Basal levels of glutathione peroxidase correlate with onset of radiation induced lung disease in inbred mouse strains

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Abstract

Biomarkers predicting for the radiation-induced lung responses of pneumonitis or fibrosis are largely unknown. Herein we investigated whether markers of oxidative stress and intracellular antioxidants, measured within days of radiation exposure, are correlated with the lung tissue injury response occurring weeks later. Mice of the eight inbred strains differing in their susceptibility to radiation induced pulmonary fibrosis, and in the duration of asymptomatic survival, received 18 Gy whole thorax irradiation and were euthanized 6h, 24h or 7d later. Control mice were not irradiated. Lung levels of antioxidants superoxide dismutase, catalase, glutathione peroxidase (GPx) and glutathione, and of oxidative damage (reactive oxygen species (ROS) and 8-hydroxydeoxyguanosine (8-OHdG)), were biochemically determined. Glutathione peroxidase was additionally measured through gene expression and immunohistochemical assessment of lung tissue; and activity in serum. ROS and 8-OHdG were increased post irradiation and exhibited significant strain and time dependent variability, but were not strongly predictive of radiation-induced lung diseases. Antioxidant measures were not dramatically changed post irradiation and varied significantly among the strains. Basal GPx activity ($r = 0.73$, $p=0.04$) in the lung and the pulmonary expression of $GPx2$ ($r = 0.94$, $p = 0.0003$) correlated with post irradiation asymptomatic survival, while serum GPx activity was inversely correlated ($r = -0.80$, $p=0.01$) with fibrosis development. In conclusion pulmonary oxidative stress and antioxidant markers were more affected by inbred strain than radiation over 7 days post treatment. Lung GPx activity, and $GPx2$ expression, predicted for survival from lethal pneumonitis, and serum GPx for fibrosis, in this panel of mice.

Key words: Inbred mouse strains; thoracic irradiation; predictive biomarker; pneumonitis; fibrosis
Introduction

Late lung tissue responses of pneumonitis and fibrosis are the most serious dose-limiting side effects of thoracic radiotherapy, which is an essential treatment modality for a wide spectrum of malignant tumors affecting the thorax region (1). Whereas pneumonitis is an acute inflammatory response, fibrosis is characterized by progressive scarring of the lung, with vascular cell damage and collagen deposition in the interstitium (5, 13). If developing, pneumonitis and fibrosis have a considerable impact on patient morbidity and mortality. Biomarkers, whose levels pre-treatment or in the pre-symptomatic post-treatment interval can predict for the occurrence of these radiotherapy side effects, are not yet available (6).

The pathogenesis of the radiation-induced lung diseases is not yet mechanistically understood (5, 13) but it is known that radiation therapy produces reactive oxygen species (ROS), which if not neutralized, can result in the altered function or death of cells through oxidative damage to proteins, lipids and DNA (2, 7, 20, 25, 35). Oxidative damage to DNA can be measured by production of 8-hydroxydeoxyguanosine (8-OHdG), which is the most abundantly generated DNA oxidation product following radiation exposure (36). In order to neutralize ROS and to prevent subsequent oxidative damage to DNA and other bio-molecules, the cells have in place an endogenous antioxidant defense system comprised of superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase and glutathione (GSH) (9). Whereas SOD catalyses the conversion of highly reactive superoxide radicals to less reactive hydrogen peroxide, GPx and catalase aid in enzymatic detoxification of hydrogen peroxide. Glutathione not only directly scavenges ROS but is also required for the catalytic activity of GPx. These cellular antioxidants, however, may not be enough to overcome the primary oxidative damage
caused by radiation exposure because of the excessive generation of ROS, a condition termed as oxidative stress (3, 18, 21). It is therefore possible that an individual’s susceptibility towards radiation induced lung diseases could depend on how efficiently the lung cells are able to manage this primary injury (21).

Inbred strains of mice recapitulate the lung pathologies of pneumonitis and fibrosis, following exposure to whole thorax irradiation (10, 19, 22, 26, 27) and have been reported to vary in tissue intracellular antioxidant levels (33). In addition to differing in their fibrotic response to radiation exposure, we identified inbred mouse strains to vary in the time post treatment to the onset of distress, or in asymptomatic survival time (22). As it has been shown that asymptomatic survival time correlated with pneumonitis in mice (10), we have included this parameter in the current investigation. Inbred strains of mice are thus a resource with which to investigate whether variation in the oxidative stress or antioxidant response to radiation-induced primary injury influences susceptibility to the late lung tissue injury response of pneumonitis and fibrosis.

Herein we assessed whether the pulmonary levels of oxidative injury markers ROS and 8-hydroxydeoxyguanosine (8OHDG), or the activity levels of endogenous antioxidants SOD, GPx, catalase and GSH, measured during the acute radiation response, are strain dependent and if so, whether these levels are predictive of the tissue response of pneumonitis or fibrosis in a panel of inbred strains.

Materials and Methods
Mice: Female mice of eight inbred strains (C3H/HeJ, C57BL/6, A/J, AKR/J, 129S1/SvlmJ, CBA/J, NZW/LacJ and KK/HIJ) were purchased from the Jackson Laboratory (Bar Harbor, USA) and housed in the animal facility of the Meakins-Christie Laboratories. These eight strains were selected for investigation as they are representative of fibrosis susceptible (C57BL/6, 129S1/SvlmJ, NZW/LacJ and KK/HIJ) and fibrosis resistant (C3H/HeJ, A/J, AKR/J, and CBA/J) responses to thoracic irradiation. Secondly, they collectively exhibit substantial variation in post irradiation survival time, which ranges from 10-14 weeks in C3H/HeJ, AKR/J, CBA/J and KK/HIJ mice and from 21-26 weeks in C57BL/6, A/J, 129S1/SvlmJ and NZW/LacJ mice, and is independent of the development of fibrosis, in these strains (22). All mice were handled according to guidelines and regulations of the Canadian Council on Animal Care, under a protocol approved by the Animal Care Committee of McGill University.

Radiation treatment: Eight week old mice, partially shielded with 3 cm of lead, received whole thorax radiation exposure (18 Gy; dose rate 0.54Gy/minute) using a Faxitron X ray machine (14). After irradiation, the animals were housed under normal laboratory conditions, and groups of 5 mice per strain were euthanized at 6h, 1d or 7d after irradiation. The control mice of each strain were not treated and were euthanized at the 7 day time point.

Serum and lung tissue collection: After drawing >500µl of blood through cardiac puncture, the mice were euthanized with overdose of sodium pentobarbital and the lungs were rapidly removed and rinsed with cold PBS. The left lobe from each mouse was perfused with 10% neutral buffered formalin and preserved for immunohistochemical analysis. The right lungs were
divided into aliquots for ROS estimation, gene expression and biochemical assays. The blood was allowed to clot at room temperature for 1h and centrifuged at 1500g for 10 minutes. The serum was collected and stored at -80 degrees C until analysis.

**ROS estimation:** A portion of right lung was cut in pieces and treated for 45 min at 37°C with 1 mL of 5 mg/mL DNase1 (Sigma chemicals) and 1 mL of 5 mg/mL Collagenase D (Sigma chemicals) in 3 mL of phosphate buffered saline containing 1% albumin bovine serum (PBS/1% BSA). Cells were released by mincing lung tissue and filtering the resulting suspension through a nylon 70-mm cell strainer. The filtrate was treated with a solution of 150 mM NH4Cl, 10mM NaHCO3, and 10mMEDTA to lyse RBCs, and washed once in PBS/1% BSA. Cells were counted with a hemacytometer and suspended to appropriate concentrations in PBS to be used for reactive oxygen species (ROS) estimation.

Intracellular levels of ROS were estimated using a cell permeable oxidation sensitive probe 2',7'-dichlorodihydrofluorescein diacetate acetyl ester (DCFDA), whose fluorescence intensity increases after oxidation to dichlorofluorescein (DCF) by ROS. In brief, lung cells (1 x 10^6) in 100 µl of PBS incubated with 5 μM of DCFDA (Invitrogen, USA) for 30 min at 37 °C and quantified for DCF fluorescence using fluorescence plate analyzer (excitation at 480 nm and emission at 530 nm). The DCF fluorescence intensity was normalized with respect to appropriate control treated with probe without adding cells. The levels of intracellular ROS are represented as the mean fold increase in DCF fluorescence intensity.

**Assessment of antioxidant enzymes and GSH:** The remainder of the right lung was perfused each of three times with 1 mL of cold PBS to remove any trapped blood and edema and was then
homogenized (10 % weight/volume) in 100 mM TRIS buffer, pH 7.4, containing 0.5% Triton X-100, 5 mM butylated hydroxytoluene and 100 µM phenylmethylsulphonyl fluoride using a Tissuemiser (Fisher Scientific, USA). The homogenate was centrifuged at 10000 × g for 5 min and the supernatant, called tissue extract, was used for the estimation of oxidative damage marker, 8 hydroxydeoxyguanisine (8OHdG) and antioxidants glutathione peroxidase (GPx), catalase, superoxide dismutase (SOD) and total glutathione (GSH).

The activity levels of GPx, catalase and SOD were determined using kits from Biovision, USA and of total GSH using a kit from Sigma Chemicals (St. Louis, MO, USA) as per the manufacturer's instructions. The activity levels of antioxidant enzymes and GSH were normalized with respect to protein content in the lung homogenate which was estimated using Bio-Rad protein assay kit. Serum levels of GPx activity were measured with a Bio-vision kit as per the manufacturer's instructions.

8-hydroxydeoxyguanosine (8OGdG) assay: Genomic DNA from the lung homogenate was isolated using DNeasy blood and tissue kit (Qiagen, Valencia, CA) according to the manufacturer's instructions with some modifications. DNase-free RNase (Sigma-Aldrich, St. Louis, MO) was added to the samples before addition of Buffer AL, to digest the RNA as per supplier’s recommendations. The RNA-free DNA (20 µg) thus obtained was converted to single-stranded DNA by incubating the samples at 95°C for 5 min and rapidly chilling on ice. After denaturation, the pH of denatured DNA was adjusted to 5.2 with 3 M sodium acetate (pH 5.2) and subjected to digestion with P1 (1 U) for 2 h at 37°C (28). Following this, pH of the reaction was adjusted back to 7.4 using 1 M Tris-HCl (pH 8.0), the reaction mixture was incubated with 1 U of alkaline phosphatase for 1 h at 37°C and centrifuged for 5 min at 6000 x g. The supernatant
obtained was used for 8-OHdG estimation using Oxiselect oxidative DNA damage ELISA-kit (Cell Biolabs, San Diego, CA) according to manufacturer's instructions. The levels of 8-OHdG were normalized with respect to per mg of genomic DNA.

Quantitative real-time PCR: A portion of the right lung from each mouse was homogenized in 1.0 ml of Trizol reagent using a Tissuemiser (Fisher Scientific, USA) and stored at -70°C until gene expression assessment by quantitative real-time PCR. Total RNA from the Trizol homogenate was isolated according to manufacturer’s instructions and 4 µg of total RNA was used for the synthesis of cDNA by reverse transcription using Superscript™ II RNase H–Reverse Transcriptase (Invitrogen, Carlsbad, CA). Quantitative real-time PCR assays were performed using Applied Biosystems International Prism 7500 Sequence Detection System using the TagMan Gene expression assays Mm00656767_g1 for glutathione peroxidise 1 (GPx1), Mm01286848_gH for glutathione peroxidise2(GPx2), Mm00492427_m1 for glutathione peroxidise3 (GPx3), Mm00515041_m1 for glutathione peroxidise4(GPx4). GAPDH (gapdh, Assay Mm99999915_g1) was used as the reference gene as in prior studies (14) and data analysis was also as in a prior study (14).

Immunohistochemistry: Immunohistochemical detection was completed on paraffin tissue sections (5µm thick). These sections after dewaxing in xylene and rehydration in graded alcohols were boiled in antigen unmasking solution (Vector Laboratories, Germany) for 50 min to expose antigenic determinants and blocked with 4 % goat serum. Afterwards sections were incubated with primary anti-GPx1 (Abcam, USA), anti-GPx2 ((Abcam, USA) or anti-GPx4 (Cayman Chemical, USA) antibodies, followed by biotinylated goat anti-rabbit or anti-rat secondary
antibody (Vector Laboratories, Germany). Sections were labeled using avidin-biotin-alkaline phosphatase and red alkaline phosphatase substrate kits (Catalog No – SK5100, Vector Laboratories, Germany) as per the manufacturer’s instruction. Finally sections were counterstained with methylene blue, mounted with non-aqueous media.

Statistical analysis: The results are presented as mean ± SEM (4-5 mice per strain at a time point). The statistical significance of the variability among the means of treatment groups was determined by one way ANOVA followed by Tukey’s post-test, p < 0.05. The correlation analysis of biochemical parameters with disease phenotypes was performed using the Pearson’s rank correlation test and p values < 0.05 were considered as statistically significant. The radiation-induced lung disease phenotypes of average fibrosis score and asymptomatic survival time for the 8 strains were taken from a previous report (22).

Results

Acute oxidative damage response in eight inbred strains

To determine whether whole thorax irradiation induces a strain dependent oxidative damage response in the lungs of exposed mice, we treated mice of 8 strains with 18 Gy and assessed lung tissue up to 7 days later. As shown in Figure 1 (A&B), radiation treatment produced a significant increase in ROS and 8-OHdG levels, although the time to the peak response was strain dependent. At each of the four time points (control, 6h, 24 h, 7days) the
extent of oxidative damage varied significantly (p<0.0001) among the strains, and measures of 8-
OHdG agreed well with those for ROS.

To assess the predictive value of these assays for lung disease correlation analyses were
completed. The values of 8-OHdG at day 7 were suggestively correlated with asymptomatic
survival time (Pearson coefficient = -0.64, p = 0.086) and no other measures were significantly
correlated with extent of radiation induced fibrosis (p>0.15) or asymptomatic survival time, apart
from 8-OHdG at day 7, (p>0.3, data not shown).

Acute antioxidant response in eight inbred strains

To determine whether whole thorax irradiation induces a strain dependent anti-oxidant
response in the lungs of exposed mice specific parameters were measured in lung homogenates
taken from the 8 strains over the time course. In contrast to the oxidative damage response,
thoracic irradiation did not profoundly alter lung tissue levels of SOD, GPx or total GSH in the
majority of strains, as shown in Figure 2 (A, B & C). Tissue catalase levels were affected by
radiation in six of eight strains (Fig. 2D). Each of GPx, total GSH and tissue catalase activity,
was, however, significantly dependent on strain (p<0.0001) at the control, 6h, 24h and 7 day
timepoints, as were the levels of SOD (p<0.04) at all times except 24 hours. As the values of
GSH and GPx in KK/HJ mice, appeared to differ from those of the other strains, the analysis of
variance was repeated with this strain removed. The significant strain dependence in GPx values
was retained, except for levels measured at 6 hours post irradiation. For GSH, the strain
dependence, without KK/HJ mice, was significant only at 24 hours and 7 days post irradiation.
Given this strain dependence we assessed the correlation of the antioxidant measures to the tissue injury response and identified basal (control tissue) levels of GPx to be significantly correlated with asymptomatic survival time in the eight inbred strains, (Pearson coefficient = 0.73, p = 0.04) as shown in Figure 3. The correlation of pulmonary GPx and asymptomatic survival time was also evident, and comparable to that at baseline, at each of 6h, 24h and 7 days post irradiation (supporting information). Tissue levels of catalase, superoxide dismutase and total glutathione were not significantly correlated with extent of radiation-induced lung disease measured as fibrosis (p>0.25, data not shown) or as asymptomatic survival (p>0.11, data not shown).

To evaluate whether basal GPx activity in the serum was reflective of that in the lung, and in turn correlated with lung disease, measures in this fluid were completed. As shown in Figure 4A, the activity of GPx in the serum was not significantly correlated with that of the lung (p=0.24). Serum GPx activity was however, significantly negatively correlated with extent of radiation-induced pulmonary fibrosis in this panel of strains (Pearson coefficient = -0.80, p = 0.01) as shown in Figure 4B. Serum levels of GPx were not significantly correlated with extent of radiation-induced lung disease measured as asymptomatic survival (p>0.62, data not shown).

Glutathione Peroxidase Expression

As at least four different GPx family members (GPx1-4), of different functionality, are expressed in lung (31), we investigated whether their expression was predictive of radiation-induced lung disease. The expression of GPx1-4 in the lungs of un-irradiated control mice was evaluated with RT-PCR and by immunohistochemistry. GPx3 was not assessed by
immunohistochemistry as it is known to be secreted by lung cells (12, 31). In agreement with a previous report (31), the pulmonary expression of GPx1 and GPx3 was abundant compared to that of GPx2 and GPx4 in each of the eight strains analysed (Fig. 5A). Significant (P<0.0002) strain dependent variability was observed in the basal expression of each of GPx1, Gpx2 and GPx3 (Fig. 5A). These data were supported by immunohistochemical staining as shown in Figure 5B and supporting information. Analyses to correlate the mRNA expression of GPx1-4 to lung disease indicated that the expression of GPx2 was significantly (Pearson coefficient = 0.94, p = 0.0003) correlated with asymptomatic survival time in the eight strains (Fig. 6).

Discussion

In present study using murine models of radiation induced lung disease, we demonstrated that the duration of asymptomatic survival time prior to the lung response of pneumonitis, with or without fibrosis, following thoracic radiation exposure, was significantly correlated with pulmonary GPx, both by activity and mRNA expression levels. Secondly, the radiation-induced pulmonary fibrosis response of the lung was correlated with the basal serum GPx activity. Neither the asymptomatic survival time nor the fibrosis response was predicted by the levels of other intracellular antioxidants SOD, catalase or GSH within seven days of thoracic irradiation in this panel of mice.

The pulmonary activity of GPx, which showed little change with time but significant variability among the strains, positively correlated with the lung disease phenotype of post-irradiation asymptomatic survival time. The correlation between the GPx activity and asymptomatic survival time, although significant at all evaluated time points, was not improved post radiation exposure compared to the basal activity, thus baseline measures of this enzyme
appear adequate for prediction of tissue radiation response. Secondly, as baseline GPx activity in the serum was correlated with tissue fibrosis in these mice, serum levels of this enzyme may be a useful biomarker, although they were not predictive of the pulmonary activity of the same enzyme. These findings are supported by one clinical report, wherein the lower levels of RBC GPx activity, both pretreatment and after radiotherapy, were observed in lung cancer patients developing pneumonitis compared to those in patients who did not develop pneumonitis following radiotherapy (21).

The association of higher GPx activity with the better post irradiation survival time, and reduced fibrosis, suggests a protective function for GPx against the onset of radiation induced lung pathologies. In line with this observation, we have previously demonstrated that the post irradiation treatment with a organoselenium compound, 3,3'-diselenodipropionic acid (DSePA), which increased the pulmonary GPx activity, significantly delayed the onset of pneumonitis in C3H/HeJ mice (14). In another such study, Liu et al., (15) showed that the administration of quercetin liposomes (known to activate antioxidant enzymes) 2h before and for 4 weeks after 16 Gy whole thorax irradiation increased the lung GPx level and decreased radiation-induced fibrosis in mice. Finally, mice deficient in Nuclear factor-E2-related factor 2 (Nrf2), a redox sensitive transcription factor which regulates antioxidant response element-dependent genes including GPx2 (28), succumb earlier than wild type mice to lung disease post thoracic radiotherapy (32) in a response that may be related to tissue glutathione peroxidase levels, although this has not been shown.

Among the four GPx isoforms expressed in the lung the basal expression levels of GPx2, specifically, were correlative of the tissue injury response of survival post irradiation. These
results are consistent with developing evidence that GPx2 has anti-inflammatory functions (4), however, the more detailed mechanism by which GPx2 may modulate the inflammatory response in lung following thoracic exposure remains to be elucidated. In contrast, GPx1, a major cytosolic isoform accounting for most of the pulmonary GPx activity (4,31); GPx3 an extracellular isoform secreted into bronchoalveolar fluid and blood (29); and GPx4 a cytosolic isoform that specifically neutralizes the phospholipid hydroperoxides at the expense of thioredoxin instead of GSH (31), were not individually correlative of lung disease in these mice.

In this work the potential radiation response biomarkers assessed were taken from components of the oxidative stress response as classically defined. In addition to this pro oxidant antioxidant definition, Jones (11) has redefined oxidative stress to include “a disruption of redox signalling and control”. Additional pathways included in this definition of the oxidative stress would be those through which peroxiredoxin and thioredoxin enzymes function (11). There are 6 mammalian peroxiredoxin enzymes, which function to degrade hydroperoxides to water, and their cellular levels may be an indication of the oxidative stress response (23,24). Mathew et al. (17) have reported the expression of peroxiredoxin-1 to be increased in the lung within weeks of irradiation. The thioredoxin antioxidant system is comprised of three thioredoxin reductase enzymes and 2 of thioredoxin which, through reductase activity, serve to defend against oxidative stress (16). Although not evaluated here, components of this expanded definition, acting individually or in composite, and possibly with the innate GPx activity may also be predictive of radiation response in the lung.

Among the remaining antioxidants assayed, catalase, SOD and GSH showed strain dependant variability in their pulmonary levels over the time following thoracic irradiation but none was significantly correlated with asymptomatic survival time or the lung disease phenotype.
of fibrosis, and thus are not suggested by these data to be useful predictive biomarkers. In support of this finding, RBC catalase and GSH measures were independent of the pneumonitis response in radiotherapy patients (21), although high SOD was associated with pneumonitis in this group of patients. Regarding the strain dependence of the biomarkers, we are not aware of other studies documenting this variability for lung tissue, although multiple groups have reported inbred strain background to have a major effect on GSH levels in the liver (8,30,34,37) and the kidney (37), and on liver catalase levels (30). Indeed GSH activity levels in the liver differing by 3 fold over a panel of strains have been reported (37), which is similar to the range in values measured here for the lung. The specific genetic differences among inbred strains which result in varied GSH levels are unknown at present, but recent studies have identified candidate genes for this trait based on association studies in mice (8,37). Finally, early levels of the primary radiation injury of reactive oxygen species generation or oxidative stress were not strongly correlated with the later lung disease in mice. In the lungs of the mice of this panel this early radiation response was not indicative of the later pathological response.

In conclusion our studies showed that among oxidative stress and antioxidant biomarkers assayed, higher control levels of pulmonary GPx activity and of \( GPx2 \) expression in the lung correlated with better survival of mice exposed to thoracic irradiation while greater levels of serum GPx were associated with sparing of a fibrosis response.

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Disclosures:

No conflicts of interest, financial or otherwise, are declared by the authors.

References


**Figure Captions**

**Fig. 1.** Effect of thoracic irradiation on the pulmonary reactive oxygen species and oxidative stress in eight inbred strains. Mice received 18 Gy whole thorax irradiation and lung tissue was evaluated at 6h, 24h and 7 days post-irradiation. Control mice were not irradiated. (A) Intracellular levels of ROS were estimated using a cell permeable oxidation sensitive probe. (B) Oxidised guanine bases (8-OHdG) in DNA were measured using commercially available kits. Mean ± S.E.M (n = 5). Line above the groups indicates significance by one way ANOVA. Fl = fluorescence; DCF=dichlorofluorescein.

**Fig. 2.** Effect of thoracic irradiation on anti-oxidant levels in eight inbred strains. Mice received 18 Gy whole thorax irradiation and lung tissue was evaluated at 6h, 24h and 7 days post-irradiation. Control mice were not irradiated. (A) SOD (B) GPx (C) total GSH and (D) catalase activities were measured using commercially available kits and levels normalized to total protein in lung homogenate. Mean ± S.E.M (n = 5). Line above the groups indicates significance level by one way ANOVA. NS= Not significant.
Fig. 3. Scatter plot of asymptomatic survival time of mouse strains post 18 Gy whole thorax irradiation on the mean pulmonary GPx activity levels of unirradiated control. r=Pearson correlation coefficient, P value indicates significance of correlation by Pearson’s rank correlation test.

Fig. 4. Scatter plots of (A) mean pulmonary and serum GPx activity levels of unirradiated control mice and (B) average fibrosis score of mouse strains post 18 Gy whole thorax irradiation on the mean serum GPx activity levels of unirradiated control. r=Pearson correlation coefficient, P value indicates significance of correlation by Pearson’s rank correlation test.

Fig. 5. Pulmonary expression of GPx1, GPx2, GPx3 and GPx4 in unirradiated mice of eight inbred strains: (A) The fold change in mRNA expression of each GPx gene in the right lungs of mice, relative to that of a reference gene (Gapdh) is presented (mean ± S.E.M, n = 5). Line above the groups indicates significance by one way ANOVA. (B) Representative lung tissue sections immunostained with anti-GPx1, anti-GPx2 and anti-GPx4; 200X magnification.

Fig. 6. Scatter plots of asymptomatic survival time of mouse strains post 18 Gy whole thorax irradiation on the mean pulmonary mRNA expression level of (A) GPx1 (B) GPx2 (C) GPx3 (D) GPx4. r=Pearson correlation coefficient, P value indicates significance of correlation by Pearson’s rank correlation test.
Fig. 2. (A) Superoxide dismutase (U/mg protein) levels in various strains at different time points after IR.

(B) GSH (μmol/mg protein) levels in various strains at different time points after IR.

(C) Glutathione peroxidase (mU/mg protein) levels in various strains at different time points after IR.

(D) Catalase (mU/mg protein) levels in various strains at different time points after IR.

Key:
- Control
- 6h post IR
- 24h post IR
- 7d post IR

Statistical significance:
- P<0.001
- P=0.0086
- P=0.0005
- P=0.012
- NS
Fig. 3

r = 0.73, P (two tailed) = 0.040

Av. Asymptomatic survival time (days)

Glutathione peroxidase (mU)/mg protein, Basal
Fig. 6

(A) $r = 0.70, P$ (two tailed) $= 0.0508$

(B) $r = 0.94, P$ (two tailed) $= 0.0003$

(C) $r = -0.16, P$ (two tailed) $= 0.687$

(D) $r = -0.011, P$ (two tailed) $= 0.979$