



Research paper

Protection against gamma-radiation injury by protein tyrosine phosphatase 1B



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ABSTRACT

Protein tyrosine phosphatase 1B (PTP1B) is widely expressed in mammalian tissues, in particular in immune cells, and plays a pleiotropic role in dephosphorylating many substrates. Moreover, PTP1B expression is enhanced in response to pro-inflammatory stimuli and to different cell stressors. Taking advantage of the use of mice deficient in PTP1B we have investigated the effect of γ -radiation in these animals and found enhanced lethality and decreased respiratory exchange ratio vs. the corresponding wild type animals. Using bone-marrow derived macrophages and mouse embryonic fibroblasts (MEFs) from wild-type and PTP1B-deficient mice, we observed a differential response to various cell stressors. PTP1B-deficient macrophages exhibited an enhanced response to γ -radiation, UV-light, LPS and S-nitroso-glutathione. Macrophages exposed to γ -radiation show DNA damage and fragmentation, increased ROS production, a lack in GSH elevation and enhanced acidic β -galactosidase activity. Interestingly, these differences were not observed in MEFs. Differential gene expression analysis of WT and KO macrophages revealed that the main pathways affected after irradiation were an up-regulation of protein secretion, TGF- β signaling and angiogenesis among other, and downregulation of Myc targets and Hedgehog signaling. These results demonstrate a key role for PTP1B in the protection against the cytotoxicity of irradiation in intact animal and in macrophages, which might be therapeutically relevant.

1. Introduction

PTP1B is a ubiquitously expressed protein tyrosine phosphatase that has emerged as a key regulator of several signaling pathways activated by members of the tyrosine kinase superfamily [1–4]. Studies on PTP1B inhibitors have received attention in different areas of research and the number of inhibitors of this phosphatase is continuously increasing [5]. In the field of diabetes, due to the ability of PTP1B to dephosphorylate and inactivate the insulin receptor, these inhibitors have been considered as potential therapeutic drugs [6–8]. Indeed, PTP1B-deficient

mice are a unique model of insulin hypersensitivity due to enhanced insulin action [9–11], since these mice are protected against diet [9,11] and age-induced obesity and insulin resistance [12,13]. PTP1B is also involved in the regulation of cytokine signaling pathways, controlling in this way cell outcomes [14–17]. Moreover, PTP1B mRNA levels are regulated by different pro- and anti-inflammatory stimuli, such as LPS or IL4 [18–21]. Through these mechanisms PTP1B has been involved in atherogenesis and cardiovascular diseases [4,22,23], liver diseases [1], neuroinflammation [3] and in endoplasmic reticulum stress [24]; however, conflictive data have been reported regarding its involvement

Abbreviations: DE, Differentially Expressed Gen; FDR, False Discovery Rates; GSH, glutathione; GSNO, S-nitroso-glutathione; GSEA, Gene Set Enrichment Analysis; JNK, c-Jun N-terminal kinase; KEGG, Kyoto encyclopedia of genes and genomes; LPS, lipopolysaccharide; KO, knockout; MAPK, mitogen-activated protein kinase; MnTBAP, Mn-7-hydroxyflavone complex; NAC, N-acetyl-L-cysteine; qRT-PCR, quantitative real-time (RT) polymerase chain reaction; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; WT, wild-type

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in cancer [18,25–28].

We have previously described that PTP1B is highly expressed in monocytes and that mice lacking PTP1B, as well as human monocytes depleted of this protein, exhibited enhanced pro-inflammatory responses, and an attenuated capacity to express anti-inflammatory and pro-resolution mediators, resulting in a low-grade pro-inflammatory phenotype [20]. Indeed, other groups have reported an increase in myeloid precursors in the spleen and bone marrow in PTP1B-deficient mice, an effect probably due to the sustained activation of the M-CSF receptor [21]. Experiments intended to ablate the bone marrow from PTP1B-deficient mice showed that these animals were more sensitive to irradiation-induced injury than the corresponding wild-type (WT), resulting in significant higher death rates [20]. In the present work, we have investigated the effect of γ -radiation on the overall metabolic behavior of WT and PTP1B-deficient mice. In addition to this and taking into account that PTP1B is highly expressed in the myeloid lineage, we determined the impact of different stressors (γ -radiation, UV-light, LPS and GSNO) in the response of macrophages. Our data show a depressed metabolic activity of PTP1B-deficient animals after irradiation and a lesser capacity of macrophages to regulate oxidative stress. Moreover, ROS scavengers protected against the drop in GSH levels in PTP1B-deficient macrophages, preserving cell viability. These data suggest a relevant role for PTP1B in the mechanisms of defense against γ -radiation.

2. Materials and methods

2.1. Materials

Common reagents were from Sigma-Aldrich-Merck (St Louis, MO, USA) or Roche (Darmstadt, Germany). Murine or human cytokines were obtained from PeproTech (London, UK). Antibodies were from Ambion (Austin, TX, USA), Abcam (Cambridge, UK) or Cell signaling (Danvers, MA, USA). Reagents for electrophoresis were from Bio-Rad (Hercules, CA, USA). Tissue culture dishes were from Falcon (Lincoln Park, NJ, USA), and serum and culture media were from Invitrogen (Life Technologies/Thermo-Fisher, Madrid, Spain).

2.2. Animal care and preparation of macrophages

Male and female PTP1B heterozygous mice [29], maintained on mixed genetic background (C57BL/6 \times 129sv), were intercrossed to yield three genotypes of mice (WT, HET, KO). In this study, we used 12-week-old WT and PTP1B KO male mice housed under 12 h light/dark cycle and food and water was provided *ad libitum*. Animals were cared for according to the protocol approved by the Ethical Committee of our institution (following directive 2010/63/EU of the European Parliament). Bone marrow derived macrophages (BMDMs) were obtained from male mice by flushing pelvises, femurs, and tibiae with DMEM. Bone marrow mononuclear phagocytic precursor cells were propagated in suspension by culturing in DMEM containing 10% FBS, 100 U/ml penicillin, 100 mg/ml streptomycin, and 0.2 nM recombinant murine M-CSF (PeproTech) in tissue-culture plates. The precursor cells became adherent within 7 days of culture. BMDMs were maintained in RPMI 1640 medium supplemented with 10% FBS for 14 h prior to use.

2.3. Animal and cell irradiation

PTP1B WT and KO male mice were irradiated with the indicated doses in a two-sources Cs-137 irradiator (JL Shepherd, San Fernando, CA, USA; model Mark-1 30 A; 29,6 TBq of total activity). When PTP1B was inhibited, the drug was intraperitoneally administered 1 h prior to irradiation (15 mg/kg) followed by daily administrations for 3 days. Cells were irradiated in a similar way.

2.4. Evaluation of animal metabolic profile

Animals were maintained in Phenomaster Metabolic Cages (TSE Systems International Group) for two days prior to irradiation and then evaluated for 5 additional days. The movement, drinking, eating, respiration (O_2 and CO_2), weight and heat production were monitored.

2.5. Determination of myeloid cell populations

Animals were irradiated (5 Gy) and the distribution of myeloid cells was determined by flow cytometry at 24 h in the spleen and bone marrow. This dose of irradiation preserved viability of the animals. The markers used were: CD45, CD115, CD11b (monocytes), Ly6G (neutrophils), Ly6C^{low} (patrolling/tissue repair) Ly6C^{medium/high} (pro-inflammatory) and F4/80 (macrophages) and combinations of fluorescence dyes was used to evaluate different cell subsets.

2.6. Measurement of cell viability

Macrophage viability was determined by flow cytometry as previously described [20] in a BD-Canto flow cytometer (BD Biosciences; San Jose, CA). In addition to this, cells were stained with annexin V-PE (Immunostep; Salamanca, Spain).

2.7. Measurement of oxidized guanine species

Cells were treated with the indicated stressors and the amount of 8-hydroxyguanine, 8-hydroxyguanosine and 8-hydroxy-2-deoxyguanosine were determined at 72 h using a DNA/RNA oxidative damage kit (Cayman-Vitro, Madrid, Spain).

2.8. Comet assay

Cells were irradiated and the DNA was analyzed at 72 h using the OxiSelect Comet assay kit (Cell Biolabs, San Diego, CA), following the instructions of the supplier.

2.9. Measurement of GSH and GSSG levels

Cells were treated with the indicated stressors and the amount of GSH and GSSG were determined at 24 h using a commercial kit (Enzo, Farmingdale, NY).

2.10. Determination of senescence

Cells were treated with the indicated stressors for the indicated times and the staining for acidic β -galactosidase activity, or the measurement of the β -galactosidase activity were determined using specific kits (Merck-Millipore), following the instructions of the supplier.

2.11. Determination of glutathione synthetase (GS) activity

GS was measured in macrophage extracts that were filtered through Sephadex G-25 columns to remove low-weight metabolites. The reaction was carried out at 25 °C in the presence of 1 mM γ -glutamylcysteine, 1 mM glycine and 1 mM MgATP at pH 7.4, and measuring the synthesis of GSH at 10 min. The ATP was regenerated from the ADP formed in the presence of 0.5 mM phospho(enol)pyruvate. One unit of GS is the amount of enzyme that synthesizes one μ mol of GSH per 10 min.

2.12. Preparation of total protein cell extracts

Macrophages were homogenized in a buffer containing 10 mM Tris-HCl, pH 7.5; 1 mM $MgCl_2$, 1 mM EGTA, 10% glycerol, 0.5% CHAPS, 1 mM β -mercaptoethanol and a protease and phosphatase inhibitor

cocktail (Sigma). The extracts were vortexed for 30 min at 4 °C and after centrifuging for 20 min at 13,000 g, the supernatants were stored at – 20 °C. Protein levels were determined using Bradford reagent (Bio-Rad).

2.13. RNA isolation and RT-PCR analysis

RNA was extracted with TRIzol Reagent (Thermo-Fisher, Carlsbad, CA) and reverse transcribed using Transcriptor First Strand cDNA Synthesis Kit for RT-PCR following the indications of the manufacturer (Thermo-Fisher). Real-time PCR was conducted with SYBR Green Master (Thermo-Fisher) on a MyiQ Real-Time PCR System (Bio-Rad). Primer oligonucleotide sequences are available on request. Validation of amplification efficiency was performed for each pair of primers [30]. PCR thermocycling parameters were 95 °C for 10 min, 40 cycles of 95 °C for 15 s, and 60 °C for 1 min. Each sample was run in duplicate and was normalized vs. 36B4. The fold induction (FI) was determined in a $\Delta\Delta Ct$ based fold-change calculation.

2.14. Western blotting

Protein extracts were boiled in loading buffer (250 mM Tris–HCl; pH 6.8, 2% SDS, 10% glycerol and 2% β -mercaptoethanol) and 30 μ g of protein were subjected to 8–10% SDS-PAGE electrophoresis gels. Proteins were transferred into polyvinylidene difluoride (PVDF) membranes (GE Healthcare). Membranes were incubated for 1 h with low-fat milk powder (5%) in PBS containing 0.1% Tween-20. Blots were incubated for 2 h or overnight at 4 °C with primary antibodies at the dilutions recommended by the suppliers. The blots were developed with ECL Advance protocol (GE Healthcare) and different exposure times were performed for each blot in an ImageQuant analyzer (LAS 500, GE) to ensure the linearity of the band intensities. Blots were normalized for lane charge using antibodies against GAPDH.

2.15. Microarray analysis

Normalized expression data in macrophages after 24 h of stimulation were obtained in our core facility, using a mouse microarray platform (Agilent-014868 Whole Mouse Genome Microarray 4 × 44K G4122F). Quality of the samples was ensured in a Bioanalyzer 2100. Processing, normalization and differential expression was performed using Limma Bioconductor package [31]. Enrichment of gene sets of interest from KEGG and REACTOME was accomplished using the GSEA software as described by Mootha et al. [32].

2.16. Statistical analysis

The values in graphs correspond to the means \pm SD. The statistical significance was determined with Student *t*-test for unpaired observation. Data were analyzed by the SPSS for Windows statistical package, v21.

3. Results

3.1. PTP1B protects against γ -radiation

Since previous data suggested that animals lacking PTP1B were more sensitive to irradiation than the WT counterparts [20] we investigated the contribution of PTP1B to this protection. To this end, PTP1B WT and KO mice were irradiated with 10 Gy in a single dose. As Fig. 1A shows, animals lacking PTP1B or treated with a PTP1B inhibitor prior to irradiation exhibited a higher morbidity than the WT counterparts. When the 10 Gy were administered in two sessions of 5 Gy each in an 18 h-interval, animal survival increased, but still a statistically significant difference between the WT and the PTP1B inhibited or the PTP1B KO condition was observed (Fig. 1A, right panel). However,

irradiation with a single dose of 5 Gy did not affect viability in any of the groups (results not shown). Next, we evaluated the response of PTP1B WT and KO animals to 5 Gy irradiation by performing indirect calorimetry. As Fig. 1B,C show, after 5 Gy irradiation PTP1B KO animals exhibited lower respiratory exchange ratio (RER), and decreased locomotor activity, food intake (Fig. 1D) and drinking (Fig. 1E) compared to the WT counterparts. The relative mRNA levels of *Ptpn1* in different tissues after exposure to 5 Gy were determined (Fig. 2A). Interestingly, 5 Gy irradiation increased *Ptpn1* mRNA levels mainly in immune organs and cells.

Irradiated PTP1B KO animals (5 Gy) exhibited a significant spleen enlargement (Fig. 2B), with a high content of apoptotic cells (Fig. 2C), although no statistically significant differences were observed in the cell populations analyzed. Moreover, analysis of myeloid precursors in the bone marrow showed a statistically significant increase of apoptotic cells, suggesting a higher sensitivity of PTP1B KO cells to irradiation (Fig. 2D).

3.2. Macrophages from PTP1B-deficient mice sense γ -radiation, UV and oxidative/nitrosative stress

To evaluate the molecular mechanisms responsible for the enhanced sensitivity of PTP1B-deficient mice to different stressors compromising animal survival or cell function, macrophages –as an immune cell type expressing high levels of *Ptpn1*- and MEFs –as a non-immune cell with significant levels of *Ptpn1*- were used. As Fig. 3A-B shows, bone marrow-derived macrophages (BMDMs) from PTP1B WT mice show an increase of *Ptpn1* mRNA and protein levels after exposure to γ -radiation, UV or LPS, and to a lesser extent after incubation with the NO-donor GSNO. At the same time, PTP1B-deficient macrophages exhibited higher apoptotic rates (48 h) under these conditions, including after GSNO incubation (Fig. 3C). When ROS production was measured we found that whereas irradiated cells from WT animals were not affected (Fig. 3D), those from PTP1B KO mice exhibited a significant increase and this effect occurred after LPS challenge. PTP1B KO macrophages treated with GSNO exhibited a decrease in ROS, probably due to the formation of peroxynitrite between O_2^- and NO. Since γ -radiation and LPS exposure produced the major impact on ROS synthesis, we determined the levels of GSH and GSSG under the different conditions assayed. As Fig. 3E shows, GSH levels increased in irradiated WT macrophages but not in those deficient in PTP1B. Moreover, GSSG concentrations were higher in the corresponding PTP1B KO macrophages than in the WT cells after irradiation or UV exposure. None of these effects were found in MEFs (results not shown). To evaluate the role of ROS on the apoptosis of PTP1B KO macrophages, cells were incubated with ROS scavengers (NAC, MnTBAP and ebselen) and GSH in the extracellular medium. As Fig. 3F shows, viability was significantly improved in irradiated PTP1B KO macrophages treated with NAC and the permeable superoxide dismutase mimetic MnTBAP, but not by ebselen (probably due to scavenging NO) nor GSH. Moreover, the intracellular levels of GSH were higher in NAC- and MnTBAP-treated cells (Fig. 3G). Analysis of the mRNA levels of the enzymes involved in GSH biosynthesis did not show significant changes in glutamate-cysteine ligase catalytic subunit (*Gclc*), a decrease in the regulatory subunit (*Gclm*), and a lesser increase in glutathione synthetase (*Gss*), compatible with modest differences in the enzymatic activity of glutathione synthetase (GS) from irradiated PTP1B KO macrophages (Suppl. Fig. S1A,B).

3.3. Absence of PTP1B promotes senescence in macrophages after challenge with different stressors

Since morphological differences were observed between WT and PTP1B KO macrophages challenged with different stressors, such as cell and nuclear enlargement (data not shown), as well as molecular markers of senescence (*Fos* and *Cdkn2a*; Fig. 4A), the occurrence of

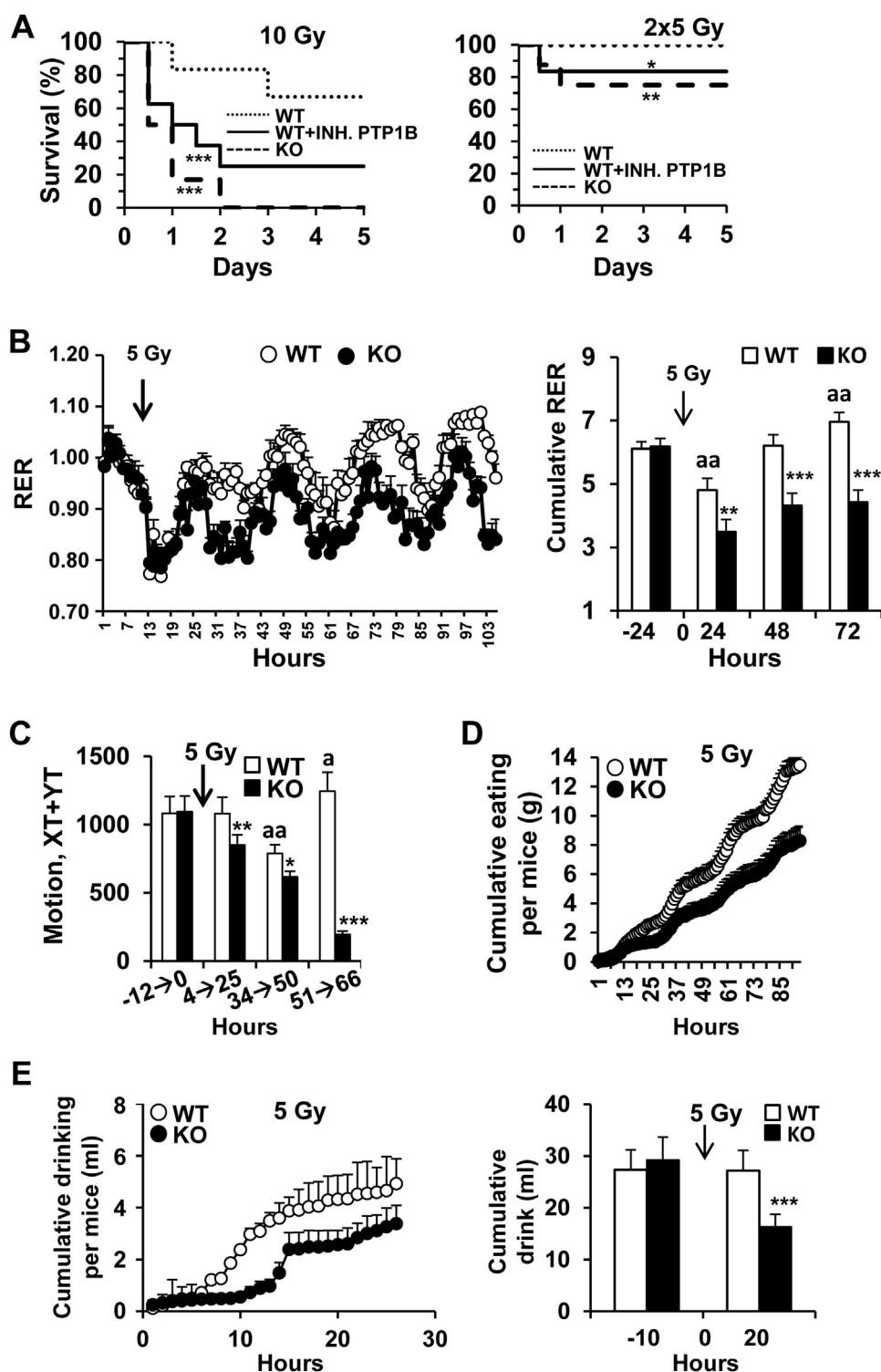


Fig. 1. *PTP1B* deficiency increases the susceptibility to γ -radiation, depresses RER and drinking in mice after 5 Gy irradiation. (A) *PTP1B* WT animals (n = 12) untreated or treated with *PTP1B* inhibitor XXII (n = 8; 15 mg/kg) or *PTP1B* KO mice (n = 12) were submitted to 10 Gy irradiation administered in a single dose or in two doses of 5 Gy in a 18 h period and survival of the animals was evaluated. (B) *PTP1B* WT or KO mice (n = 10) were submitted to 5 Gy irradiation and the plural effects on RER, motion (C), eating (D) and drinking (E) were determined in ‘Phenomaster Metabolic Cages’. Results show the mean \pm SD of two independent experiments. *P < 0.05; **P < 0.01; ***P < 0.005 vs. the same condition in *PTP1B* WT animals; ^aP < 0.05; ^{aa}P < 0.01 vs. the ‘untreated’ condition of WT animals.

senescence was investigated under these conditions. Exposure to γ -radiation and UV and, to a lesser extend to GSNO resulted in an increase in senescence-associated acidic β -galactosidase activity in macrophages lacking *PTP1B* or in WT cells after inhibition of *PTP1B* (Fig. 4B–D). In addition to these changes, increased DNA fragmentation 72 h after irradiation was observed in *PTP1B* KO macrophages (Fig. 5A). However, the phosphorylation of histone H2AX (γ -H2AX) as an indicator of DNA damage was observed at 24 h after irradiation and was similar in WT and *PTP1B* KO macrophages (Fig. 5B). This is despite the increased

DNA damage in *PTP1B* KO macrophages as determined at 72 h by the Comet assay (Fig. 5C), and by the increase in 8-hydroxyguanine, 8-hydroxy-2'-deoxyguanosine and 8-hydroxyguanosine species (8-HO-G) after 5 Gy irradiation (Fig. 5D). These results indicate that the absence of *PTP1B* favors an increase in oxidative stress, but also a lack in the capacity to prevent the harmful effects associated to these radicals as evidenced by the DNA damage and the accumulation of oxidized nucleotide species.

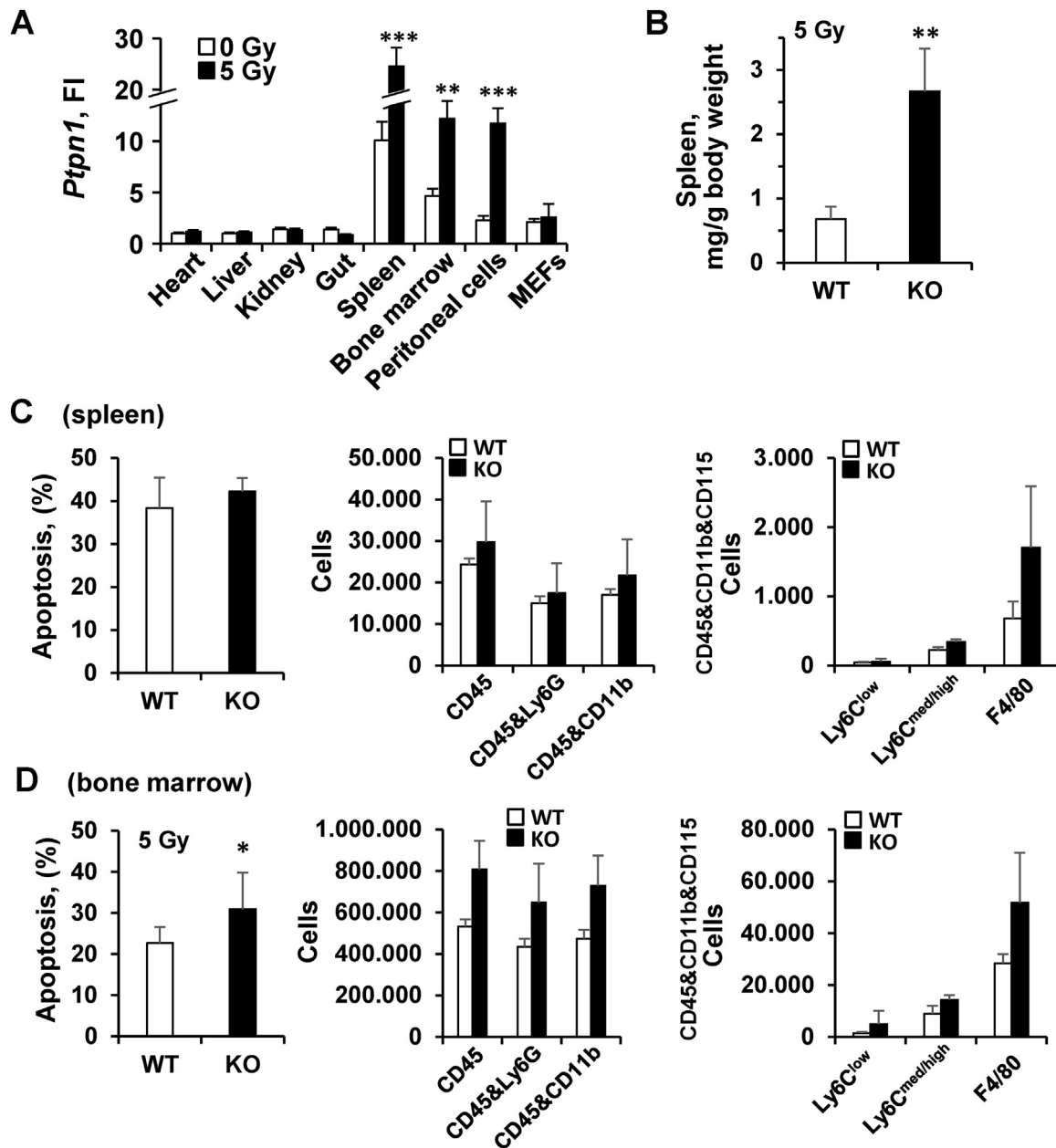


Fig. 2. *PTP1B* is highly expressed in immune cells and its absence decreases viability of myeloid cells after γ -irradiation. (A) *PTP1B* mRNA basal levels of different organs from WT animals ($n = 5$) or MEFs isolated from these animals, or at 6 h after exposure to 5 Gy. The indicated tissues were isolated, the RNA extracted and analyzed for the levels of *Ptpn1* vs. the corresponding unexposed ($n = 5$) counterparts (Fold Induction, FI, was calculated vs. the liver *Ptpn1* levels from untreated animals). (B) Spleens of irradiated animals were excised off and expressed vs. the body weight of the animals (24 h). (C,D) The percentage of apoptotic cells in the spleen (per mg of tissue) and bone marrow, as well as the distribution of different subsets of myeloid cells were determined 24 h after 5 Gy irradiation of WT or *PTP1B* KO mice ($n = 4-5$). Results show the mean \pm SD of two independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$ vs. the same condition in WT animals.

3.4. *PTP1B* deficiency results in a *Bcl-2* decrease and increase in *Bax* levels in response to different stressors in macrophages

The loss in viability of *PTP1B*-deficient macrophages after irradiation reported in Fig. 2C was associated with a significant decrease in *Bcl-2* and a rise in *Bax* levels 24 h after 5 Gy exposure (Fig. 6A). Moreover, after UV challenge *Bax* levels increased similarly in WT and *PTP1B*-deficient macrophages whereas a dose-dependent increase in *Bcl-2* was observed only in WT cells (Fig. 6B). In the same way, the rise in *Bax* levels after treatment with LPS or GSNO was higher in *PTP1B* KO cells vs. WT (Fig. 6C,D), resulting in a higher increase in the *Bax/Bcl-2* ratio (Fig. 6E). Regarding *p53* levels, whereas UV treatment enhanced its expression mainly in WT cells, this effect was attenuated in *PTP1B*

KO macrophages. In addition, incubation with LPS resulted in higher levels of *p53* in *PTP1B* KO cells, probably due to the increased production of reactive oxygen and nitrogen species (Fig. 6C,D, and Fig. 3D). Regarding MAPKs signaling, we have analyzed P-ERK and P-p38 in a previous work [20] and they exhibited minimal changes in response to LPS. Here we show that P-JNK1/2 levels were higher after irradiation or GSNO treatment of *PTP1B* KO macrophages, but remained similar in WT and *PTP1B* KO cells exposed to UV (Fig. 6F-H).

3.5. Gene expression analysis of the response of macrophages from *PTP1B*-deficient mice to γ -radiation

Because *PTP1B*-deficient macrophages have an enhanced response

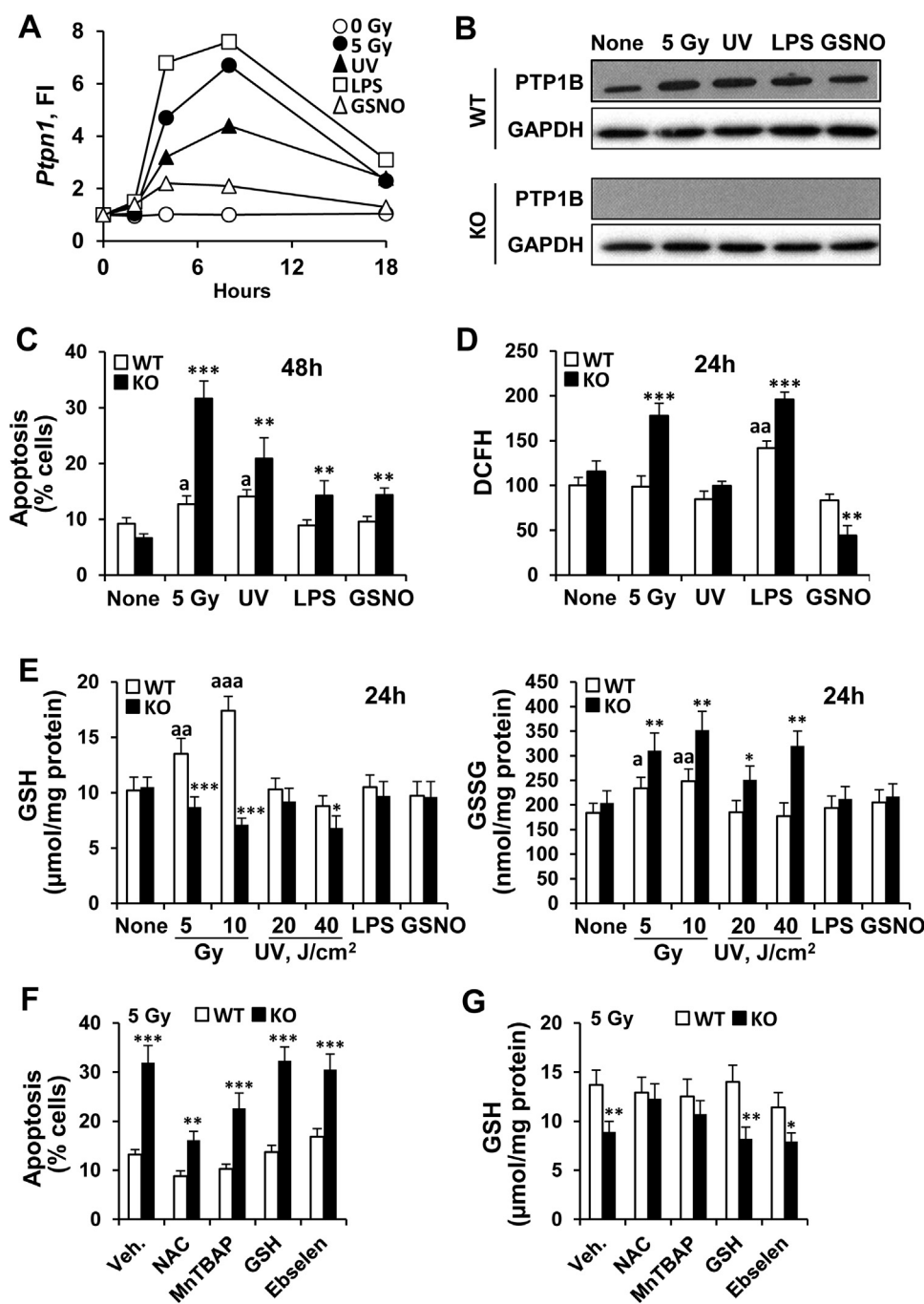


Fig. 3. Macrophages from PTP1B-deficient mice exhibit increased apoptosis and phagocytosis, and fail to restore GSH levels after 5 Gy irradiation. (A) BMDM from WT animals were exposed to 5 Gy, 20 J/cm² of UV light, 200 ng/ml of LPS or 0.5 mM GSNO and the time-course of *Ptpn1* mRNA levels was determined. (B) The protein levels of PTP1B were determined after 24 h of treatment by immunoblot. (C,D) The extent of apoptotic cells (48 h) and production of reactive oxygen species (24 h) were determined under these conditions. (E) The dose-dependent effect of γ -radiation (5 Gy and 10 Gy), UV exposure (20 and 40 J/cm²), LPS and GSNO on the GSH and GSSG levels were determined 24 h after exposure to these stressors. (F) Macrophages from WT (n = 3) or from PTP1B-deficient mice (n = 4) were isolated, incubated for 60 min with 5 mM N-acetyl-cysteine (NAC), 0.2 μ M MnTBAP, 10 mM GSH or 0.1 μ M ebselen and exposed to 5 Gy. After 48 h the percentage of apoptotic cells and the levels of GSH (G) were determined. Results show the mean of two experiments (A), a representative blot (B) and the mean \pm SD of three independent experiments (C-G), *P < 0.05; **P < 0.01; ***P < 0.005 vs. the same condition in WT cells or in the absence of challenge; ^aP < 0.05; ^{aa}P < 0.01; ^{aaa}P < 0.005 vs. the ‘none’ condition of WT cells.

to 5 Gy irradiation, a microarray gene expression analysis comparing PTP1B WT and KO macrophages was carried out. As Table 1 and Suppl. Fig. S2A–C show, 8 pathways were significantly upregulated and two down-regulated in PTP1B KO macrophages vs. the WT counterparts, after GSEA analysis. Among these pathways, the protein secretion route, the TGF- β signaling, angiogenesis and Wnt/ β -catenin pathways are of special relevance in the pathophysiological role of macrophages in different contexts (see in Discussion), and the same holds true for the down-regulated Myc and Hedgehog signaling. Indeed, *Myc* levels were determined by qPCR confirming the microarray data, and exhibited a significant decrease in irradiated PTP1B KO macrophages (Suppl. Fig. S3).

4. Discussion

Work from several groups has demonstrated that protein tyrosine phosphatases play a relevant role in the process of macrophage polarization acting, not only as negative regulators of pro-inflammatory signaling, but also as attenuators of anti-inflammatory pathways [21,33–37]. Moreover, in addition to immune regulation, an essential role for PTP1B in obesity-induced chronic low-grade inflammation and in other pathologies including diabetes, cancer, neuroinflammation, liver diseases or Rett syndrome, has been described as evidenced by the use of selective PTP1B inhibitors or animal models targeted for PTP1B [12,18,20,22,38–41].

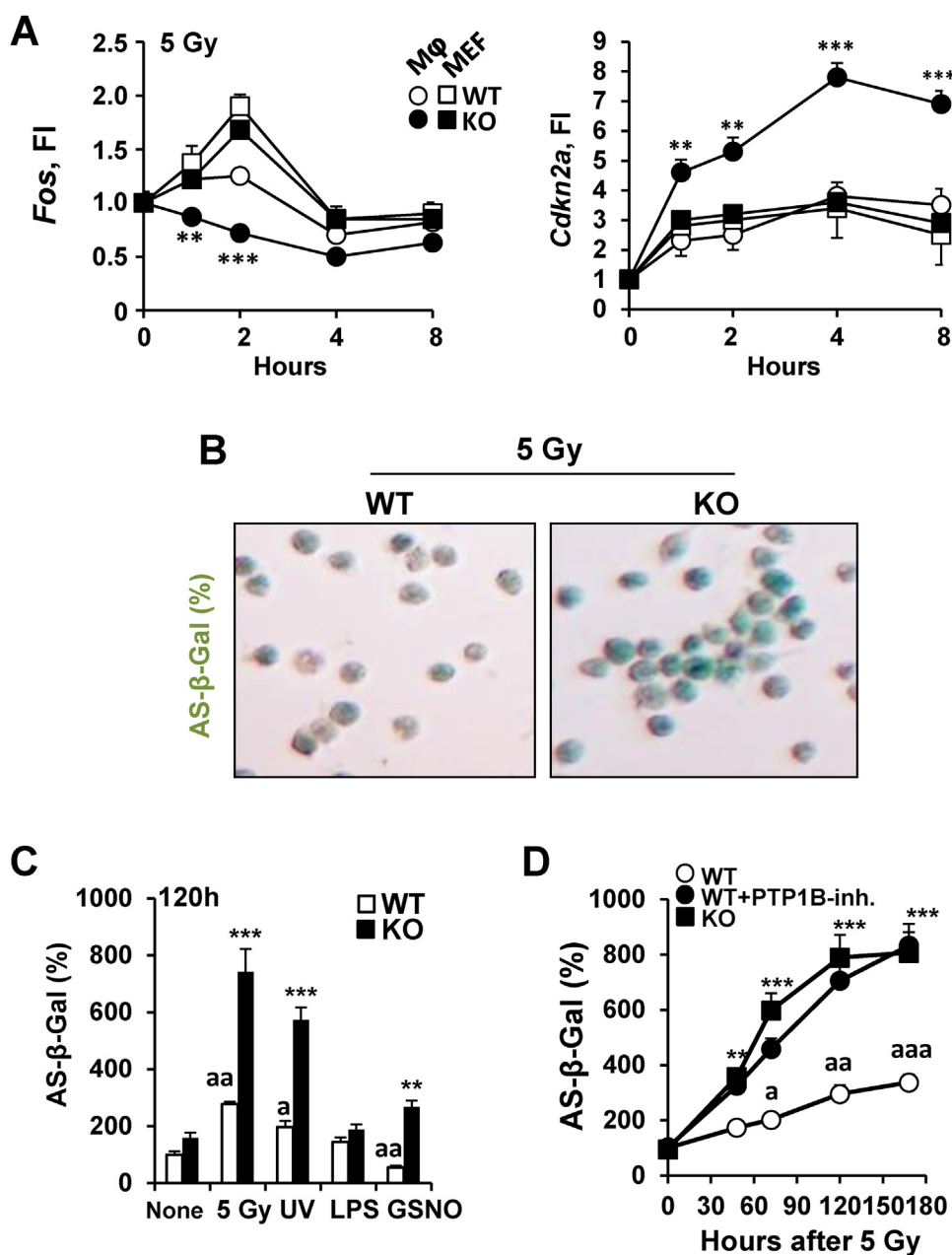


Fig. 4. PTP1B deficiency enhances senescence in macrophages submitted to 5 Gy irradiation or UV exposure. (A) BMDMs and MEFs from WT or PTP1B-deficient mice were maintained at subconfluence and then submitted to 5 Gy irradiation and at the indicated times the mRNA levels of *Fos* and *Cdkn2a* were determined by qPCR. (B) Macrophages from WT and PTP1B KO mice were irradiated (5 Gy) and the staining for acidic β -galactosidase was determined. (C-D) Macrophages from PTP1B WT mice in the absence or presence of 5 μ M PTP1B inhibitor XXII and PTP1B KO mice were submitted to 5 Gy, 20 J/cm² of UV light, 200 ng/ml of LPS or 0.5 mM GSNO and after 5 days the acidic β -galactosidase activity, and (D) results show the mean \pm SD of three independent experiments, or a representative experiment out of three (B). *P < 0.05; **P < 0.01; ***P < 0.005 vs. the same condition in the WT cells; ^aP < 0.05; ^{aa}P < 0.01; ^{aaa}P < 0.005 vs. the ‘none’ condition of WT cells. Fold induction (F.I., A).

In this work, we confirmed the enhanced sensitivity of PTP1B-deficient mice to γ -radiation. Interestingly enough, 10 Gy exerted a deleterious effect on these animals in a time shorter than 24 h. These animals exhibited hemorrhages in organs such as liver, a situation not found in their age-matched WT counterparts. Pharmacological inhibition of PTP1B partially mimicked the PTP1B-deficient genotype, with a prominent death in the initial 24 h period. In addition to this, we have evaluated metabolic parameters in animals submitted to a single 5 Gy administration, a situation in which all animals survived. Under these conditions, the severity of the irradiation was significantly higher in PTP1B-deficient mice as reflected by a reduced locomotor activity and impaired physiological functions (*i.e.*, eating and drinking), and by an attenuated RER. The latter effect could be explained by lower carbohydrate consumption due to decreases in food intake. Moreover, this mild irradiation significantly decreased myeloid cell viability in the bone marrow, in addition to splenic enlargement. Thus, PTP1B seems to be a relevant target in the protection against ionizing radiation. Indeed, absence of PTP1B reduces life-span and enhances the appearance of

lymphoproliferative disorders in mice [18]. Also, these results are in agreement with previous data on the role of myeloid cell irradiation (macrophages among them) in oncogenic treatments [42–44].

Although PTP1B is present in almost all tissues analyzed, myeloid cells, particularly monocyte/macrophages exhibited high levels of *Ptpn1* mRNA, and both mRNA and protein increased in response to different stressors, such as ionizing radiation, UV exposure or LPS challenge. It is worthy to mention that cytokines involved in the differentiation to anti-inflammatory profile of macrophages, such as IL-4, also promote elevations in PTP1B protein and enzymatic activity, suggesting a main role for this protein tyrosine phosphatase as modulator of the resolution phase of the inflammatory response [19,45]. Conversely, absence of PTP1B impairs the well-known anti-inflammatory action of IL-4, reinforcing its role in the regulation of macrophage polarization [20]. In addition to PTP1B, other protein tyrosine phosphatases, such as the T-cell protein tyrosine phosphatase (TC-PTP) that are relevant in immune cell development, play non-redundant roles in macrophage function, reflecting the singularity of these PTPs [36].

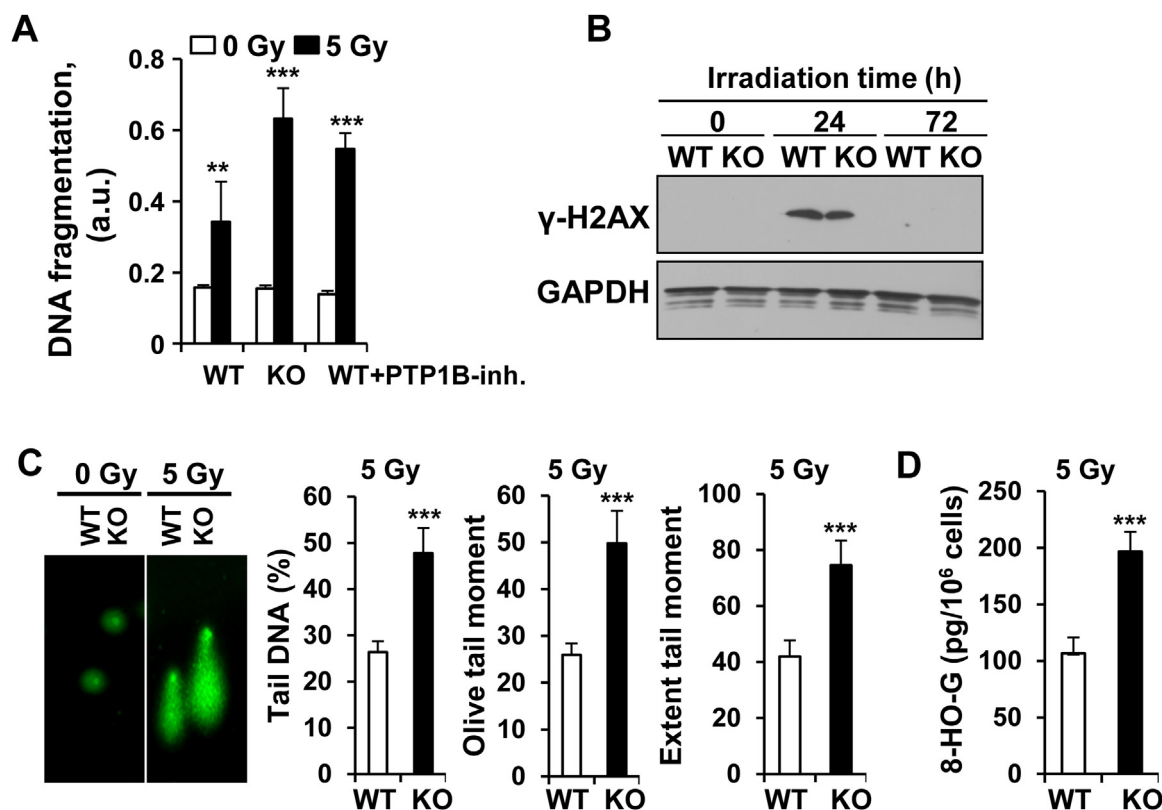


Fig. 5. *PTP1B* deficiency enhances DNA damage in macrophages submitted to 5 Gy irradiation. (A) BMDMs from WT or *PTP1B*-deficient mice were exposed to 5 Gy and the DNA fragmentation was determined at 72 h in these cells. The *PTP1B* inhibitor XXII was used at 5 μ M. (B) The phosphorylation of histone H2AX was determined by immunoblot at the indicated times. (C) Comet assay of macrophages 72 h after exposure to 5 Gy irradiation. (D) Quantification of oxidized guanine species (72 h post-irradiation). Results show the mean \pm SD of three independent experiments, or a representative experiment out of three (B,C). ** $P < 0.01$; *** $P < 0.005$ vs. the same condition in the WT cells.

Macrophages were used to gain insight on the mechanisms controlled by *PTP1B* and involved in the maintenance of cell viability after irradiation or exposure to other stressors. One of the most characteristic features of irradiated macrophages from *PTP1B* KO mice was an enhanced production of ROS together with a loss in cell viability. When GSH levels were measured, a reduced capacity of *PTP1B*-deficient macrophages to restore GSH concentration after irradiation was observed, a situation not found in the WT counterparts. Interestingly, incubation of cells with ROS scavengers significantly protected cell viability and preserved GSH levels after irradiation of *PTP1B* KO macrophages. This effect was not mimicked by GSH in the extracellular milieu. Moreover, *PTP1B* KO cells exhibited increased DNA and RNA oxidative damage as reflected by the Comet assay, by the formation of 8-hydroxy derivatives of guanine and guanosine species and by an induction of the senescence program in the surviving cells. The latter phenotype was manifested by the appearance of acidic β -galactosidase activity, and by a drop in *Fos* and a rise in *Cdkn2a* mRNA in the *PTP1B* KO macrophages. In endothelial cells, absence of *PTP1B* also promoted senescence and favored neointimal hyperplasia [46]. Since histone H2AX phosphorylation was similar in *PTP1B* WT and KO cells after irradiation, it can be concluded that the repair mechanisms are defective in the *PTP1B* KO counterparts, probably due to the decreased *Myc* signaling, as observed in the microarray analysis [47]. Indeed, the cross-talk between the c-*Myc* and p53 pathways upon irradiation are very cell dependent [48]. Here we show an up-regulation of p53 in *PTP1B* KO macrophages that may explain the decrease the repression of *Myc* after irradiation [49]. However, the situation was more complex since examination of pathways involved in DNA repair, such as p53 were defective in *PTP1B* KO macrophages exposed to irradiation or UV, but not after incubation with LPS or GSNO, suggesting the existence of

time-dependent specific mechanisms involved in the up-regulation of p53 levels in *PTP1B* KO cells after irradiation. In addition to this, Bax levels increased and Bcl-2 levels were reduced in *PTP1B* KO cells exposed to these stressors suggesting a role for these proteins in cell viability in macrophages lacking *PTP1B*.

In a previous work comparing macrophages from WT and *PTP1B*-deficient mice the microarray analysis at 24 h after LPS challenge showed main differences in gene expression in the p53 and DNA excision/repair pathways, but not in genes related to pro-inflammatory pathways, probably because they occurred at earlier times [20]. Thus, the link between the p53 pathway and the DNA excision/repair pathway fits with the observed loss of viability of *PTP1B*-deficient macrophages. This role of *PTP1B* in protecting mice against irradiation injury is a relevant issue of the present work and we suggest that strategies aimed to induce a transient *PTP1B* activity would prevent adverse effects in case of harmful exposure and, alternatively, inhibition of this activity may enhance the therapeutic action of irradiation in cancer radiotherapy. Moreover, the levels of *PTP1B* are consistently increased after stressing macrophages, probably as a compensatory response to improve cell viability under conditions of intense synthesis of reactive oxygen and/or nitrogen species that may damage the DNA. Microarray analysis of macrophages from WT and *PTP1B* KO mice 24 h after 5 Gy irradiation reflected an up-regulation of several pathways, among them protein secretion, the Wnt/ β -catenin pathway, the TGF- β signaling pathway and the IL-6/Jak/Stat3 pathway. In agreement to these changes, an increase in the Wnt/ β -catenin pathway [43] and vascular development [42,50] have been described. Although these pathways regulate many macrophage activities, it should be expected a specific phenotype in the irradiated cells lacking *PTP1B*. In the same way, the *Myc* and the Hedgehog signaling pathways are repressed in

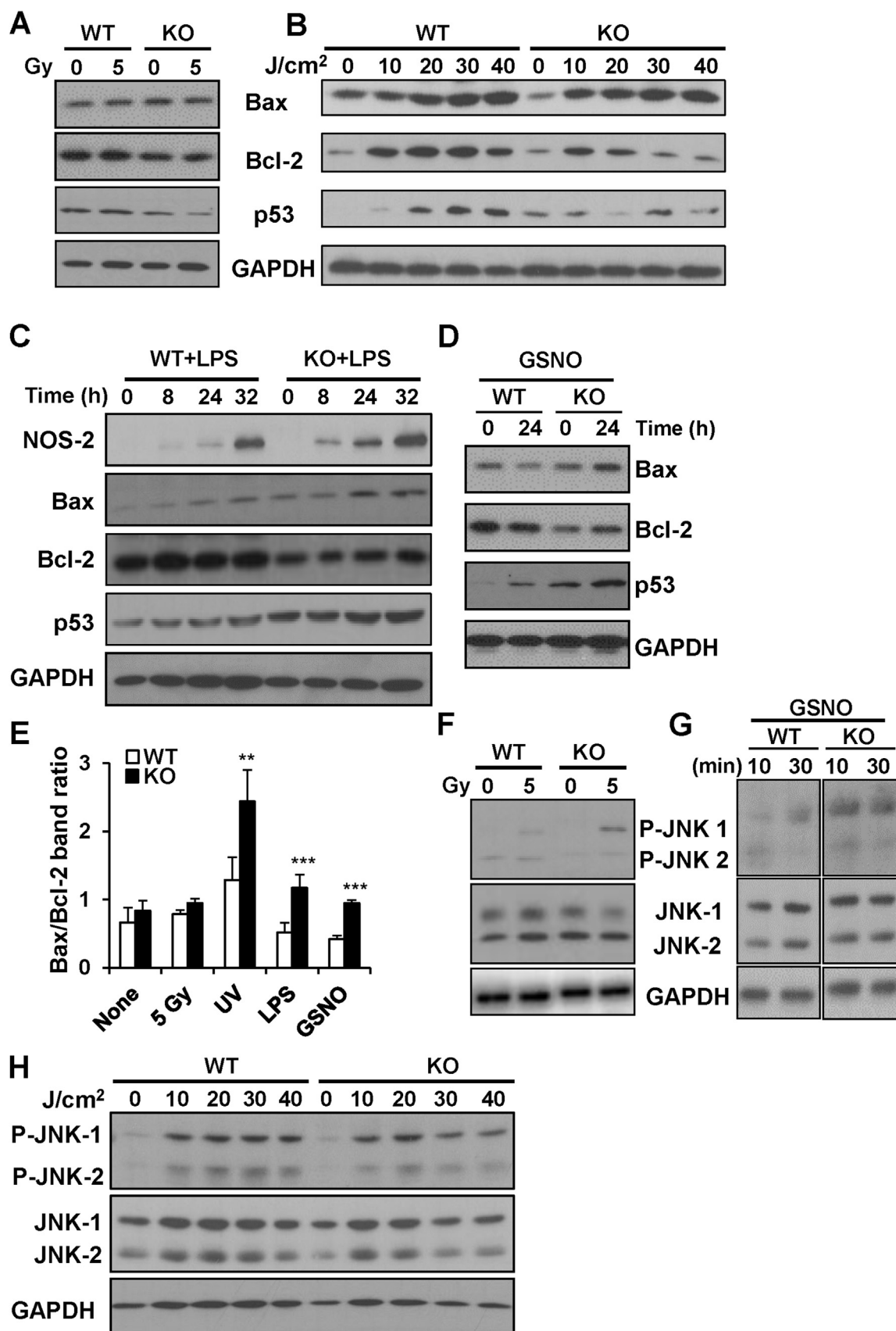


Fig. 6. PTP1B-deficient macrophages exhibit higher Bax/Bcl-2 ratios in response to different stressors. Macrophages from PTP1B WT or PTP1B KO mice were submitted to 5 Gy, UV light, 200 ng/ml of LPS or 0.5 mM GSNO and the levels of Bax, Bcl-2, p53, GAPDH and NOS-2 (in the case of LPS challenge) were determined after 24 h (A,B) or at the indicated times (C,D). (F-H) The levels p-JNK1/2 after 30 min or at the indicated times were determined. Results show a representative experiment out of three and (E) the mean \pm SD of the Bax/Bcl-2 ratios at 24 h (30 J/cm² in the case of UV light). **P < 0.01; ***P < 0.005; vs. the same condition in the WT cells.

Table 1

GSEA analysis of microarray data from macrophages from PTP1B WT and PTP1B KO mice exposed to 5 Gy irradiation. Cells from 4 independent animals for each condition were maintained in culture for 24 h and after analysis of the RNA quality in a Bioanalyzer, equal amounts of RNA were pooled and submitted to microarray hybridization. Gene Set Enrichment Analysis (GSEA) was performed and the quantitative parameters of the significant pathways (FDR < 0.25) are provided. Results show the data from two independent experiments as reflected in [Supplementary Fig. S1](#).

Pathway	Enrichment score	Normal p-value	FDR q-value
Upregulated			
Protein secretion	0.374	0.0106	0.2331
TGF- β signaling	0.418	0.0191	0.1216
Angiogenesis	0.442	0.0445	0.1124
Androgen receptor	0.336	0.0720	0.2194
Wnt/ β -Catenin	0.386	0.1013	0.2093
IFN- α response	0.316	0.0972	0.2385
Peroxisome	0.309	0.1037	0.2257
IL-6/Jak/Stat-3	0.308	0.1507	0.2338
Downregulated			
Myc targets	– 0.436	0.0111	0.0434
Hedgehog signaling	– 0.443	0.0482	0.0746

PTP1B KO macrophages, and confirm the deficient DNA repair and the relevant pathophysiological role of macrophages after irradiation [47,51,52]. Noteworthy, the p53 and the DNA excision/repair pathways that are upregulated in the LPS condition in PTP1B KO cells are not altered after irradiation; the possibility exists that the gene changes occurred earlier than the 24 h of analysis performed in our arrays.

In conclusion, our data show an enhanced sensitivity to γ -radiation and to other stressors in mice and macrophages lacking PTP1B and exposed to irradiation. These results might be relevant for patients submitted to irradiation for therapeutic purposes, or for personnel on irradiation risk. Under these circumstances, favoring PTP1B activation might reduce the deleterious effects in immune cells. Unraveling the exact contribution of PTP1B activity to these situations might help to develop both protocols for protection or increased immune damage in these interventions.

5. Conclusions

The results presented describe a relevant role for PTP1B in the protection against ionizing irradiation, and can be used either to enhance sensitivity, by using selective inhibitors, or to increase resistance after induction and/or activation of this phosphatase.

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Authors disclosure statement

None.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.redox.2018.04.018>.

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