

The spatio-temporal expression pattern of cytoplasmic Cu/Zn superoxide dismutase (SOD1) mRNA during mouse embryogenesis

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Abstract The cytoplasmic Cu/Zn-superoxide dismutase (SOD1) represents along with catalase and glutathione peroxidase at the first defense line against reactive oxygen species in all aerobic organisms, but little is known about its distribution in developing embryos. In this study, the expression patterns of SOD1 mRNA in mouse embryos were investigated using real-time RT-PCR and in situ hybridization analyses. Expression of SOD1 mRNA was detected in all embryos with embryonic days (EDs) 7.5–18.5. The signal showed the weakest level at ED 12.5, but the highest level at ED 15.5. SOD1 mRNA was expressed in chorion, allantois, amnion, and neural folds at ED 7.5 and in neural folds, notochord, neuromeres, gut, and primitive streak at ED 8.5. In central nervous system, SOD1 mRNA was expressed greatly in embryos of EDs 9.5–11.5, but weakly in embryos of ED 12.5. At EDs 9.5–12.5, the expression of SOD1 mRNA was high in sensory organs such as tongue, olfactory organ (nasal prominence) and eye (optic vesicle), while it was decreased in ear (otic vesicle) after ED 10.5. In developing limbs, SOD1 mRNA was greatly expressed in forelimbs at EDs 9.5–11.5 and in hindlimbs at EDs 10.5–11.5. The signal increased in liver, heart and genital tubercle after ED 11.5. In

the sections of embryos after ED 13.5, SOD1 mRNA was expressed in various tissues and especially high in mucosa and metabolically active sites such as lung, kidney, stomach, and intestines and epithelial cells of skin, whisker follicles, and ear and nasal cavities. These results suggest that SOD1 may be related to organogenesis of embryos as an antioxidant enzyme.

Keywords Superoxide dismutase · Antioxidant enzyme · Reactive oxygen species · Mouse embryo · In situ hybridization

Introduction

Reactive oxygen species (ROS) are continuously produced during aerobic metabolism in mammalian cells. Recent data indicate that ROS modulate signaling pathways by oxidizing and transiently inactivating phosphatases. On the other hand, excessive ROS has the ability to induce lipid peroxidation, protein oxidation and DNA damage. Therefore, mammalian cells are equipped with antioxidant enzyme defense mechanisms to minimize the cellular damage resulting from interaction between cellular constituents and ROS (Chan 1994; Allen and Tresini 2000; Valko et al. 2006).

Superoxide dismutase (SOD) catalyses the dismutation of superoxide radicals ($O_2^{\cdot-}$) to hydrogen peroxide, and either catalase or glutathione peroxidase (GPx) converts the hydrogen peroxide into water (McCord and Fridovich 1969). Three distinct isoforms of SOD have been identified in mammals. Two isoforms of SOD have Cu and Zn in their catalytic center and are localized to either intracellular cytoplasmic compartments (SOD1 or Cu/Zn-SOD) or to extracellular elements (SOD3 or EC-SOD). SOD1 is located

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in intracellular compartments including the cytosol, nucleus, and lysosome (Crapo et al. 1992). Recent studies have indicated that some SOD1 are also located in the mitochondrial intermembrane space, thereby possibly contributing to protection of this organelle against oxidative stress (Okado-Matsumoto and Fridovich 2001; Inarrea et al. 2007). The isoform of SOD has Mn (SOD2 or Mn-SOD) and is highly expressed in differentiated organs that contain a large number of mitochondria such as heart, liver, and kidney (Grankvist et al. 1981). SOD2 plays a major role in promoting cellular differentiation and tumorigenesis (St Clair et al. 1994) and in protecting against hyperoxia-induced pulmonary toxicity (Wispe et al. 1992). SOD3 was first detected in human plasma, lymph, ascites, and cerebrospinal fluids. The expression of SOD3 is highly restricted to the specific cell types and tissues where its activity can exceed that of SOD1 and SOD2 (Marklund et al. 1982).

ROS are generally produced by embryo metabolism. Embryo protection against ROS depends, in part, upon an endogenous pool of antioxidant enzymes (Goto et al. 1993; Guerin et al. 2001). Recently, we demonstrated that the cytosolic GPx (cGPx) mRNA is expressed in forelimb and hindlimb buds, central nervous system (CNS), active metabolic tissues, and developing epithelial tissues of mouse embryos (Baek et al. 2005). Defects in embryonic SOD and catalase activities may reduce the ability of the fetus to clear free oxygen radicals and expose them to an increased oxidative load, thereby causing fetal dysmorphogenesis (Sivan et al. 1997). Mice with targeted deletions of SOD2 gene die within the first 10 days of life with a dilated cardiomyopathy, accumulation of lipid in liver and skeletal muscle, and metabolic acidosis (Li et al. 1995). Cu deficiency resulted in embryos with malformations and reduced SOD enzyme activity (Hawk et al. 2003). Female mice lacking SOD1 are characterized by an increase in embryo lethality (Ho et al. 1998). Significantly, an increase in SOD activity can improve the development outcome of the embryos exposed to selective teratogenic agents (Zimmerman et al. 1994; Kotch et al. 1995; Karabulut et al. 2000).

It is, therefore, hypothesized that SOD1 is related to embryogenesis. But little is known about the spatio-temporal expression pattern of SOD1 mRNA in developing embryos. The purpose of this study is to investigate whether SOD1 gene is expressed spatio-temporally during embryo development as the first stage for understanding the function of SOD family in embryogenesis.

Materials and methods

Experimental animals

Male and female ICR mice (8–10 weeks old) were purchased from a commercial breeder, Biogenomics Co.

(Seoul, Korea). All animals were housed in polycarbonate cages and acclimated for 1 week. They were kept in an environmentally controlled room with $21 \pm 2^\circ\text{C}$ of temperature, $55 \pm 10\%$ of relative humidity, 10 times/h of ventilation, and a 12 h light/dark cycle. The animals were fed a standard mouse chow (Samyang Ltd., Incheon, Korea) and tap water *ad libitum* throughout the experimental period. All experiments were approved and carried out according to the “Guide for care and use of animals” (Chungbuk National University Animal Care Committee according to NIH #86–23).

Preparation of embryos

One male and three female mice were housed in a cage for mating. The successful mating was confirmed by the presence of a copulation plug in vagina. The day found the vaginal plug was designated as embryonic day (ED) 0.5. Under pentobarbital anesthesia, pregnant mice were sacrificed and embryos were obtained from EDs of 7.5–18.5. The embryos were used for real-time reverse-transcription polymerase chain reaction (RT-PCR) and whole mount (embryo) or tissue in situ hybridization analyses.

Real-time RT-PCR analysis

Total RNAs were extracted from mice embryos of EDs 7.5–18.5 using a Trizol reagent kit (Invitrogen, San Diego, USA). cDNA synthesis was done in a 20- μl reaction mix starting with 1 μg of total RNA using the iScriptTMcDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA, USA). Quantitative real-time RT-PCR was performed on a 7500 real-time PCR System (Applied Biosystem, CA, U.S.A.) using 2X TaqMan Universal PCR Master Mix Kit (Applied Biosystems, CA, U.S.A.) according to the manufacturer’s instructions. Gene-specific primers were designed by TIB Mol-Biol Syntheselabor (Berlin, Germany). The sequences of specific mouse SOD1 primers (Bewley 1988) and probes used were as follows: forward primer: 5'-CTT CTC GTC TTG CTC TCT CTG G-3'; reverse primer: 5'-TCC TGT AAAT TTG TCC TGA CAA CAC-3'; TaqMan probe: 5'-ACT GGT TCA CCG CTT GCC TTC TGC. The TaqMan probe was JOE-labeled. The thermal cycling conditions were 2 min at 50°C (action of uracil-N-glycosylase), 10 min at 95°C followed by 40 cycles consisting of denaturing at 95°C for 15 s, annealing at 55°C for 1 min and extension at 60°C for 1 min. Data were acquired and analyzed with a 7500 system SDS software (version 1.3.1.21; Applied Biosystem, CA, U.S.A.). Amplification kinetics was recorded in real-time mode as sigmoid process curves, for which the

fluorescence was plotted against the number of amplification cycles. GAPDH mRNA was used as an internal standard (Assay on demand #4352932E, Applied Bioscience) to normalize expression of the target transcripts.

In situ hybridization

To prepare RNA probes for in situ hybridization, a pGEM-T easy plasmid (Promega, Madison, USA) containing the mouse SOD1 cDNA clones (accession number: NM_011434, fragment; 176 bp) was linearized with *SpeI* or *NcoI* restriction enzyme. Digoxigenin (DIG; Roche Co., Germany)—labeled sense or antisense riboprobe for SOD1 was generated via in vitro transcription in the presence of T7 or Sp6 RNA polymerase (Takara Co., Japan) at 37°C for 60 min. For whole-mount in situ hybridization of embryos, EDs 7.5–12.5 mouse embryos were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) overnight and dehydrated in methanol (Correia and Conlon 2001). The embryos were kept at –20°C until used. The general in situ hybridization procedure for tissue sections was carried out as described previously (Olivier and Walter 1998). Tissue sections from mouse embryos (EDs 13.5–18.5) were placed on RNase-free glass slides and deparaffinized. The sections were incubated in PBS containing 10 µg/ml of proteinase K at 37°C for 10 min and treated with 4% paraformaldehyde in PBS for 15 min. The sections were incubated for 15 min in PBS containing 0.1% active diethylpyrocarbonate twice, equilibrated for 15 min in 5 × standard sodium citrate (SSC; 0.15 M sodium chloride and 0.015 M sodium citrate), and then prehybridized for 2 h at 58°C in the hybridization solution (50% formamide, 5 × SSC and salmon sperm DNA 40 µg/ml). The hybridization reaction was carried out at 58°C for 17 h with 50 µl of hybridization mix on each section. After the hybridization, the sections were washed for 30 min in 2 × SSC at room temperature, 1 h in 2 × SSC at 65°C, 1 h in 0.1 × SSC at 65°C, then finally equilibrated for 5 min in Buffer 1 (100 mM Tris–HCl and 150 mM NaCl, pH 7.5). The sections were then incubated for 2 h at room temperature with anti-DIG-alkaline phosphatase (Roche Co., Germany) diluted with 1:5000 in Buffer 1 containing 1% blocking reagent (Roche Co., Germany). The reaction was stopped by washing with Buffer 1 for 15 min twice and the sections were equilibrated for 5 min in detection buffer (0.1 M NaCl, 0.1 M Tris–HCl, pH 9.5). Signal detection was performed at room temperature for 30 min to 1 day in the detection buffer containing 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium solution (DAKO, Carpinteria, USA). Staining was stopped by a 10 min washing in 10 mM Tris/EDTA (pH 8.0) buffer. Non-specific staining was removed by overnight agitation in 95%

ethanol. The sections were rehydrated for 15 min in deionized water to remove the precipitated Tris and then dehydrated in a series of ethanol and xylene.

Results

Expression level of SOD1 mRNA during embryogenesis

As analyzed by quantitative real-time RT-PCR technique, SOD1 transcripts were detected throughout all embryonic stages from EDs 7.5 to 18.5 (Fig. 1). The signal showed the weakest level at ED 12.5, but the highest level at ED 15.5. The primers for mouse GAPDH were used as an internal standard in the PCR analysis.

Spatio-temporal expression of SOD1 mRNA in whole embryos

At ED 7.5, SOD1 mRNA was expressed in chorion, allantois, amnion and neural folds (Fig. 2A). At ED 8.5, the signal was detected in neural folds, notochord, neuromeres, gut, and primitive streak (Fig. 2B and C). At EDs 9.5–12.5, SOD1 mRNA showed a high expression in CNS such as forebrain, midbrain, hindbrain, and spinal cord and sensory organs including tongue, olfactory organ (nasal prominence), ear (otic vesicle) and eye (optic vesicle), while it began to decrease in the ear after ED 10.5 (Fig. 2D–G). SOD1 mRNA was greatly expressed in forelimbs at EDs 9.5–11.5 (Fig. 2D–F) and in hindlimbs at EDs 10.5–11.5 (Fig. 2E and F). The signal increased in liver, heart, and genital tubercle at EDs 11.5–12.5 (Fig. 2F and G).

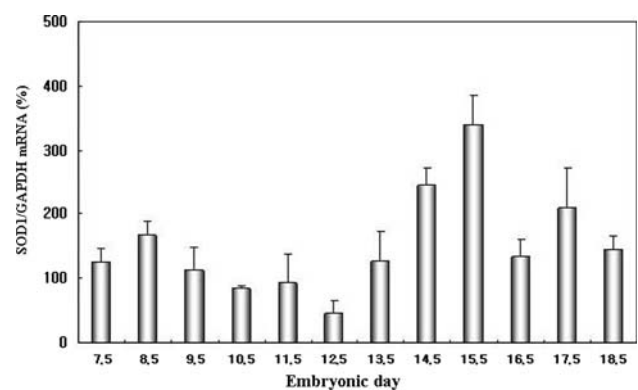


Fig. 1 Expression level of SOD1 mRNA during mouse embryogenesis. Total RNAs isolated from embryos were reverse-transcribed and the cDNAs were amplified by real-time RT-PCR using the mouse SOD1 or GAPDH primers. The detected signal of SOD1 mRNA was normalized with GAPDH mRNA. The expression of SOD1 mRNA appears throughout all the embryonic stages. The signal is observed the weakest level at ED 12.5, but the highest level at ED 15.5. Each data represent means ±SD from five mouse embryos

At ED 13.5, SOD1 mRNA was expressed in the telencephalon, diencephalon, mesencephalon, myelencephalon, spinal cord, Rathke's pocket, nasal chamber, lung, pancreas, intestine, genital tubercle, metanephros, adrenal gland, liver, and heart (Fig. 2H).

At EDs 14.5–15.5, SOD1 mRNA was persistently detected in the same regions as those at ED 13.5 (data not shown). At ED 16.5, SOD1 mRNA was detected in various tissues such as CNS, vibrissae, submandibular gland, thymus, liver, heart, pancreas, metanephric kidney,

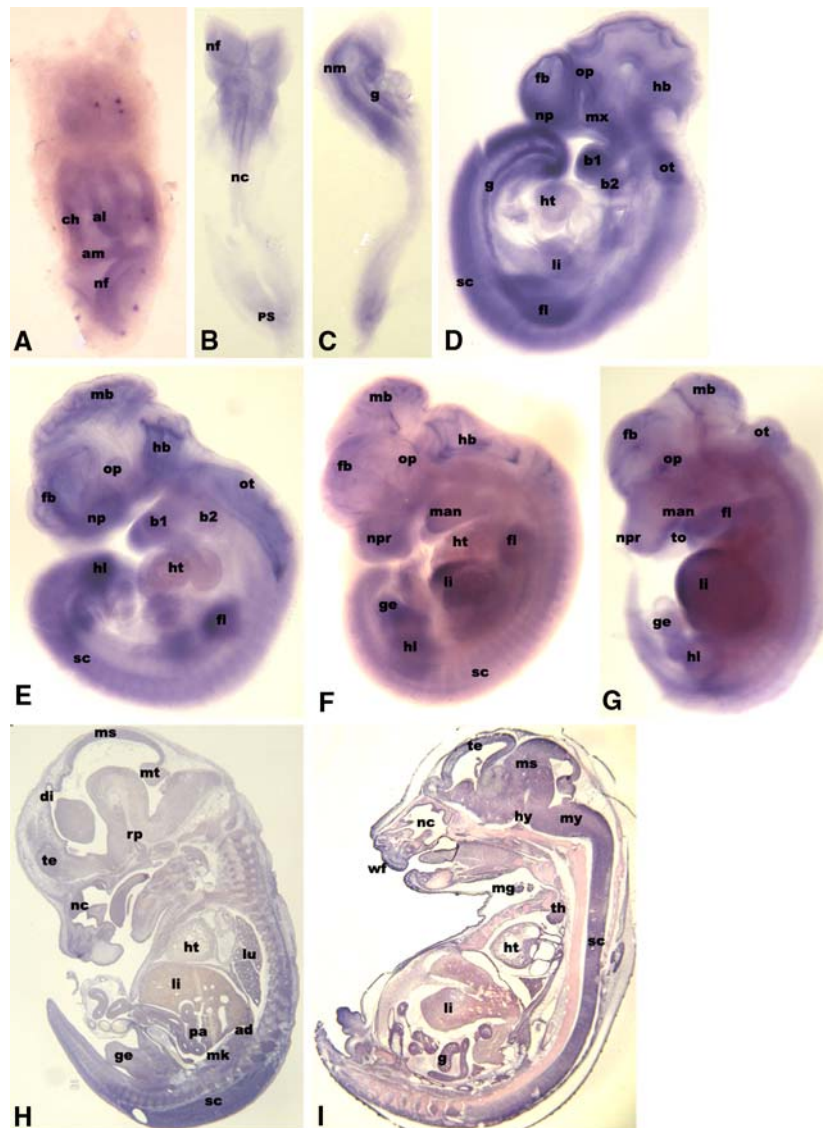


Fig. 2 Whole-mount in situ hybridization of SOD1 mRNA (A–G). (A) Embryonic day (ED) 7.5, SOD1 mRNA is expressed in chorion (ch), allantois (al), amnion (am), and neural fold (nf). (B and C) At ED 8.5, the signal is expressed in notochord (nc), neuromeres (nm), neural folds (nf), gut (g), and primitive streak (ps). (D) At ED 9.5, SOD1 mRNA is expressed in forebrain (fb), hindbrain (hb), spinal cord (sc), branchial arches (b1,b2), optic vesicles (op), otic vesicles (ot), nasal process (np), gut (g), heart (ht), liver (li) and forelimb buds (fl). (E) At ED 10.5, the signal is detected in fb, midbrain(mb), hb, sc, op, np, b1, b2, fl, hindlimb buds (hl), ot, li and ht. (F) At ED 11.5, SOD1 mRNA is expressed in fb, mb, hb, sc, op, nasal prominence (npr), mandible (man), li, genital eminence (ge), fl, and hl. (G) At ED

12.5, SOD1 mRNA is mainly detected man, maxilla, npr, tongue (to), li, brain, limbs, ht, and ge. Hybridization with a SOD1 antisense riboprobe on sagittal sections of mouse embryos (H and I). (H) At ED13.5, the signal is expressed in the developing central nerve system [CNS : telencephalon (te), diencephalon (di), mesencephalon (ms), metencephalon (mt), and sc], nasal chamber (nc), Rathke's pocket (rp), lung (lu), pancreas (pa), intestine, genital eminence (ge), metanephros (mk), and adrenal gland (ad). (I) At ED 16.5, it is detected in various tissues such as CNS including myelencephalon (my), vibrissae (wf), hypophysis (hy), submandibular gland (mg), thymus (th), li, ht, and gut (g). A, C–I : lateral view, B: dorsal view

adrenal gland, and intestine. Especially, the signal was greatly expressed in mucosa and metabolically active sites such as lung, kidney, stomach, and intestine and was detected in epithelial cells of skin, whisker follicles, and ear and nasal cavities (Fig. 2I). At EDs 17.5 and 18.5, the

expression pattern of SOD1 mRNA was similar to that of ED 16.5 (Fig. 3B, D, F, H, I, L, N, P, R, V, and X). In addition, there were no apparent signals in in situ hybridization analysis using a DIG-labeled SOD1 sense probe (data not shown).

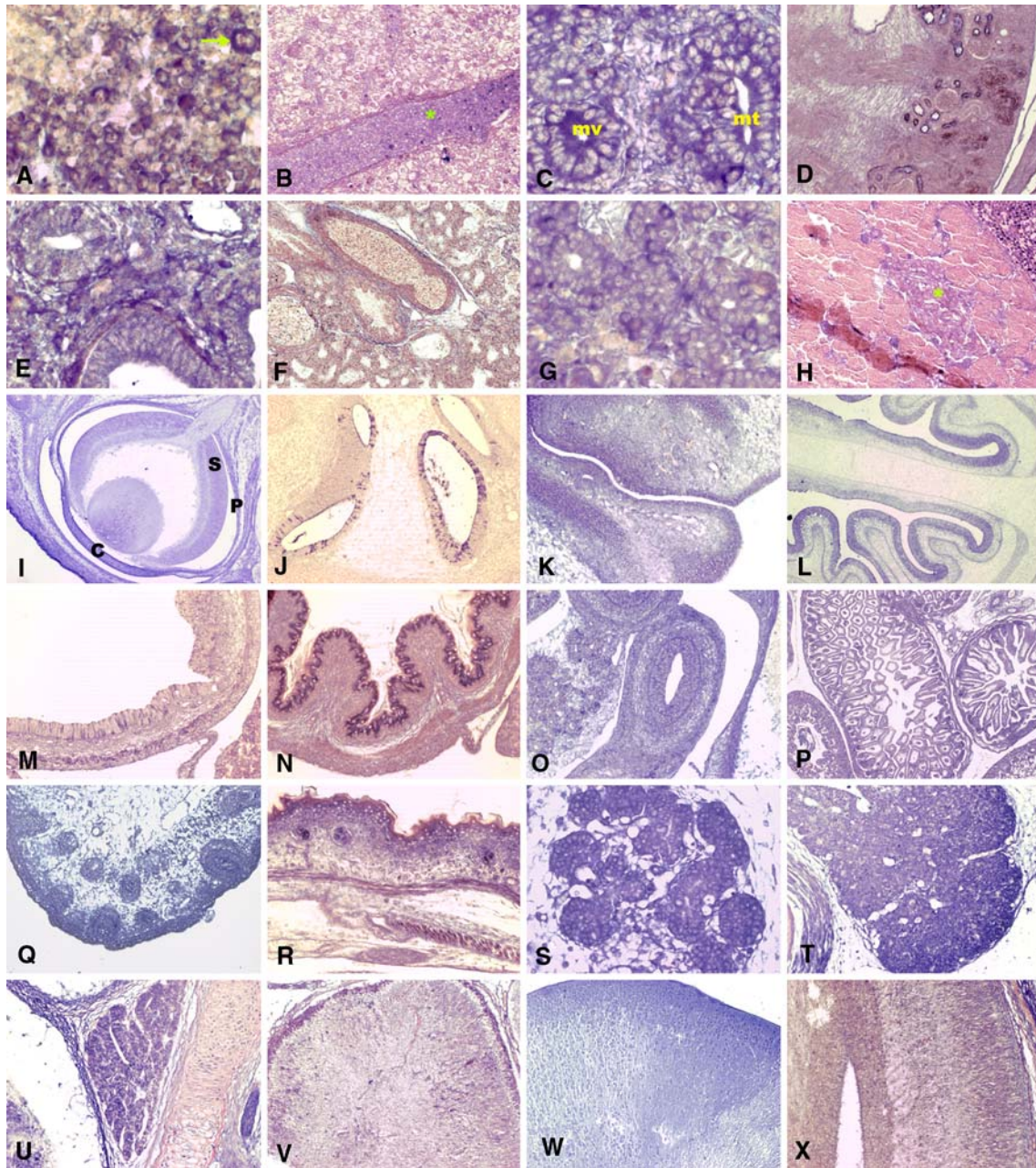


Fig. 3 Hybridization with a SOD1 antisense riboprobe on the sagittal sections of mouse embryos. (A) ED 13.5 liver: megakaryocyte (arrow), (B) ED 18.5 liver: hepatic vein (*), (C) ED 13.5 kidney: metanephric vesicle (mv) and tubule (mt), (D) ED 17.5 kidney, (E) ED 13.5 lung, (F) ED 17.5 lung, (G) ED 13.5 pancreas, (H) ED 18.5 pancreas: islet of pancreas (*), (I) ED 17.5 eye: cornea (c), sensory layer of retina (s), and pigment layer of retina (p), (J) ED 15.5 inner

ear, (K) ED 13.5 nasal prominence, (L) ED 17.5 nasal conchae, (M) ED 15.5 stomach, (N) ED 17.5 stomach, (O) ED 13.5 intestines, (P) ED 17.5 intestines, (Q) ED 16.5 skin, (R) ED 17.5 skin, (S) ED 16.5 submandibular gland, (T) ED 16.5 thymus, (U) ED 16.5 hypophysis, (V) ED 17.5 cerebellum, (W) ED 14.5 mesencephalon, (X) ED 17.5 telencephalon. Magnification: I, L, P (×40); D, F, I, K, M–O, Q, R, T–X (×100); B, H, S (×200); A, C, E, G (×400)

Tissue-specific expression of SOD1 mRNA during embryogenesis

- (1) Liver: At ED 13.5, SOD1 mRNA was expressed higher in hepatic cells and megakaryocytes than in blood cells (Fig. 3A). At EDs 14.5–17.5, SOD1 mRNA expression was higher in megakaryocytes than hepatic cells. Also, the signal was detected in white blood cells from the sinusoid (data not shown). At ED 18.5, the signal was mainly observed in megakaryocytes and white blood cells in hepatic vein and sinusoids (Fig. 3B).
- (2) Kidney: In developing kidneys, SOD1 mRNA showed a high expression in metanephric vesicles and tubules and mesenchyme at ED 13.5 (Fig. 3C). At ED 14.5, SOD1 mRNA was expressed in mesenchyme, tubular lumens, and Bowman's capsules (data not shown). At ED 17.5, SOD1 mRNA was greatly expressed in tubular epithelium of renal cortex, while it was weakly expressed in the medullary mesenchyme and surface epithelium of renal pelvis (Fig. 3D).
- (3) Lung: In developing lung tissues, SOD1 mRNA was greatly expressed in the bronchial epithelium and mesenchyme at ED 13.5 (Fig. 3E). At ED 14.5, SOD1 mRNA was weakly expressed in surface of bronchial epithelium, while it showed a high expression in mesenchyme. At ED 15.5, SOD1 mRNA was mainly detected in mesenchyme in the vicinity of bronchus (data not shown). After ED 17.5, SOD1 mRNA was expressed in alveolar epithelial cells and mesenchyme (Fig. 3F).
- (4) Pancreas: At ED 13.5, SOD1 mRNA was greatly expressed in parenchyma of pancreas, pancreatic ducts, and mesenchyme (Fig. 3G). At EDs 16.5–18.5, SOD1 mRNA showed mainly in islet of pancreas and connective tissue (Fig. 3H).
- (5) Sensory organs: In developing eyes, SOD1 mRNA was expressed higher in surface epithelium and cornea than lens fibers, pigment layer, and inner and outer nuclear layer of retina, and lacrimal gland at EDs 15.5–17.5 (Fig. 3I). SOD1 mRNA was strongly expressed in the epithelia of cochlear and vestibular membranes of inner ear at EDs 15.5–17.5 (Fig. 3J). SOD1 mRNA was expressed in the respiratory and olfactory epithelia at EDs 13.5–18.5 (Fig. 3K). The signal was expressed in basal cells, sustentacular cells, and Bowman's glands in the olfactory epithelium of nasal conchae (Fig. 3L).
- (6) Gastrointestinal tract: In stomach, SOD1 mRNA was expressed in epithelium and submucosal gland (Fig. 3M). Especially, the signal was greatly observed in the surface epithelium of cardiac part (Fig. 3N). In intestines, SOD1 mRNA was detected in the tubular epithelium and mesenchyme at ED 13.5 (Fig. 3O) and intestinal villi and surrounding muscle layer at ED 17.5 (Fig. 3P).
- (7) Skin: SOD1 mRNA was significantly expressed in the keratinized surface epithelium and hair follicles at EDs 13.5–18.5 (Fig. 3Q and R).
- (8) Submandibular gland, thymus, and hypophysis: SOD1 mRNA was strongly expressed in secretory acini and ducts of submandibular gland at EDs 13.5–17.5 (Fig. 3S). In thymus, SOD1 mRNA was expressed in thymic lobules and surrounding mesenchyme (Fig. 3T). SOD1 mRNA was observed greatly in pituitary glandular cells including Rathke's pouch and surrounding connective tissue at ED 16.5 (Fig. 3U).
- (9) Brain: During brain development, SOD1 mRNA was strongly expressed at EDs 13.5–18.5. Especially, it was observed remarkably in pyramidal cells, cortical plate and ventricular zone of cerebrum (Fig. 3W and X), and Purkinje cells and external granular layer of cerebellar primordium (Fig. 3V). The signal was greatly expressed epithelial cells in choroid plexus, spinal cord, and ganglia (Fig. 2I).

Discussion

ROS are produced consistently by metabolic reaction in cells. A finite amount of ROS is generated under the normal physiological conditions, they exist during organogenesis, and their regulation is important for maintaining viability and normal cell function (Fantel 1996). However, high amounts of ROS have been produced by high oxygen treatment during development of mouse pronuclear stage embryos and could induce the developmental blockage of embryo (Goto et al. 1993). Furthermore, high levels of ROS have been implicated as a causative agent in the pathologies of a number of teratogens, including ethanol, cocaine, thalidomide, and diabetes (Fantel et al. 1992; Chen and Sulik 1996; Parman et al. 1999; Cederberg et al. 2001). On the other hand, SOD1 encoded on human chromosome 21 is pathogenetically relevant to several disease states including Down syndrome (trisomy 21) (Gulesserian et al. 2001). Reymond et al. (2002) reported the expression patterns of all identifiable murine orthologues of human chromosome 21 genes by RNA in situ hybridization on whole mounts and tissue sections of mouse embryos. However, the exact expression profile of SOD1 gene during embryogenesis has been unknown yet.

The gene expression of antioxidant enzymes can be stimulated by oxidative stress (Allen and Tresini 2000). Fetal tissues have the capacity to form ROS at early ED 12

and significant regional and temporal differences in the rates of formation of individual ROS occur in rat embryos (Fantel et al. 1998). In *Drosophila* embryogenesis, the level of SOD1 rose rapidly, peaking in mid-embryogenesis, and then fell gradually in late embryogenesis (Radyuk et al. 2004). Recently, we demonstrated that the cGPx mRNA was expressed throughout all the embryonic stages of mouse (Baek et al. 2005). In this quantitative real-time RT-PCR analysis, SOD1 transcript was detected throughout all embryonic stages from ED 7.5 to ED 18.5. Moreover, the signal showed the weakest level at ED 12.5, but the highest level at ED 15.5. These findings suggest that SOD1 expression may be changed according to organogenic stages of embryos.

In the present study, SOD1 mRNA was expressed in chorion, allantois, and amnion at ED 7.5. After implantation, rodent embryos get oxygen and nutrients by diffusion through chorion, allantois, and amnion (Carney et al. 2004). Therefore, the expression of SOD1 mRNA in the chorion, allantois, and amnion is consistent with the hypothesis that SOD1 functions to protect the embryo from ROS generated during nutrient transport as well as the contribution to normal organogenesis of embryos.

Previous studies found that $O_2^{\cdot-}$ formation is the most sensitive in developing limb and brain of fetus (Fantel et al. 1995). Neurons in the brain consume high amounts of oxygen, thereby constantly submitted to oxidative stress. SOD1 is mainly found in neuronal cells (Peluffo et al. 2005). The brain has been characterized by comparatively high SOD activity during mouse embryogenesis (Hussain et al. 1995). In the present study, SOD1 mRNA was highly expressed in developing brains and spinal cord during embryogenesis. These findings indicate that SOD1 may play an important part in the development of CNS as a free radical scavenger.

In vivo cotreatment with SOD/catalase mimetic EUK-134 resulted in diminished apical ectodermal ridge cell death and parallel reductions in the incidence and severity of limb defects (Chen et al. 2004). The forelimb buds appear at ED 9, while the hindlimb buds appear at ED10 (Roberts 1990). In this study, SOD1 mRNA was greatly expressed in forelimbs of EDs 9.5–10.5 and hindlimbs of EDs 10.5–11.5. These findings indicate that SOD1 may be intimately related to the development and differentiation of limbs during embryogenesis.

Surai (1999) reported that in liver, the specific activity of SOD increased between days 10 and 11 of development, then significantly decreased up to day 15 and remained at the same value during the rest of the developmental period. However, Danchenko and Kalytka (2002) proposed that the activity of basic antioxidant enzymes (SOD, catalase and GPx) in liver appears already at early stages of embryogenesis and is considerably enlarged in the end of embryogenesis. In the present study, SOD1 mRNA in liver

showed a weak expression in embryos of EDs 9.5–10.5, but a high expression in embryos of EDs 11.5–12.5. After ED 13.5, the expression began to decrease in liver and was observed weakly in the liver of ED 18.5. On the other hand, SOD1 mRNA was strongly expressed in megakaryocytes in fetal liver. Megakaryocytes are highly specialized precursor cells that differentiate to produce blood platelets (Italiano and Jr Shivdasani 2003). Platelets have been shown to exert both inhibitory and stimulatory effects on superoxide anion (Ward et al. 1988).

SOD1 is observed in the cochlear tissues (Rarey and Yao 1996). Targeted deletion of SOD1 is highly susceptible to noise-induced hearing loss (Ohlemiller et al. 1999). In this study, SOD1 mRNA was strongly expressed in the epithelium of cochlear and vestibular membranes of inner ear at EDs 15.5–17.5. Multiple SOD isoenzymes are present in human eyes and SOD1 is the most abundant SOD in the lens (Noor et al. 2002). As shown in Fig. 3I, SOD1 mRNA was also detected in cornea, lens epithelium, and pigment and sensory layers of retina during fetal period. Immunoreactivity for both SOD1 and SOD2 is localized in nasal mucosa and vomeronasal organ (Kulkarni-Narla et al. 1997). In our study, the signal was detected in basal cells, sustentacular cells, and Bowman's glands in olfactory epithelium at EDs 17.5–18.5. These findings suggest that SOD1 would be a protecting outer barrier in eye, ear, and nasal tissues against ROS.

After the ubiquitous expression noted in early and mid-embryos of *Drosophila*, the SOD1 expression became localized to digestive, excretory, and peripheral nervous tissues, as well as endocrine glands and oenocytes (subcuticular abdominal cells) (Ferveur et al. 1997). In this study, the expression of SOD1 mRNA was observed in the CNS, sensory and endocrine organs, gut, liver, limb, heart, skin, and genital eminence during embryogenesis. These findings suggest that the spatio-temporal differences in the expression level of SOD1, which may play major antioxidant roles in cellular differentiation and growth regulatory signals, arise as a result of the occurrence of metabolic gradients during development. SOD1 in embryonic tissues may reflect its participation in the use of certain essential material during periods of extensive growth and differentiation as in the developing mouse embryo.

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