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Arsenite sensitizes to ferroptosis by disrupting selenium metabolism and reducing GPx4 expression

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ABSTRACT

Arsenic (As), an environmental toxicant commonly found in groundwater, exerts its toxic effects primarily through oxidative stress. Selenium (Se) plays a crucial role in counteracting oxidative stress by promoting the synthesis of Se-containing antioxidant enzymes, such as glutathione peroxidases (GPx). To elucidate the impact of As on cellular Se metabolism, we investigated the effects of inorganic arsenic on cultured cells (HT-1080, Jurkat, and SH-SY5Y). Our findings indicate that As(III) disrupts Se metabolism and inhibits Se-induced GPx expression. By comparing different Se sources (selenoprotein P, selenocysteine, and selenite), we determined that As(III) primarily interferes with Se metabolism downstream of selenite, an inorganic form of Se. Notably, exposure to As(III) reduced Se incorporation into RNA, suggesting inhibition of Sec-tRNA^{Sec} synthesis, a critical step in selenoprotein biosynthesis. Additionally, As(III) increased cellular susceptibility to ferroptosis, a form of oxidative stress-driven lipid peroxidation-mediated cell death primarily regulated by GPx4. Supporting this, genetic deletion of PRDX6, a recently identified regulator of cellular Se metabolism, further suppressed selenoprotein expression and exacerbated As(III)-induced ferroptosis. These findings provide new insights into the toxicological mechanisms of As compounds, highlighting their role in disrupting Se metabolism and potentially mitigating the side effects associated with arsenic-based anticancer therapies.

1. Introduction

Arsenic (As) contamination in groundwater, especially in Asia, has become a worldwide health issue (Podgorski and Berg, 2020). An estimated 94–200 million people are potentially exposed to high levels of As-containing groundwater (Podgorski and Berg, 2020). Groundwater contains mainly pentavalent and trivalent arsenic (As(V) (arsenate) and As(III) (arsenite)). Among these, As(III) is the most toxic, and chronic exposure leads to adverse health effects, including keratinization and skin cancer (Rahman and Wu, 2025; Tao and Wang, 2024). However, arsenic has also been used medicinally for centuries, such as in the treatment of syphilis, and is currently employed as an anticancer agent. Despite its therapeutic potential, arsenic-based treatments are associated with significant side effects, including cardiotoxicity. Oxidative stress is recognized as a key factor in arsenic toxicity; however, the precise mechanisms underlying its diverse toxic effects remain unclear,

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as arsenic interacts with multiple cellular proteins and signaling pathways.

Selenium (Se) is an essential trace element that plays a crucial role in maintaining redox homeostasis and protecting against oxidative stress. In biological systems, Se is incorporated as selenocysteine (Sec) into selenoproteins, a family of 25 proteins in humans (Kryukov et al. 2003). The primary dietary source of Se is plant-derived selenomethionine (SeMet), which undergoes desulfurization to release inorganic Se within cells (Hu et al. 2021). Another key source is Sec, which is metabolized by selenocysteine lyase (Scly) to release inorganic Se (Mihara et al. 2000). Additionally, selenoprotein P (SeP), the most abundant selenoprotein in human plasma, serves as a crucial Se transporter between organs (Saito, 2021; Saito et al. 2004; Saito and Takahashi, 2002). SeP, which contains 10 Sec residues, is predominantly synthesized in the liver and secreted into the bloodstream. It is taken up by cells via the apolipoprotein E receptor 2 (ApoER2; also known as LRP8) and degraded in lysosomes to release Sec (Mizuno et al. 2023). The inorganic Se derived these sources is subsequently converted to selenophosphate ($Se\text{-}PO_3^{2-}$) by selenophosphate synthase 2 (SPS2). This selenophosphate is then used by O-phosphoseryl-tRNA(Sec) selenium transferase (SepSecS) to synthesize selenocysteinyl-tRNA ($Sec\text{-}tRNA^{Sec}$) (Manta et al. 2022). $Sec\text{-}tRNA^{Sec}$ enables the incorporation of Sec at UGA codons in selenoprotein transcripts through a stem-loop structure known as the selenocysteine insertion sequence (SECIS) in the 3′ untranslated region.

Among selenoproteins, glutathione peroxidases (GPx) play essential roles in maintaining intracellular redox homeostasis and protecting against oxidative stress induced by environmental metals such as methylmercury, cadmium, and arsenic (Branco et al. 2012; Yao et al. 2015). A particularly important function of selenoproteins is the regulation of ferroptosis, a form of cell death characterized by iron-dependent lipid peroxidation. Ferroptosis has gained significant attention due to its implications in various diseases, and its relationship with environmental metals is actively being investigated (Mishima and Conrad, 2022). Notably, glutathione peroxidase 4 (GPx4) is a key regulator of ferroptosis, as it directly reduces harmful lipid hydroperoxides. The cellular expression of GPx4 is tightly linked to Se metabolism, as Se availability dictates its synthesis. For instance, we previously reported that selenium levels in fetal bovine serum influence ferroptosis susceptibility by modulating GPx4 expression in cultured cells (Takashima et al. 2024). Recently, our group and others identified peroxiredoxin 6 (PRDX6) as a novel regulator of Se metabolism, acting as a cellular selenium acceptor protein that is essential for efficient cellular Se utilization (Chen et al. 2024; Fujita et al. 2024; Ito et al. 2024). Therefore, PRDX6 is currently gaining attention as a key ferroptosis regulator and a potential molecular target for drug development.

Several studies have suggested the link between ferroptosis and As (III) toxicity (Dong et al. 2024; Xiao et al. 2021). For example, the toxicity of arsenic trioxide (ATO; an As(III)-releasing molecule), a chemotherapeutic agent used to treat leukemia, has been implicated in relevant to ferroptosis in cultured cancer cells (Chen et al. 2023; Jiang et al. 2023), and is reported to transcriptionally repress GPx4 (Su et al. 2024). However, the precise molecular mechanisms linking As exposure to ferroptosis remain unclear. Previously, we demonstrated that certain dietary electrophiles covalently modify SeP, inhibiting its Se transport activity and affecting oxidative stress responses (Ye et al. 2023; Ye et al. 2025). Given that As is also an electrophile, it may similarly interfere with Se metabolism. Since Se metabolism is closely associated with ferroptosis, we hypothesized that As exposure alters ferroptosis susceptibility by disrupting cellular Se metabolism. In this study, we aimed to elucidate the effects of As on Se metabolism, including its interaction with PRDX6, and to investigate its role in arsenic-induced toxicity.

2. Materials and methods

2.1. Resources and reagents

SH-SY5Y was obtained from KAC (EC94030304-F0; Kyoto, Japan). Jurkat cells were obtained from Cell Resource Center for Biomedical Research Cell Bank, Tohoku University (TKG0209). HT-1080 was obtained from ATCC (CCL-121). *PRDX6* knockout (KO) HT-1080 were established in the previous study (Ito et al. 2024). Purified selenoprotein P was prepared according to the previously described method (Saito et al. 1999). Recombinant PRDX6 was prepared as previously reported (Ito et al. 2024). As(III) was purchased from WAKO pure chemical (191–01241, Osaka, Japan). As(V) was purchased from Sigma (A-6756, SA, USA)

2.2. Cell culture

Cells were cultured in DMEM (08458–16; Nacalai Tesque, Kyoto, Japan) supplemented with 10 % (v/v) FBS (Sigma, Lot. BCCC5944; containing 8.3 ppb Se) and 1 % (v/v) penicillin-streptomycin mixed solution. Cultures were maintained in a humidified incubator at 37°C with 5 % CO_2. For PRDX6 KO HT-1080 cells, the culture medium was supplemented with 20 nM sodium selenite to support cell maintenance, because these cells are unable to grow under our experimental condition using FBS with limited selenium content (Takashima et al. 2024). Therefore, experiments with PRDX6 KO cells were conducted in a condition with a base level of 20 nM selenite, which is a minimal concentration that support same growth compared with WT cells. In the experiments shown in Figs. 3 and 4, HT-1080 WT and PRDX6 KO cells were cultured with a 10 nM selenite under basal conditions. This condition was set at 10 nM as the minimum concentration of selenite that would not affect the experiment (not involved in the inhibition of ferroptosis by Se itself). Cells were seeded onto appropriate culture plates 24 h before the experiments.

2.3. SDS-PAGE and Western blotting

After the stimulus, the cells were washed by PBS and harvested by lysis buffer (0.05 M Tris-HCL (pH6.8) and 2 % SDS), then incubated for 10 min at 95°C. Protein concentration was determined using the DC Protein Assay Kit (Bio-Rad, Hercules, CA, USA). An aliquot of the protein was loaded onto a sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel, and subsequently transferred to a PVDF membrane (Immobilon, Merck Millipore, Darmstadt, Germany). The membrane was incubated with primary antibodies (diluted 1:2000–4000 in CanGet Solution1, Toyobo, Osaka, Japan), followed by secondary antibodies (diluted 1:10,000 in CanGet Solution2, Toyobo) for 1 h at room temperature. The membrane was washed with TTBS (50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05 % Tween 20), treated with ImmunoStar LD (Wako Pure Chemical, Osaka, Japan), and detected using a LuminoGraph I (ATTO, Saitama, Japan). The following antibodies were used: anti-GPx1 antibody (ab108427), anti-GPx4 antibody (ab125066), anti-SPS2 antibody (ab96541), and anti-PRDX6 antibody (ab59543) from Abcam (Cambridge, UK); anti-GAPDH antibody from Wako (015–25473, Osaka, Japan); and the anti-SeP antibody, which was previously established by our group (Mita et al. 2017).

2.4. Determination of RNA-binding selenium by inductively coupled plasma mass spectrometry (ICP/MS)

RNA was extracted using Isogen II (Wako) according to the manufacturer's instructions. Briefly, the cells were washed twice with PBS and added the aliquot of Isogen II (750 μL). After adding the RNase free water (300 μL), pellets (proteins) were removed by centrifugation. RNA containing supernatant was mixed with isopropanol and the pelleted RNA was washed with 70 % EtOH, then dissolved in 250 μL of 70 %

nitric acid (sg 1.42) (143–09741; Wako), and incubated at 50°C overnight for ashing. The sample was then diluted with 750 μL and subjected to ICP-MS (Agilent 8900 Triple Quadrupole ICP-MS, Agilent, Santa Clara, CA, USA). The ICP-MS analysis for selenium was performed in collision/reaction cell mode with He and O_2 gas to omit the interferences of Ar-Ar polyatomic ions to Selenium ions (monitoring the isotopes ^{78}Se and ^{80}Se). Standard curve for selenium solution (Se 1000) was from Kanto Kagaku (Tokyo, Japan). The internal standard solution, which contains Be, Y, In, Te, and Bi, was injected into the ICP-MS online with samples and corrected the value. Selenium content in RNA was quantified as total Se (ng/μg of RNA), with RNA concentration measured using a NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA).

2.5. Cell viability

After treatment of the cells in 96-well plate, the medium was removed and replaced with the medium containing 10 % alamarBlue Cell Viability Reagent (Thermo Fisher Scientific). After incubation at 37°C for 1–2 h, fluorescence (λ ex/em = 544/585 nm) was measured by SpectrMax iD5 (Molecular Devices, San Jose, CA, USA).

2.6. Production of lipid radicals

The cells were seeded on 6 well plate and stimulated with 100 μM of Trolox or 5 μM ofαT for 1 hr. And then, stimulated with 2.5 μM of As(III) for 12 hr.After that, Lipiradical Green (1 μM) (Funakoshi, Tokyo, Japan), a fluorescence probe for lipid radicals, were added. The cells were washed and collected with BSA buffer(1 mg/mL) and subjected to Flow cytometer (CytoFLEX, Beckman Coulter Life Sciences, Brea, CA, USA). Mean fluorescence intensity of cells was used for the quantification.

2.7. Statistical analysis

Band intensity was quantified by ImageJ 1.53i (National Institutes of Health, Bethesda, MD). Data analysis was performed by GraphPad Prism (ver10.2.2., San Diego, CA, USA). A multiple comparison test (one-way ANOVA, post hoc test Tukey's method) was used to detect significant differences. The significance between the two groups was determined by Student's *t*-test. Data are expressed as mean ± standard deviation (S.D.) and the difference was significant when the p-value was 5 % or less.

3. Results

3.1. Inhibition of selenium metabolism by arsenic and its underlying mechanism

To examine the effect of arsenic on selenium metabolism, we assessed the induction of GPx expression by selenium treatment in multiple cell lines (human sarcoma HT-1080, human T lymphocyte Jurkat, and human neuroblastoma SH-SY5Y). Treatment with selenite or selenocystine ($SeCys_2$) induced GPx1 and GPx4 expression, which are involved in intracellular redox regulation (Fig. 1A-C). GAPDH was used as an internal control. Co-treatment with As(III) (arsenite) and As(V) (arsenate) inhibited GPx induction in a concentration-dependent manner in HT-1080 cells (Fig. 1A). In the Jurkat and SH-SY5Y cells, As(III) also inhibited GPx induction, whereas As(V) showed low potency in inhibiting GPx induction at the same concentration (Fig. 1B, C). These findings suggest that As(III) especially inhibits the selenium metabolism involved in GPx induction and that the impact of As(V) depends on the cell species.

To address the underlying mechanism, we next examined whether As (III) enhances GPx degradation or inhibits its synthesis by altering the timing of selenium and As(III) treatment (Fig. 2A, upper panel). Since cells utilize organic selenium (i.e., SeP and $SeCys_2$) through different pathways than selenite, which is an inorganic form of selenium, we tested whether As(III) affects these organic selenium pathways as well. The results showed that iAs(III) inhibited the induction of GPx by all selenium sources and the most efficient inhibition of GPx induction was achieved when arsenic was administered first, followed by selenium treatment (Post-Se condition) or co-treatment of selenium and As(III) (Co-Se condition). Conversely, when GPx was induced by selenium prior to As(III) exposure (Pre-Se condition), the effect of As(III) was limited and it was detected if SeP was used as a selenium source. This result suggested that As(III) inhibits the *de novo* synthesis of GPx induced by

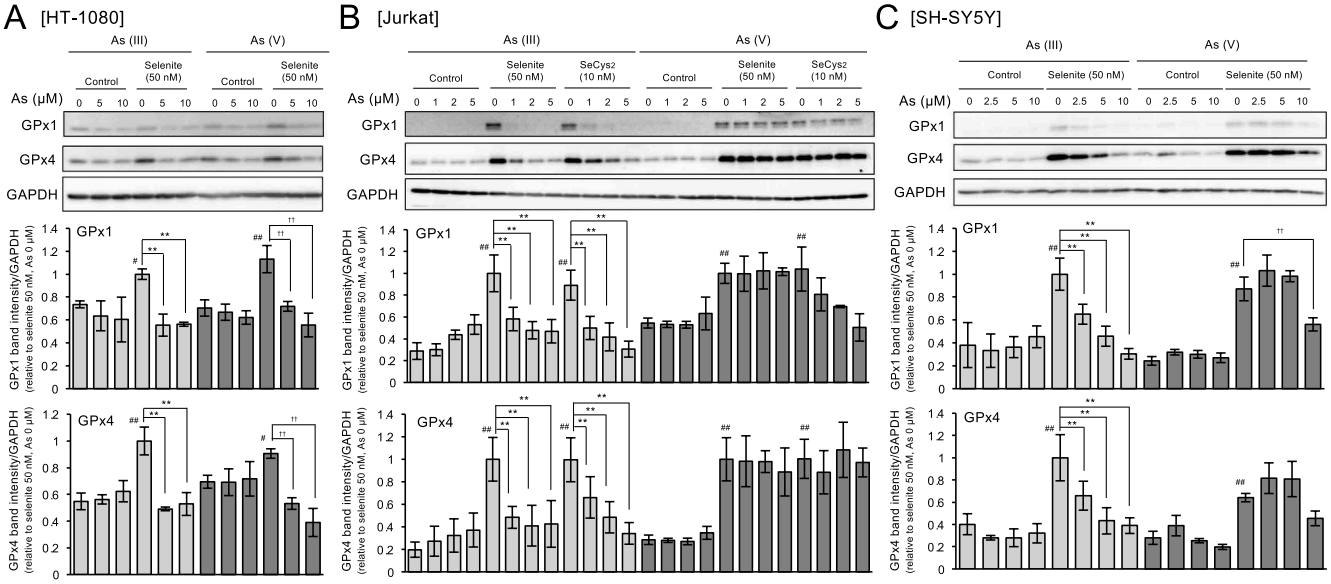

Fig. 1. Effect of arsenicals on glutathione peroxidases expression, which is induced by selenium. Cultured cells (A; HT-1080, B; Jurkat) were seeded and precultured for 24 hr. Then the cells were pre-treated with As(III) and As(V) for 4 hr and treated with selenite (50 nM) and selenocystine (10 nM) for 8 hr. C; SH-SY5Y cells precultured for 24 hr, and the cells were treated with As(III) and selenite (50 nM) for a further 24 hr. The cells were harvested and subjected to Western blotting and detected by indicated antibodies. The data were a representative image of n = 3 and the quantitative data were shown in lower panel. Data were shown as mean±S. D. Significance was determined by Tukey's multiple comparisons test, and shown as #(vs control "As 0 μM ", $p < 0.05$), ##(vs control "As 0 μM ", $p < 0.01$), **(vs selenite (50 nM) "As(III) 0 μM ", $p < 0.01$), and ††(vs selenite (50 nM) "As(V) 0 μM", $p < 0.01$).

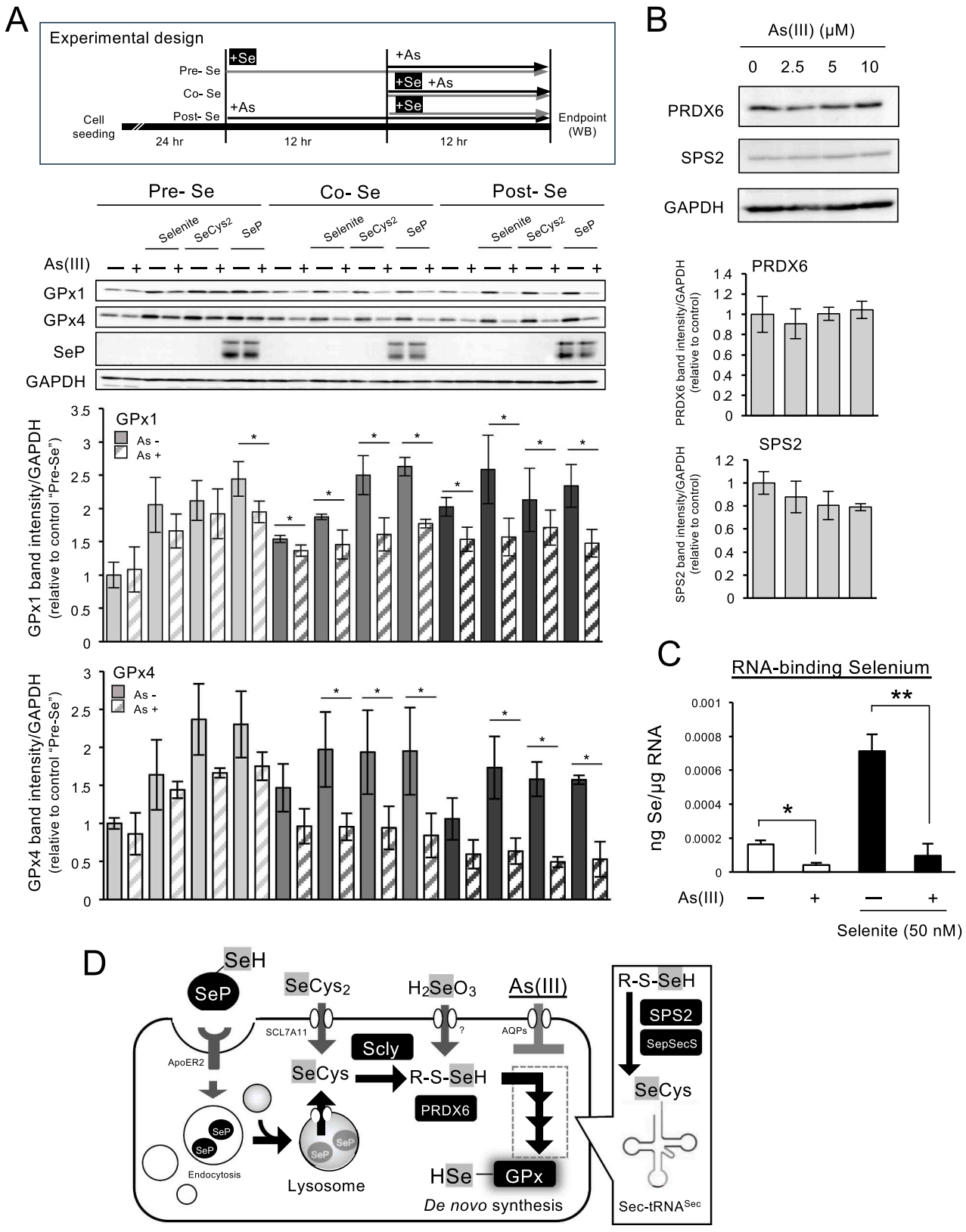

Fig. 2. Mechanism underlying the inhibition of Se metabolism by arsenite. (A) The upper panel indicates experimental design. HT-1080 cells were treated with selenite (50 nM), selenocystine; $SeCys_2$ (5 nM), SeP (0.5 μg/mL), and As(III) (5 μM) at the indicated time points (Pre-, Co-, Post-). The lower panel indicated the result of Western blotting at the endpoint. (B) The cells were treated with indicated concentration of As(III) for 24 hr and subjected to Western blotting. (A, B) The data were a representative image of n = 3 and the quantitative data were shown in lower panel. Data were shown as mean±S.D. Significance was determined by *t*-test, and shown as *p < 0.05 vs As(-). (C) HT-1080 cells were treated with 50 nM selenite with As(III) (2.5 μM) for 24 hr, and RNA-binding selenium was measured by ICP-MS. Mean±S.D., n = 3, *P < 0.05, **P < 0.01, Tukey's multiple comparisons test.

selenium, mainly (Fig. 2A, lower panel), and GPx induction by SeP and $SeCys_2$ was also inhibited by As(III) treatment, suggesting that As(III) disrupts cellular selenium metabolism after the generation of inorganic selenium, by interfering with the degradation of organic selenium. SeP incorporation into the cells was not altered by As(III) treatment at any exposure-timing (Fig. 2A).

Once organic selenium is converted into its inorganic form, or when inorganic selenium is taken up, it binds to the Cys47 residue in PRDX6 and is transferred to SPS2 and SepSecS for the synthesis of Sec-tRNASec. As(III) did not affect PRDX6 or SPS2 expression, which are responsible for selenium metabolism (Fig. 2B), but significantly reduced selenium binding to RNA. This suggests that As(III) inhibits Sec-tRNASec synthesis on tRNA by modulating the activity of SPS2 and/or SepSecS (Fig. 2C, D). However, this measurement was performed on total RNA, and it was difficult to isolate and detect selenium on tRNA due to sensitivity, so further improvements are needed and this is a limitation of this experiment.

3.2. Effect of arsenite on ferroptosis and the involvement of PRDX6 in arsenite-induced ferroptosis

Since decreased GPx4 expression contributes to cellular susceptibility to ferroptosis, we investigated the effect of As(III) on ferroptosis sensitivity. Treatment of HT-1080 cells with 1 μM As(III) had no effect on cell viability, but As(III) treatment increased sensitivity to ferroptosis induced by the GPx4 inhibitor RSL3 (Fig. 3A), indicating that As(III) enhances ferroptosis sensitivity.

PRDX6, a key factor in selenium metabolism, regulates ferroptosis sensitivity by maintaining the production of selenoproteins, including GPx4. PRDX6 knockout (KO) HT-1080 cells exhibited significantly increased susceptibility to As(III) toxicity (Fig. 3B), indicating that selenium metabolism plays a protective role in As(III) toxicity. In PRDX6 KO cells, basal GPx induction was lower, and selenite-induced GPx expression was more profoundly inhibited by As(III) compared to wild-type (WT) cells (Fig. 3C). Notably, the increased As(III) sensitivity observed in PRDX6 KO cells was rescued by an iron chelator, deferoxamine (DFO), and the ferroptosis inhibitor, ferrostatin-1 (Fer1) (Fig. 3D). Furthermore, since selenium-induced GPx4 suppresses ferroptosis, we verified its effect and found that arsenic toxicity was significantly reduced in PRDX6 KO cells by selenite (Supplemental figure 1). This inhibitory effect appeared to be dependent on the suppression of ferroptosis, as no additional suppression was observed with ferroptosis inhibitors. While it is challenging to draw definitive conclusions due to the confound between GPx4 expression induction inhibition and GPx4-mediated ferroptosis suppression, these factors are implicated in arsenic-induced ferroptosis. These results suggest that PRDX6 mitigates As(III)-induced ferroptosis by maintaining selenium metabolism, and supplementation of higher level of selenium to induce GPx4 in PRDX6 KO condition can rescue As(III) induced ferroptosis.

3.3. Mitigating arsenic toxicity in PRDX6 KO cells by vitamin E, a lipophilic antioxidant

Since our results demonstrated that As(III) increases ferroptosis susceptibility and disrupts selenium metabolism, we considered the possibility that decreased GPx4 expression by As(III) may be mediated by increased lipid radicals, a trigger of starting ferroptosis. To test this, we evaluated the effect of vitamin E (Trolox, a vitamin E derivative, and α-tocopherol; αT), a radical-scavenging antioxidant that prevents lipid peroxidation, on As(III)-induced inhibition of selenium metabolism. However, neither form of vitamin E treatment affected the reduction of GPx expression by As(III) (Fig. 4A).

In PRDX6 KO cells, vitamin E ameliorated arsenic-induced toxicity, indicating that it mitigates As(III) toxicity associated with ferroptosis (Fig. 4C). In contrast, vitamin E did not alleviate arsenic toxicity in WT cells (Fig. 4B), suggesting that its protective effect is specific to ferroptosis-prone conditions, such as in PRDX6 KO cells. Lipid radicals, which are involved in triggering ferroptosis, were increased by arsenite,

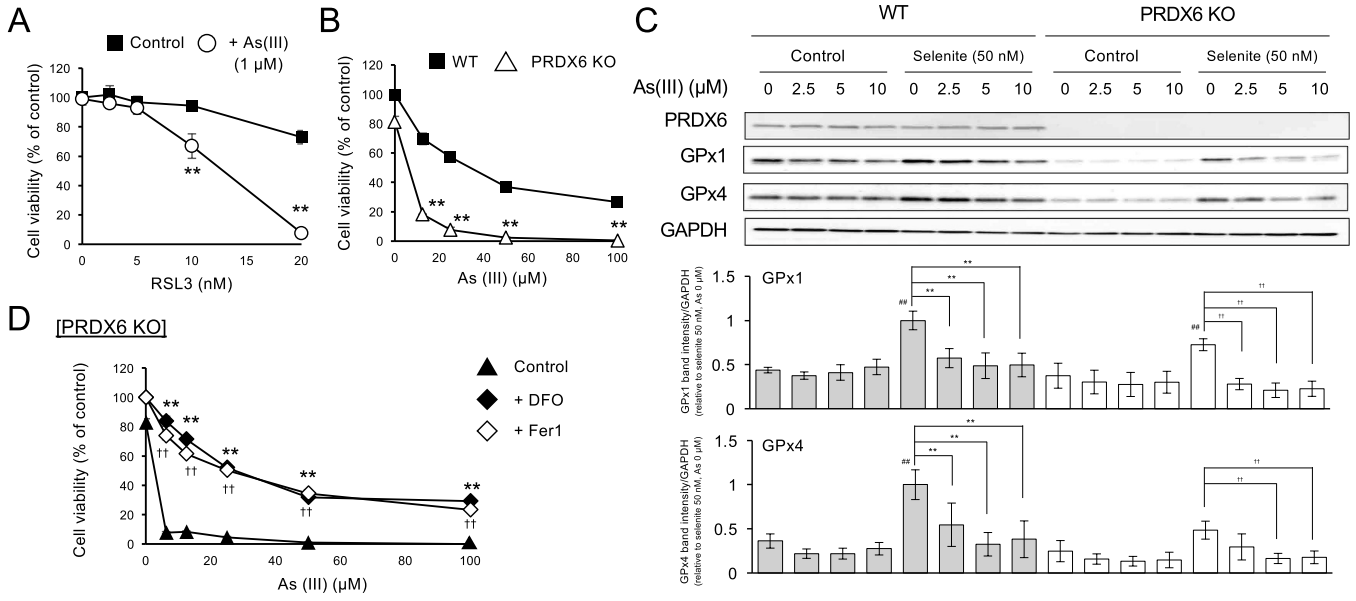

Fig. 3. Involvement of PRDX6 in arsenite-induced ferroptosis. (A) HT-1080 cells were seeded and precultured for 24 hr, then the cells were exposed to 1 μM of As(III) for 6 hr and treated with or without of indicated concentration of RSL3 for 18 hr. After that cell viability was measured. Mean±S.D., n = 3, *P < 0.05, **P < 0.01, Tukey's test was used. (B) HT-1080 WT or PRDX6 KO cells were treated with As(III) for 24 hr and cell viability was measured. Mean±S.D., n = 3, *P < 0.05, **P < 0.01, (C) HT-1080 WT and PRDX6 KO cells were treated with selenite and the indicated concentration of As(III) for 24 hr. Then the cells were collected and subjected to Western blotting. The data were a representative image of n = 3 and the quantitative data were shown in lower panel. Data were shown as mean±S.D. Significance was determined by Tukey's multiple comparisons test, and shown as ## (vs control "As(III) 0 μM ", p < 0.05), **(vs selenite (50 nM) "As(III) 0 μM ", p < 0.01), and ††(vs selenite (50 nM) "As(V) 0 μM", p < 0.01). (D) PRDX6 KO HT-1080 cells were pretreated with DFO and Fer1 for 1 hr and exposed to As(III). After 24 hr, cell viability was measured. Control data were shown as the means of multiple plates. Mean±S.D., n = 3 (DFO and Fer1), n = 6 (control). **P < 0.01 (Control vs DFO), ††P < 0.01 (control vs Fer1).

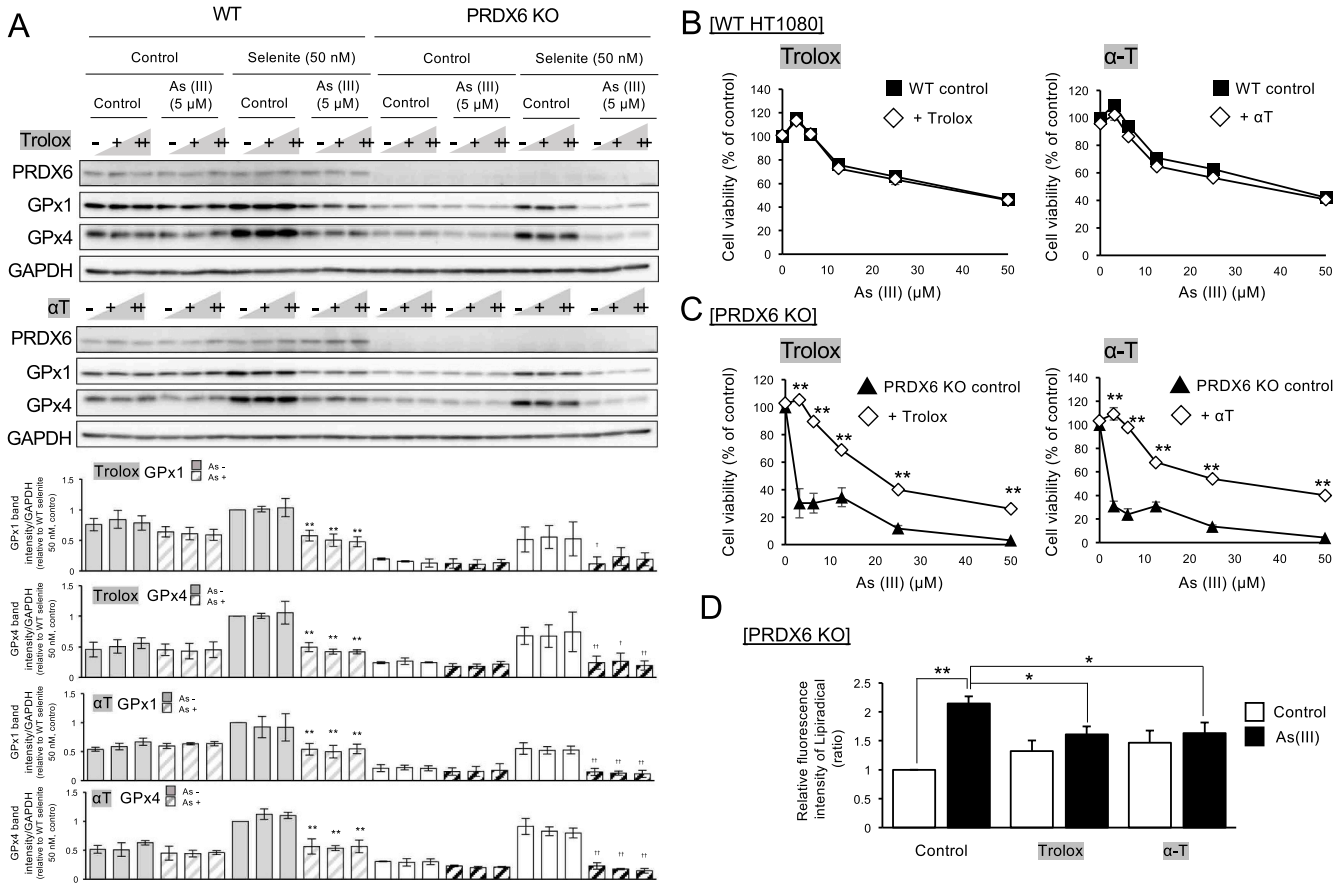

Fig. 4. Effect of vitamin E on Se-metabolism and arsenite-induced ferroptosis in PRDX6 KO cells. (A) HT-1080 (WT) or its PRDX6 KO cells were pretreated with Trolox (-; 0 μM +; 50 μM, ++; 100 μM) or αT (-; 0 μM +; 2.5 μM, ++; 5 μM) for 1 hr and exposed to As(III) (5 μM) for 4 hr, then added selenite (50 nM) for 8 hr. The cells were subjected to Western blotting. The data were a representative image of n = 3 and the quantitative data were shown in lower panel. Data were shown as mean±S.D. Significance was determined by Tukey's test, and shown as **(vs WT selenite (50 nM), $p < 0.01$), †(vs PRDX6 KO selenite (50 nM), $p < 0.05$) and ††(vs PRDX6 KO selenite (50 nM), $p < 0.01$). (B) HT-1080 cells were pretreated with Trolox (50 μM) or αT (2.5 μM) and exposed to the indicated concentration of As(III) for 24 hr. After that cell viability was measured. Mean±S.D., n = 3. **$P < 0.01$, Tukey's test was used. (C) PRDX6 KO cells were treated as same as (B). (D) The cells were pretreated with 100 μM of Trolox or 5 μM of αT for 1 hr, after that the cells were stimulated wiht 2.5 μM of As(III) for 12 hr, which is a sub-cytotoxic condition. Accumulation of lipid peroxide and effect of vitamin E were evaluated by Lipiradical and mean fluorescent value detected by flow cytometer was shown. Mean±S.D., n = 3. *$P < 0.05$, **$P < 0.01$, Tukey's test was used.

which was inhibited by Trolox and αT in PRDX6 KO cells (Fig. 4D). These findings imply that As(III) toxicity becomes more pronounced under impaired selenium metabolism conditions and can be counteracted by nutritional supplementation with antioxidants, such as vitamin E.

4. Discussion

This study demonstrates that As(III) reduces selenoprotein GPx expression by inhibiting cellular selenium utilization and decreasing the synthesis of Sec-tRNA^{Sec}. Through this mechanism, As(III) treatment sensitizes cells to ferroptosis and even induces ferroptosis in cells with impaired selenium utilization, such as PRDX6 KO cells. Our findings also indicate that decreased selenoprotein expression and increased ferroptosis sensitivity contribute to arsenic toxicity (Fig. 5).

Furthermore, our study revealed that As(III)-induced ferroptosis is exacerbated in PRDX6-deficient cells. Analysis of the TCGA database indicated that PRDX6 expression is significantly lower in cancerous tissues/cells from patients with acute myeloid leukemia (Supplemental Figure 3). Given that arsenic trioxide (ATO), an As(III) releasing molecule, is used for the treatment of acute myeloid leukemia (Lengfelder et al. 2012), further investigation into the relationship between PRDX6 expression and the therapeutic efficacy of ATO could be important. Our findings suggest that ATO may be more effective in tumors with low

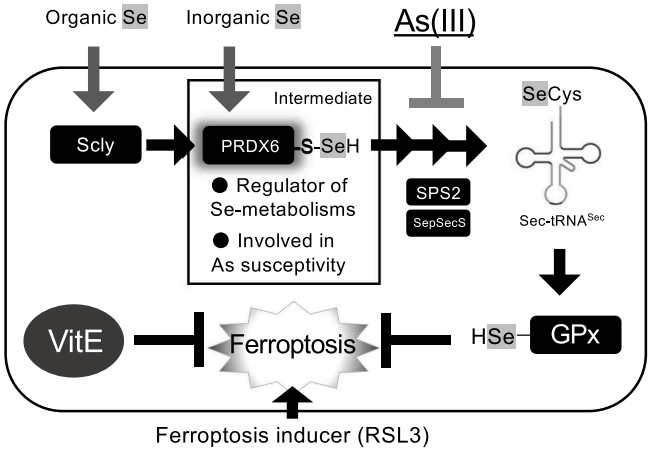

Fig. 5. A mechanism underlying the inhibition Se metabolism by arsenite. As (III) could inhibit Se-metabolism through inhibition of the production of mature Sec-tRNA^{Sec}. This enhances the susceptibility to ferroptosis. Deficiency of PRDX6 enhances this process. Vitamin E (VitE) protects against As(III)-induced ferroptosis instead of GPx.

PRDX6 expression. Conversely, PRDX6 expression was found to be

elevated in glioblastoma (Supplemental Figure 3), a cancer type in which we previously demonstrated that enhanced selenium metabolism contributes to ferroptosis resistance (Zheng et al. 2024). From this perspective, PRDX6 expression may serve as a potential biomarker of resistance to arsenic-based anticancer therapies. Future studies aimed at developing PRDX6-specific inhibitors could expand the therapeutic application of arsenic compounds in oncology, and some trials for the development of PRDX6 inhibitors were already reported (Chen et al. 2021; Inague et al. 2024). However, substances that inhibit PRDX6, as well as populations with low genetic expression of PRDX6, may increase susceptibility to arsenic toxicity in the environment.

Here, the study used various cell lines, including SH-SY5Y cells from neuroblastoma, Jurkat cells from leukemia, and HT-1080 cells from fibrosarcoma. Arsenite inhibited selenium metabolism in all cell lines, however the possibility that the action is cell-type-dependent cannot be ruled out, nor can its action in normal cells be determined. This point is a limitation of the study. In Fig. 1, each cell type has a different maximum GPx induction time with selenium and sensitivity to arsenic, so treatment conditions were optimized for each cell type to see inhibition of selenium metabolism. Therefore, the actual *in vivo* organ in which inhibition of selenium metabolism occurs would be expected to depend on selenium supply, arsenic accumulation, and the arsenic sensitivity of that organ. Future *in vivo* analysis of selenium metabolism inhibition is expected to clarify this point. It is also possible that arsenic inhibited selenium metabolism and caused ferroptosis due to selenium deficiency because the original selenium utilization was limited, and PRDX6-deficient cells, which die of selenium deficiency, were used. This suggests that arsenic toxicity is enhanced under some selenium deficient conditions.

A cross-sectional study of 292 primary school children in rural Vietnam reported that 75.6 % exhibited low serum selenium levels (Nhien et al. 2008). If arsenic exposure occurs in such selenium-deficient populations, the associated health risks could be exacerbated. Identifying potential epidemiological links between selenium deficiency and arsenic exposure could provide critical insights from an environmental health perspective.

Although GPx4 is known as the master regulator of ferroptosis, GPx1 is known to be implicated in the suppression of apoptosis (Faucher et al. 2005). In the present condition, enhanced toxicity of As(III) by PRDX6 KO was almost completely canceled by ferroptosis inhibitors (Figs. 3D and 4). These results suggest that the PRDX6-GPx4 axis is dominantly involved in ferroptosis in the cells. However, higher concentrations of As (III) caused WT cell death independent of ferroptosis, which indicates another type of cell death. Although the apoptotic pathway was not examined in this study, it is likely that this reported subtype of cell death is involved, which is independent of selenium metabolism. Again, there is a possibility of time-dependent changes in the subtype of cell death, which needs to be verified.

Despite these findings, the precise mechanism by which As(III) disrupts selenium metabolism remains unclear, representing a limitation of this study. Arsenic may, at least in part, affect the activity of SPS2 and SepSecS, and further investigation into these mechanisms will provide a more comprehensive understanding of how arsenic inhibits selenium metabolism. Our previous study reported that the dietary component sulforaphane forms adducts with SeP, thereby reducing its selenium utilization efficiency (Ye et al. 2025). To determine whether As(III) similarly forms adducts with SeP, we employed the biotin-$PEAC_5$ maleimide labeling method (BPML), which enables the detection of adduct formation at cysteine and selenocysteine residues (Toyama et al. 2013). However, BPML analysis revealed minimal binding of As(III) to SeP (Supplemental Figure 2 A). Additionally, As(III) treatment did not appear to interfere with SeP endocytosis, as SeP uptake was observed under arsenic exposure conditions (Fig. 2A). Previous studies have reported that arsenic induces peroxiredoxin expression via Nrf2 activation (Aono et al. 2003), while also inhibiting peroxiredoxin activity (Olahova et al. 2008). However, our findings indicate that As(III) does not affect PRDX6 expression, and BPML analysis showed no direct binding of As (III) to PRDX6 (Supplemental Figure 2B), suggesting that PRDX6 inhibition is unlikely to be responsible for the suppression of selenium metabolism by As(III). Considering that GPx induction by $SecCys_2$ was also inhibited, it is likely that As(III) disrupts selenium metabolism at a stage downstream of inorganic selenium formation within cells.

In summary, our results highlight the impact of As(III) exposure on selenium metabolism and its role in increasing ferroptosis sensitivity. Additionally, ferroptosis inhibitors, including vitamin E supplementation, may help mitigate arsenic toxicity. These findings have implications not only for understanding arsenic toxicity in environmental health but also for the development of potential arsenic-based anticancer therapies.

CRediT authorship contribution statement

Marcus Conrad: Resources, Methodology. **Daigo Sumi:** Writing – review & editing, Methodology. **Yoshiro Saito:** Writing – review & editing, Resources, Funding acquisition. **Tomioka Yoshihisa:** Resources, Methodology. **Junya Ito:** Resources, Methodology. **Hiroki Taguchi:** Investigation, Data curation. **Yasutoshi Akiyama:** Methodology. **Takenaka Yoshika:** Methodology, Data curation. **Reiko Makino:** Investigation, Data curation. **Hayato Takashima:** Investigation, Data curation. **Takashi Toyama:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Eikan Mishima:** Resources, Methodology.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Takashi Tyama reports financial support was provided by Japan Society for the Promotion of Science. Yoshiro Saito reports financial support was provided by japan society for the promotion of science. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tox.2026.154409.

Data availability

Data will be made available on request.

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