Mitochondrial superoxide dismutase 2 mediates γ-irradiation-induced cancer cell invasion

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Abstract
Sublethal doses of γ-rays promote cancer cell invasion by stimulating a signaling pathway that sequentially involves p53, sulfatase 2 (SULF2), β-catenin, interleukin-6 (IL-6), signal transducer and activator of transcription 3 (STAT3), and Bcl-XL. Given that Bcl-XL can increase O2− production by stimulating respiratory complex I, the possible role of mitochondrial reactive oxygen species (ROS) in γ-irradiation-induced cell invasion was investigated. Indeed, γ-irradiation promoted cell invasion by increasing mitochondrial ROS levels, which was prevented by metformin, an inhibitor of complex I. γ-Irradiation-stimulated STAT3 increased the expression of superoxide dismutase 2 (SOD2), a mitochondrial enzyme that catalyzes the conversion of O2− to hydrogen peroxide (H2O2). In contrast to O2−, H2O2 functions as a signaling molecule. γ-Irradiation consistently stimulated the Src-dependent invasion pathway in a manner dependent on both complex I and SOD2. SOD2 was also essential for the invasion of un-irradiated cancer cells induced by upregulation of Bcl-XL, an intracellular oncogene, or extracellular factors, such as SULF2 and IL-6. Overall, these data suggested that SOD2 is critical for the malignant effects of radiotherapy and tumor progression through diverse endogenous factors.

Introduction
Ionizing radiation (IR), such as γ-irradiation, is a major therapeutic modality for treating cancer. In most patients, IR offers a significant survival benefit, but in some patients, local recurrence or distal metastasis following radiotherapy is a major therapeutic challenge. These undesirable consequences may reflect the regrowth or spread of cancer cells that survived radiotherapy. Studies using cultured cells and animal models have shown that sublethal doses of IR increase the mobility, invasiveness, and metastatic potential of cancer cells1,2, suggesting that IR promotes malignant behavior in postradiation tumors. Therefore, the cellular components involved in the malignant effects of IR should be defined to develop new strategies for improving the therapeutic effects of IR.

Mitochondria have emerged as central regulators of cancer cell invasion and metastasis, and reactive oxygen species (ROS) produced via the mitochondrial respiratory chain have been implicated as stimulators of various cellular pathways leading to cell migration and invasion3. The production of mitochondrial ROS is regulated by Bcl-2 family proteins4. Although they were originally identified as key regulators of cell death5, certain Bcl-2 family members also regulate cell migration, invasion, and cancer metastasis4. A well-characterized example is the group of pro-survival Bcl-2 family members, including Bcl-XL, Bcl-2, and Bcl-w, which stimulate complex I, a major source of ROS in the mitochondrial respiratory chain, to produce additional ROS. The ROS produced the following overexpression of Bcl-w, or Bcl-XL promote cell invasion by stimulating Src and its downstream signaling components6.

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We have previously shown that sublethal doses of IR increase sulfatase 2 (SULF2) expression via the p53 transcription factor. SULF2 is an extracellular sulfatase that modulates the signaling activities of diverse cell surface receptors, and IR-induced SULF2 mediates the pro-invasive activity of IR by stimulating the signaling pathway that sequentially involves β-catenin, interleukin-6 (IL-6), and signal transducer and activator of transcription 3 (STAT3). STAT3 is a transcription factor that induces Bcl-XL expression. Consistently, sublethal doses of IR increase the messenger RNA (mRNA) and protein levels of Bcl-XL in several cancer cell types, and Bcl-XL knockdown abolishes the pro-invasive activity of IR, suggesting a role for Bcl-XL in IR-induced cell invasion. These results suggest the involvement of mitochondrial ROS in IR-induced cancer cell invasion. However, this possibility has not been directly addressed.

ROS include free radicals, such as superoxide anion (O$_2^-$) and hydroxyl radical (HO•), as well as nonradical molecules, such as hydrogen peroxide (H$_2$O$_2$). Among these free radicals, H$_2$O$_2$ has a relatively long half-life and can freely diffuse to induce signaling. Therefore, it is thought that H$_2$O$_2$ is the effector ROS that modulates the signaling activities of diverse cell surface receptors. However, the mitochondrial respiratory chain produces O$_2^-$ that needs to be converted to H$_2$O$_2$ to modulate signaling. Superoxide dismutase (SOD) is a metalloenzyme that catalyzes the conversion of O$_2^-$ to H$_2$O$_2$. In mammals, there are three distinct types of SOD as follows: Cu/ZnSOD (SOD1), MnSOD (SOD2), and extracellular Cu/ZnSOD (SOD3). SOD1 is the major intracellular form of SOD, and it is localized primarily in the cytosol. In contrast, SOD2 is exclusively localized to the mitochondrial matrix. This feature of SOD2 suggests that it may be involved in the conversion of mitochondrial O$_2^-$ to H$_2$O$_2$, thus contributing to cell invasion. Hence, the present study investigated the potential role of SOD2 in IR-induced cell invasion. The possibility was indeed supported by our data. The present findings demonstrated that SOD2 also mediates the invasion of un-irradiated cancer cells induced by upregulation of diverse oncogenic components, supporting the role of SOD2 in tumor progression. Therefore, SOD2 is a potential target for preventing cancer cell invasion following radiotherapy and suppressing tumor progression under diverse conditions.

Materials and methods
Antibodies and recombinant proteins
The following antibodies were used in the present study: anti-SOD2 from Enzo Life Sciences (Farmingdale, NY, USA); anti-IL-6 and anti-β-catenin from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-Bcl-XL, anti-STAT3, anti-Src, and anti-phospho-Src from Cell Signaling Technology (Danvers, MA, USA); and anti-β-actin from Sigma-Aldrich (St. Louis, MO, USA). Recombinant human IL-6 was purchased from Millipore (Darmstadt, Germany).

siRNA and shRNAs
Small interfering RNA (siRNAs) targeting IL-6 (S7312) and Bcl-XL (120717) were purchased from Ambion (Austin, TX, USA). siRNAs targeting SOD2 (sc-41655), β-catenin (sc-44275), and STAT3 (sc-29209) as well as lentiviruses expressing small hairpin RNAs (shRNAs) targeting SOD2 (sc-41655-V), Bcl-XL (sc-43630-V), and SULF2 (sc-63088-V) were obtained from Santa Cruz Biotechnology.

Cell culture, transfection, infection, and treatments
All cell lines used in this study, except for HCT116/p53$^{wt}$ and HCT116/p53$^{−/−}$ colon cancer cells (a generous gift from Dr. Bert Vogelstein), were obtained from the American Type Culture Collection (Manassas, VA, USA). Cells were cultured in RPMI-1640 (A549, H1299, and H460 lung cancer cells) or DMEM (MCF-7 breast cancer and HCT116 cells) medium supplemented with 10% heat-inactivated fetal bovine serum (FBS). Expression constructs for SOD2, SULF2, and Bcl-XL were prepared using the pcDNA3 vector (Invitrogen, Carlsbad, CA, USA). Expression constructs and siRNAs were introduced into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. The transfectants were used for experiments after 48 h of recovery. For long-term gene silencing, cells were infected with lentiviruses containing shRNAs targeting SOD2 in the presence of polybrene (5 µg/mL) according to the manufacturer’s protocols. Infected cells were selected with puromycin (2 µg/mL). For irradiation, cells were exposed to the specified doses of γ-rays from a $^{137}$Cs γ-ray source (Atomic Energy of Canada, Chalk River, Canada) at a dose rate of 3 Gy/min. Alternatively, cells were treated with the indicated concentrations of IL-6.

Western blotting
Cell lysates were prepared using previously described methods. Proteins in the lysates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, electrotransferred onto nitrocellulose membranes (Millipore), and analyzed using the specified antibodies and an ECL detection system (Bio-Rad, Hercules, CA, USA).

RT-PCR and quantitative real-time PCR
Reverse transcription-PCR (RT-PCR) was performed by amplifying complementary DNA (cDNA) in Premix PCR solution (Takara, Shiga, Japan) with SOD2 primers (5′-GGA-AGC-CAT-CAA-ACG-TGA-CTT-3′ and 5′-GGTG-CTC-CCA-CAC-ATC-AAT-CC-3′). Quantitative
real-time PCR was performed using the SYBR Fast Universal qPCR Kit (Kapa Biosystems, Wilmington, MA, USA) and SOD2 primers (5′-GGCG-CTA-GGA-CA-GCT-GGA-3′ and 5′-GTT-GAA-CAT-GCT-TGG-CCA-3′). GAPDH was amplified in both PCR assays with the following primers as an internal control for normalization: 5′-CAT-CTC-TGCC-CCC-CTC-TGC-TG A-3′ and 5′-GGA-TGA-CCT-TGC-CCA-CAG-CCT-3′. The RT-PCR and real-time PCR results were analyzed by agarose gel electrophoresis and an IQ-5 Real-Time System (Bio-Rad), respectively.

**Invasion assay**

As described previously, cells in serum-free medium were seeded onto the upper surfaces of Matrigel-coated Transwell chambers (BD Biosciences, San Jose, CA, USA). The lower compartments of the chambers were filled with medium supplemented with 10% heat-inactivated FBS. After 16 h of incubation, cells that invaded the lower surface of the filter were stained with the Diff-Quick Kit (Fisher Scientific, Waltham, MA, USA) and counted under a microscope.

**Analysis of mitochondrial ROS levels**

Cells were exposed to 10 μM MitoSOX Red (Invitrogen) or 5 μM Peroxy Orange-1 (Tocris Bioscience, Bristol, UK) for 30 min, and cell-associated fluorescence was analyzed by flow cytometry.

**Clonogenic assay**

Various numbers of cells infected with the specified lentiviruses were seeded in triplicate into 60 mm dishes (100, 200, 400, and 800 cells/dish). After 24 h of incubation, cells were exposed to different doses of γ-rays (1, 3, 5, and 7 Gy). Irradiated and untreated control cells were cultured for 14 days. The number of colonies was counted with a colony counter (Imaging Products, Hollywood, CA, USA), and clonogenic survival was calculated as described previously.

**Statistical analysis**

All experiments were performed at least three times to obtain means and standard deviations. Statistical significance was determined with one-way analysis of variance (GraphPad Software, La Jolla, CA, USA), and *p* values <0.05 were considered significant.

**Results**

**Sublethal doses of IR increase SOD2 expression via the p53/SULF2/β-catenin/IL-6/STAT3 pathway**

To investigate the potential involvement of SOD2 in IR-induced cell invasion, p53<sup>wt</sup>-expressing (H460 and A549 lung cancer cells as well as HCT116 colon cancer cells) and p53<sup>null</sup> cells (H1299 lung cancer cells) were irradiated with sublethal doses of γ-rays. Irradiation elevated protein levels of SOD2 in the p53<sup>wt</sup>-expressing cells but not in the p53<sup>null</sup> cells (Fig. 1a). Consistently, knockout of p53 in HCT116 cells abolished IR-induced SOD2 accumulation. It has been previously confirmed that p53 protein levels in p53<sup>wt</sup>-expressing cells are elevated upon γ-irradiation, but that p53 expression is not detected in p53<sup>null</sup> or p53-knockout cells even after γ-irradiation. These findings suggested that the γ-irradiation mediated increase in SOD2 levels is p53 dependent.

p53 mediates IR-induced cell invasion by stimulating cellular pathways sequentially involving SULF2, β-catenin, IL-6, and STAT3. To investigate the relationship between this pathway and SOD2 induction, SULF2 was knocked down in H460 and A549 cells using a specific siRNA, which abolished or attenuated IR-induced SOD2 accumulation (Fig. 1a). Consistently, SOD2 protein levels were increased following overexpression of SULF2 in unirradiated cells (Fig. 1b), confirming that SULF2 increases SOD2 protein levels. Moreover, knockdown of β-catenin, IL-6, or STAT3 using specific siRNAs reduced IR-induced accumulation of SOD2 protein (Fig. 1c), suggesting that IR increases SOD2 levels via the p53/SULF2/β-catenin/IL-6/STAT3 pathway.

In addition, IR elevated mRNA levels of SOD2, and this increase was abolished by knockdown of SULF2 (Fig. 1d) or STAT3 (Fig. 1e), suggesting that IR induces SOD2 protein accumulation by increasing its mRNA levels via STAT3 stimulated by the SULF2 pathway. The ability of STAT3 to induce SOD2 gene expression has also been reported in a mouse model of ischemic brain injury. Moreover, the promoter region of SOD2 has been shown to contain STAT3-binding sites.

**SOD2 mediates IR-induced cell invasion**

To investigate the potential role of SOD2 in IR-induced cell invasion, SOD2 was overexpressed in H460 and A549 cells. The invasiveness of both cell lines was enhanced by SOD2 overexpression (Fig. 2a). Consistently, IR-induced cell invasion was reduced by knockdown of SOD2 (Fig. 2b), suggesting that SOD2 mediates IR-induced cell invasion. Consistent with the role of SOD2, its knockdown also abolished cell invasion induced by SULF2 overexpression (Fig. 2c) or IL-6 treatment (Fig. 2d).

**IR induces activation of the mitochondrial ROS/Src-dependent invasion pathway**

In response to IR, STAT3 increases the mRNA and protein levels of Bcl-X<sub>L</sub>, which is essential for IR-induced cell invasion. To eliminate the possibility of Bcl-X<sub>L</sub> influencing SOD2 expression, Bcl-X<sub>L</sub> was overexpressed in H460 cells. Bcl-X<sub>L</sub> overexpression did not significantly affect SOD2 mRNA and protein levels (Fig. 3a), confirming the irrelevance of Bcl-X<sub>L</sub> in SOD2 induction. These data
suggested that STAT3 promotes the expression of Bcl-X<sub>L</sub> and SOD2 in response to γ-irradiation. Notably, these two proteins co-accumulated at 24 and/or 48 h postradiation (Fig. 3b). This phenomenon was observed in four different p53<sup>wt</sup> cancer cell lines, including lung (H460 and A549), colon (HCT116/p53<sup>wt</sup>), and breast cancer cells (MCF-7), and the post-irradiation peak accumulation time was cell type-dependent. The co-induction of Bcl-X<sub>L</sub> and SOD2 implied their cooperation in IR-induced cellular responses.

Bcl-X<sub>L</sub> overexpression increases the ability of complex I to produce ROS, which, in turn, stimulates the Src-dependent invasion pathway<sup>6</sup>. Given that IR upregulates Bcl-X<sub>L</sub><sup>10</sup>, it may also stimulate the ROS/Src pathway. Because complex I produces O<sub>2</sub><sup>•−</sup>, the levels of mitochondrial O<sub>2</sub><sup>•−</sup> levels in control and irradiated cells were compared using MitoSox Red, a probe specific for mitochondrial O<sub>2</sub><sup>•−</sup>. IR increased mitochondrial O<sub>2</sub><sup>•−</sup> levels, which was prevented by N-acetylcysteine (NAC), an ROS

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**Fig. 1 IR induces SOD2 expression via the p53/SULF2/β-catenin/IL-6/STAT3 pathway.**

- **a** Western blotting and RT-PCR were performed 48 h after γ-irradiation. H460 and A549 lung cancer cells (p53<sup>wt</sup>) were infected with lentiviruses expressing control (nontargeting sequence) or SULF2-specific shRNA. These transfectants, along with H1299 lung cancer cells (p53<sup>null</sup>) and p53<sup>wt</sup>-expressing or p53-knockout HCT116 colon cancer cells, were irradiated with the indicated doses of γ-rays, and SOD2 levels were compared by western blot analysis using β-actin as a loading control. SULF2 expression was compared by RT-PCR using GAPDH as a loading control.

- **b** A549 and H460 cells were transfected with an empty or SULF2 expression vector, and SOD2 protein and SULF2 mRNA levels were compared.

- **c** H460 cells treated with a control or an siRNA targeting β-catenin, IL-6, or STAT3 were irradiated with 2 Gy of γ-rays, and the levels of the indicated proteins were compared.

- **d** H460 cells infected with the lentiviruses indicated in **a** were irradiated, and SOD2 mRNA levels were analyzed by RT-PCR.

- **e** H460 cells treated with a control or a STAT3-targeting siRNA were irradiated, and SOD2 mRNA levels were compared by quantitative real-time PCR at 24 and 48 h after irradiation.
scavenger, or metformin, an inhibitor of complex I \(^20\) (Fig. 3c), suggesting that complex I contributes to IR-induced \(\text{O}_2^-\) production. IR also increased Src phosphorylation, and both IR-induced cell invasion and Src phosphorylation were abolished by NAC or metformin (Fig. 3d). Similar effects on IR-induced Src phosphorylation were observed following Bcl-XL knockdown (Fig. 4a, left). These results suggested that \(\text{O}_2^-\) produced by the Bcl-XL/complex I pathway contributes to the ability of IR to promote the Src-dependent invasion pathway.

**SOD2 mediates IR-induced Src phosphorylation in a cooperative manner with Bcl-XL**

While complex I produces \(\text{O}_2^-\), \(\text{H}_2\text{O}_2\) is the ROS that stimulates Src phosphorylation \(^21\). Therefore, SOD2 may mediate IR-induced cell invasion by converting \(\text{O}_2^-\) to \(\text{H}_2\text{O}_2\). IR-induced Src phosphorylation was attenuated by SOD2 knockdown (Fig. 4a, right). SOD2 knockdown also abolished Bcl-XL-induced cell invasion (Fig. 4b, left), a result consistent with the view that SOD2 acts downstream of Bcl-XL to promote cell invasion. However, cell invasion induced by SOD2 overexpression was also prevented by Bcl-XL knockdown (Fig. 4b, right), suggesting that SOD2 requires Bcl-XL for cell invasion. The functional interdependence of Bcl-XL and SOD2 supports the view that both \(\text{O}_2^-\) production (by Bcl-XL) and its conversion to \(\text{H}_2\text{O}_2\) (by SOD2) are critical for cell invasion. The functional cooperation between Bcl-XL and SOD2 was further supported by the finding that overexpression of both Bcl-XL and SOD2 was superior to overexpression of either alone for inducing Src phosphorylation and cell invasion (Fig. 4c).

To directly confirm the role of SOD2 in the conversion of IR-induced \(\text{O}_2^-\) to \(\text{H}_2\text{O}_2\), the levels of \(\text{O}_2^-\) and \(\text{H}_2\text{O}_2\) were determined using MitoSox Red and Peroxy Orange \(^22\), respectively. SOD2 knockdown increased the amount of \(\text{O}_2^-\) and decreased \(\text{H}_2\text{O}_2\) levels in both control and irradiated cells (Fig. 4d). Consistently, SULF2 overexpression and IL-6 treatment increased \(\text{H}_2\text{O}_2\) levels, and this increase was prevented by SOD2 knockdown.
Therefore, these findings clearly demonstrated that SOD2 contributes to the conversion of $O_2^{-}$ generated via the IR-induced SULF2/IL-6 pathway to $H_2O_2$.

**SOD2 promotes cell invasion without altering radiosensitivity**

SOD2 contributes to radioresistance in certain but not all cell types\(^{23,24}\). Colony-forming assays revealed that SOD2 knockdown did not significantly influence the radiosensitivity of H460 and A549 cells (Fig. 5). However, IR-induced cell invasion was inhibited by SOD2 knockdown (Fig. 2b), suggesting that SOD2 mediates IR-induced cell invasion without altering cellular radiosensitivity.

**Discussion**

In the present study, overexpression or knockdown SOD2 in lung cancer cells showed that SOD2 promotes cancer cell invasion. As similar results have been reported for other types of cancer, such as fibrosarcoma\(^{25}\), salivary\(^{26}\), and tongue carcinomas\(^{27,28}\), the pro-invasive activity of SOD2 appears to be a common feature of diverse cancers. This view is consistent with the report that SOD2 is upregulated in many cancer types, including...
lung, breast, colon, brain, thyroid, gastric, and salivary cancers, and especially late-stage metastatic cancers. SOD2 upregulation in cancer cells has been correlated with distant metastasis, poorer prognosis, and lower overall and disease-free survival. Therefore, SOD2 upregulation is critical for tumor progression.

An important finding of the present study was that SOD2 functions as a key mediator of IR-induced cancer cell invasion, which was initially indicated by the finding that SOD2 mRNA and protein levels were elevated in cancer cells that survived γ-irradiation. In addition, the ability of IR to increase SOD2 expression has been reported in human fibroblasts as well as in mouse brain and gut. In the present study, the IR-induced SOD2 expression was mediated by the p53/SULF2/β-catenin/IL-6/STAT3 pathway, which has been previously shown to mediate IR-induced cancer cell invasion. Moreover, prevention of IR-induced cancer cell invasion by SOD2 knockdown directly supports the role of SOD2 in the malignant effects of sublethal doses of IR. This finding and our previous findings demonstrate that IR-stimulated STAT3 promotes cancer cell invasion by inducing the expression of SOD2 and Bcl-XL. Based on the recent report that Bcl-XL upregulation increases the ability of complex I to produce ROS, which then stimulate the Src-dependent invasion pathway, the ability of IR to utilize the mitochondrial pathway and promote cancer cell invasion was investigated. The findings demonstrated that IR increased mitochondrial ROS production and Src phosphorylation. Notably, ROS production, Src phosphorylation, and IR-induced cell invasion were prevented by treatment with an ROS scavenger (NAC) or a complex
I inhibitor (metformin), thereby supporting the view that IR stimulates the Src-dependent invasion pathway by promoting complex I-dependent ROS production. Thus, SOD2 likely supports IR-induced cell invasion by converting O$_2^{-}$ generated by complex I to H$_2$O$_2$, a signaling molecule that stimulates Src phosphorylation 21, which was supported by the finding that SOD2 knockdown abolished IR-induced Src phosphorylation, which was supported by the finding that SOD2 knockdown abolished IR-induced Src phosphorylation. Similar effects were observed when Bcl-XL was knocked down, indicating that both Bcl-XL and SOD2 are required for IR-mediated induction of the Src-dependent invasion pathway. Thus, IR-induced Bcl-XL may increase the ability of complex I to produce O$_2^{-}$, which is then converted to H$_2$O$_2$ by SOD2 (Fig. 6). The ability of SOD2 to convert IR-induced O$_2^{-}$ to H$_2$O$_2$ was further confirmed by determining levels of O$_2^{-}$ and H$_2$O$_2$ using specific dyes, namely, MitoSox Red and Peroxy Orange-1, respectively. According to the present model, SOD2 acts downstream of Bcl-XL in an IR-induced signaling pathway, leading to cell invasion. However, despite this hierarchy, the functional relationship of Bcl-XL and SOD2 was cooperative. Both Bcl-XL and SOD2 were required to promote cell invasion, and their co-expression induced Src phosphorylation and cell invasion much more effectively than either alone, suggesting that both O$_2^{-}$ production and its conversion to H$_2$O$_2$ are required for the pro-invasive activity of IR. The present data also suggested that IR fulfils these two requirements by co-inducing Bcl-XL and SOD2 via STAT3. This co-induction was verified in lung, colon, and breast cancer cells. Therefore, the model depicted in Fig. 6 is a general mechanism applicable to diverse cancer types.

The ability of p53 to induce SOD2 expression has been reported by other investigators 31,32. Although the consensus p53-binding sequences have been identified in the promoter region of SOD2 31, they are not required for the transcriptional activation of SOD2 by p53 32, suggesting that p53 induces SOD2 expression indirectly, which is consistent with the present model (Fig. 6).

Some investigators have reported that SOD2 protects cells from IR 23, while others have reported that SOD2 is irrelevant in radioresistance 24. While the reason for this discrepancy is still unclear, the present study demonstrated that SOD2 did not significantly influence the radiosensitivity of H460 and A549 cells. Considering that SOD2 plays an essential role in the IR-induced invasion of H460 cells, it is clear that SOD2 mediates IR-induced cell invasion without altering cellular radiosensitivity.

The present data also suggested that the pro-invasive role of SOD2 is not restricted to extrinsic treatments, such as radiotherapy, as it was also involved in intrinsic factor-induced cell invasion. In the present study, SOD2 was essential for cell invasion induced by Bcl-XL, an oncogene upregulated in many cancers 4. In addition, SOD2 mediated cell invasion induced by extracellular factors, such as IL-6 and SULF2, suggesting that SOD2 plays an essential role in cell invasion induced in response to tumor microenvironment changes. Thus, SOD2 is critical for tumor progression under diverse conditions.

Metformin is a first-line medication for patients with type 2 diabetes because it reduces hyperglycemia by suppressing hepatic gluconeogenesis 33. However, accumulating evidence has supported the potential of
metformin for cancer therapy. Metformin has been shown to inhibit the growth of many cancer types, and among cancer patients with diabetes, metformin users show better survival than non-users. Metformin also improves tumor responses to radiotherapy by acting as a radiosensitizer, but also by preventing malignant actions of IR. The present study showed that SOD2 is a key mediator of IR-induced cancer cell invasion, thereby supporting the critical role of mitochondria in cancer cell invasion and metastasis. Considering the short half-life of $\mathrm{O}_2^-$, mitochondrial SOD2 may have an advantage for converting mitochondrial $\mathrm{O}_2^-$ to $\mathrm{H}_2\mathrm{O}_2$. Therefore, mitochondrial components may be potential therapeutic targets for overcoming the malignant effects of IR.

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Conflict of interest

The authors declare that they have no conflict of interest.
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