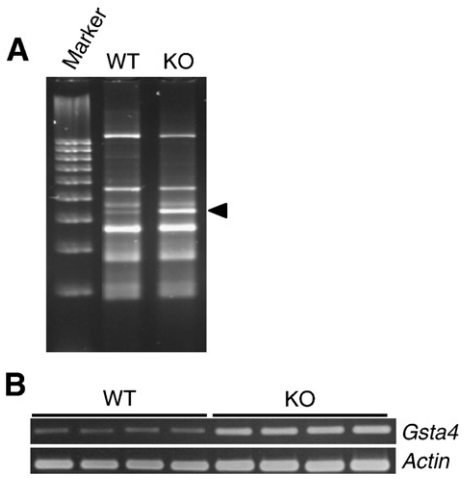

Fig. 1. Differential gene expression in SOD1 KO and WT mouse kidneys. (A) Differential banding patterns of genes isolated from SOD1 KO and WT mouse kidneys. The arrowhead indicates the more highly expressed gene in SOD1 KO mice. (B) RT-PCR analysis of *Gsta4* expression. Total RNAs were isolated from 3-week-old male SOD1 KO and WT mouse kidneys. PCR products were separated on a 2% agarose gel and stained with SYBR Green I.

The kidney plays an important role in the excretion of metabolic wastes and reabsorption of nutrients through blood filtration. The renal tubules tend to suffer oxidative stress from such metabolites including harmful agents through the active transport. Although SOD1 deficiency may further increase renal oxidative stress, clear abnormalities are not observed in young SOD1 KO mouse kidneys. Also, few differences in renal function between wild-type (WT) and SOD1 KO mice after ischemia/reperfusion at a young age were reported [6]. Accumulating studies have shown that SOD isoenzymes such as SOD2 and SOD3 probably do not cover a SOD1 deficiency [13] and that SOD1 and SOD3 have no overlapping roles, as seen with SOD1 and SOD3 double-KO mice [13]. These facts lead to the hypothesis that some unidentified antioxidative proteins protect against oxidative stress, compensating for SOD1.

In this study, we performed differential display analysis, using 3-week-old SOD1 KO and WT mouse kidneys, to search for compensatory working proteins. We found that the GSTA4 gene, *Gsta4,* which encodes the GSTA4 subunit, is remarkably induced in young SOD1 KO mouse kidneys. Thus, we focused on the investigation of the expression and roles of GSTA4 in SOD1 KO mouse kidneys by comparison with the expression of other antioxidative enzymes. In addition, we investigated whether ROS production mimicking SOD1 deficiency induces GSTA4 expression and whether GSTA4 overexpression has a protective effect against ROS-induced cell death. The results show that oxidative stress induces GSTA4 expression even in wild-type mouse kidneys and that the GSTA4-expressed cells have tolerance to 4-HNE- and hydrogen peroxide-induced cell death. Moreover, we found iron deposits in SOD1 KO mouse kidneys, which are probably related to the GSTA4 induction.

Experimental procedures

Materials

All chemicals used in this study were purchased from Wako Pure Chemical Industries Ltd., Nacalai Tesque, Inc., or Sigma–Aldrich, unless specified otherwise, and were of the highest grade available.

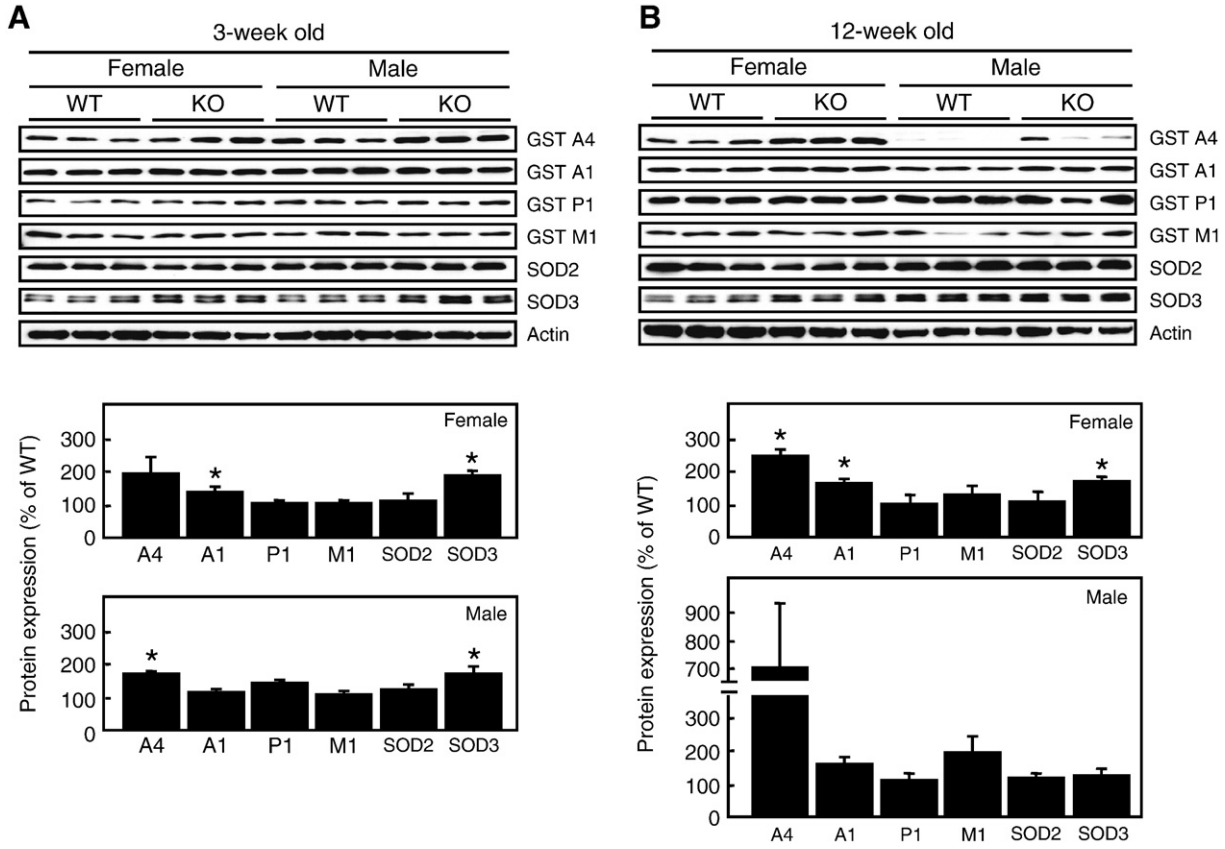

Fig. 2. GSTA4, GSTA1, GSTP1, GSTM1, SOD2, and SOD3 protein expression in SOD1 KO and WT mouse kidneys, at (A) 3 and (B) 12 weeks of age. The top shows the protein expression levels determined by Western blot analysis. The bottom (bar graphs) shows the fold (%) changes calculated against the protein levels in WT mice by densitometric scanning of the Western blots. The data are normalized to the expression levels of β-actin and expressed as means ± SEM (*$p<0.05$, compared with WT mice).