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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 × 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 florescence 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 synthetizes one μmol 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