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Selenium-binding protein 1: Its physiological function, dependence on aryl hydrocarbon receptors, and role in wasting syndrome by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

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ABSTRACT

Background: Selenium-binding protein 1 (Selenbp1) is suggested to play a role in tumor suppression, and may be involved in the toxicity produced by dioxin, an activator of aryl hydrocarbon receptors (AhR). However, the mechanism or likelihood is largely unknown because of the limited information available about the physiological role of Selenbp1.
Methods: To address this issue, we generated Selenbp1-null [Selenbp1 (−/−)] mice, and examined the toxic effect of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in this mouse model.
Results: Selenbp1 (−/−) mice exhibited only a few differences from wild-type mice in their apparent phenotypes. However, a DNA microarray experiment showed that many genes including Notch1 and Cdk1, which are known to be enhanced in ovarian carcinoma, are also increased in the ovaries of Selenbp1 (−/−) mice. Based on the different responses to TCDD between C57BL/6J and DBA/2J strains of mice, the expression of Selenbp1 is suggested to be under the control of AhR. However, wasting syndrome by TCDD occurred equally in Selenbp1 (−/−) and (+/+) mice.
Conclusions: The above pieces of evidence suggest that 1) Selenbp1 suppresses the expression of tumor-promoting genes although a reduction in Selenbp1 alone is not very serious as far as the animals are concerned; and 2) Selenbp1 induction by TCDD is neither a pre-requisite for toxicity nor a protective response for combating TCDD toxicity.
General significance: Selenbp1 (−/−) mice exhibit little difference in their apparent phenotype and responsiveness to dioxin compared with the wild-type. This may be due to the compensation of Selenbp1 function by a closely-related protein, Selenbp2.

1. Introduction

Selenium-binding protein 1 (Selenbp1) is a cytosolic protein which was partially characterized in 1989 [1]. Of the selenium-containing proteins reported so far, Selenbp is distinct from others in its mode of selenium binding. For example, while glutathione peroxidase (GPx), thioredoxin reductase and other enzymes are selenocysteine-containing proteins [2,3] (see also a review [4]), Selenbp1 is assumed to associate with selenium in a different manner [5]. A number of studies have suggested a tumor-suppressive role for Selenbp1. This is based on the fact that the expression of Selenbp1 is reduced in tumor tissues in the lung [6], colon [7,8], esophagus [9], ovary [10,11] and liver [12]. Conversely, elevated expression of Selenbp1 has been demonstrated in the brain of

Abbreviations: AhR, aryl hydrocarbon receptor; ALT, alanine aminotransferase; AST, aspartate aminotransferase; bp, base pair; Cyp, cytochrome P450; GPx, glutathione peroxidase; RT-PCR, reverse transcription-polymerase chain reaction; Selenbp, selenium-binding protein; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TBARS, thiobarbituric acid-reactive substances

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schizophrenic patients [13]. Selenbp1 may also have a role in anti-aging [14,15], cell expansion [16], modification of oxidative stress status [17,18], and protein transportation or degradation in cells [19,20]. Selenbp2 is highly homologous with Selenbp1 (the homology of the amino acid sequence is ca 98%) [21]. Although Selenbp2 has been identified as a protein targeted by reactive metabolite of acetaminophen [22,23], a later study showed that the acetaminophen reactive metabolite binds to Selenbp1 as well as Selenbp2 [24]. These selenoproteins are also modified by covalent binding with electrophilic metabolites formed from 3-methylindole, bromobenzene, naphthalene, and 2,6-di-*tert*-butyl-4-methylphenol (BHT) [25–28]. Thus, the covalent modification of Selenbp1/2 by chemicals is assumed to be a possible mechanism associated with their toxicity. In spite of these studies, the physiological functions of Selenbp1 and 2 remain largely unknown. Thus, one of the objectives of the present study focused on this issue, and we generated Selenbp1 gene-deficient [Selenbp1 (−/−)] mice to examine their phenotypes.

In our previous study, we observed that Selenbp1 was induced by 3,3′,4,4′,5-pentachlorobiphenyl, one of the toxic dioxins, in rats [29]. However, because the physiological significance of Selenbp1 remains obscure, the relevance of the above induction to toxicity is also unknown. In general, many forms of dioxin toxicity are believed to occur via activation of aryl hydrocarbon receptors (AhR), a transcription factor [30,31]. AhR is activated upon dioxin binding to alter the expression of more than 200 genes, and among the changes in gene expression are thought to seriously affect cellular function [32,33]. Although many more studies are needed to identify the genes responsible for dioxin toxicity, if Selenbp1 is one of the genes governed by AhR, Selenbp1 would be a candidate. However, it has not yet been clarified whether AhR is involved in the regulation of Selenbp1 expression. To address this issue, the present study examined Selenbp1 induction by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) using different strains of mice expressing AhR with higher (C57BL/6J) and lower (DBA/2J) affinity for TCDD [34,35]. If Selenbp1 induction is pre-requisite for the occurrence of dioxin toxicity, Selenbp1-null mice should resist TCDD toxicity. This study also examined this hypothesis.

2. Materials and methods

2.1. Chemicals

TCDD was obtained from AccuStandard, Inc. (New Haven, CT). 3-Methylcholanthrene (3MC) and 2-thiobarbituric acid were purchased from Sigma-Aldrich (St. Louis, MO). The other reagents were of the highest grade commercially available.

2.2. Animals and treatments

All experiments were approved by the Institutional Animal Care and Experiment Committee of Kyushu University. Male mice (3 weeks-old) of C57BL/6J and DBA/2J strains were obtained from CLEA Japan (Tokyo, Japan). They were fed on a standard chow (CE-2; CLEA Japan, Tokyo, Japan), allowed sterilized water *ad libitum*, and kept in an environmentally-controlled room maintained at 22 ± 5 °C and 50 ± 15% relative humidity under a 24 h light/dark cycle (light period, 7:00 AM–7:00 PM). After acclimatization for 1 week, they were given a single intraperitoneal injection of TCDD dissolved in corn oil (10 ml/kg). The dose of TCDD is described in the Results section. Control mice were given corn oil alone. The weight of tissues was recorded 5 days after the treatment, and the liver was subjected to mRNA analysis. In a separate experiment, mice were injected intraperitoneally with 3MC at a dose of 10, 50 or 100 mg/kg/10 ml corn oil for 3 consecutive days. The tissues were removed 24 h after the last treatment, weighed and subjected to mRNA analysis.

2.3. Selenbp1-KO mice

For disrupting the Selenbp1 gene, the region from the middle of the 2nd exon to the middle of the 12th exon was replaced with a neomycin-resistant gene cassette designated as 'Neo' by homologous recombination (Supplementary Fig. 1). A 3.0 kbp short arm, which spans the middle of intron 1 and a part of exon 2, was obtained by polymerase chain reaction (PCR) amplification using a C57BL/6 mouse bacterial artificial chromosome (BAC) clone (ID: RP23-431C22 or RP23-63K4; Invitrogen Corp., Carlsbad, CA) as a template and a primer pair into each of which Sac II and Not I restriction sites were introduced (Sac II–Not I fragment). The restriction sites of Sac II and Not I were introduced into the 5′-end of the forward primer and the 3′-end of the reverse primer, respectively. Similarly, a 7.5 kbp long arm covering a part of exon 12 and its downstream region was obtained by PCR with a primer pair attached to Cla I and Sal I restriction sites at the 5′- and 3′-ends of the forward and reverse primers, respectively (Cla I–Sal I fragment). The 3.0 kbp short arm (Sac II–Not I fragment) was cloned into Neo-connecting pBS vector (pBS-Neo vector, UNITECH Ltd., Chiba, Japan) as Neo was located at the 3′-flanking region of short arm (Construct 1), and the 7.5 kbp long arm was cloned into Cla I and Sal I sites of pBluescriptII SK(+) vector and termed pBSIISK + long. Construct 1 was inserted into pBSIISK + long vector at the digestion sites of Sac II and Cla I (Construct 2), and then the Diphtheria Toxin-A fragment (DTA) from the pBS-DTA vector (UNITECH Ltd., Chiba, Japan) was inserted into the Construct 2 to generate a targeting vector. This was injected into C57BL/6 embryonic stem cells. After G418 selection, the genomic DNAs from 3 surviving clones were digested with BamHI or Acc65 I to identify a correct homologous recombination by targeting vector. For example, homologous recombination at the 5′-side was confirmed by southern blotting using the genomic DNA fragments digested with BamHI and a '5′-probe' (479 bp, see the Supplementary Fig. 1 for the approximate location). Similarly, homologous recombination at the 3′-side and insertion of the Neo gene were checked using Acc65 I-digested genomic DNA, and a '3′-probe' (469 bp) and a 'Neo-probe' (668 bp). Two correctly-targeted clones selected by the above genotyping were injected into BALB/c blastocysts and these were transferred to the uteri of pseudopregnant ICR females. Both parents gave chimeric mice. Three males of chimeric progeny with black hair were mated with wild type C57BL/6 females (male: female = 1: 2) for germline transmission of the Selenbp1-target allele in the C57BL/6. After identification of the germline by the hair color and PCR, heterozygous F1 mice were inbred to generate F2 progeny. The deletion of the Selenbp1 gene in F2 progeny was screened by PCR (see the next section).

2.4. Genotyping

Litters from Selenbp1 (+/−) dams were examined for their genotypes when they were 3 weeks-old. Genomic DNA was extracted from the tail end (ca 5 mm) or a similar size of cut ear and diagnosed, using a KAPA mouse genotyping kit (KAPA Biosystems, Inc., Woburn, MA). The genotype in terms of Selenbp1 gene was determined by amplifying the diagnostic regions of the DNA by PCR. For detecting the wild-type allele, an 829 bp region spanning the 5th and 7th introns was amplified, using the following primers: forward, 5′-TTGTTTCCC ATCCACTGTCA-3′; and reverse, 5′-CATAGCTGAGTGTTGGGGGT-3′. For detecting the disrupted allele, a 668 bp region located within the neomycin-resistant gene was amplified using the following primers: forward, 5′-GAACAAGATGGATTGCACGCAGGTTCTCCG-3′; and reverse, 5′-GTAGCCAACGCTATGTCCTGATAG-3′. Genomic DNA solution (2 μl) was subjected to PCR (final vol., 10 μl) using the following conditions: 95 °C for 3 min, 30 cycles of amplifying step (95 °C for 15 s–58 °C for 15 s–72 °C for 15 s), and 72 °C for 3 min. The reaction solution (5 μl) was subjected to agarose gel electrophoresis to determine the size of the amplified DNA.