Heat Killed Salmonella typhimurium protects intestine against radiation injury through Wnt signaling pathway

Yuanyuan Chen
Second Military Medical University https://orcid.org/0000-0002-6142-5913
Kun Cao
Second Military Medical University
Xuguang Hu
Second Military Medical University First Hospital: Changhai Hospital
Tingting Liu
Second Military Medical University
Bailong Li
Second Military Medical University
Jianguo Cui
Second Military Medical University
Jian ming Cai
Second Military Medical University
Fu Gao
Second Military Medical University
Yanyong Yang (yyyang2010@163.com)
Second Military Medical University

Research

Keywords: Heat Killed Salmonella typhimurium, intestinal injury, ionizing radiation, DNA damage response, Wnt signaling

Posted Date: October 28th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-96695/v1

Version of Record: A version of this preprint was published at Journal of Oncology on June 18th, 2021. See the published version at https://doi.org/10.1155/2021/5550956.

Abstract

Purpose: Gastrointestinal (GI) toxicity caused by ionizing radiation (IR) appears to be a limited factor in radiotherapy and a great threat for soldiers on nuclear-related military missions. However, there are currently no approved medical strategies to effectively prevent or mitigate the damage on intestine induced by IR. The present study aimed to elucidate the protective activity of Heat Killed Salmonella typhimurium (HKST) on intestine against ionizing radiation exposure.

Materials and Methods: Mouse intestinal crypts and intestinal organoids were isolated from C57BL/6J mice. Mice and HIEC cells were exposed to ^{60}Co γ-radiation. The dosage was set as following: whole body radiation of 8 Gy; mouse intestinal organoids were irradiated with 0 Gy, 4 Gy, and 6 Gy of γ-rays at dose rate of 1 Gy/min. Cells or mice were pretreated with Heat Killed *Salmonella typhimurium* (HKST) (10^7 cells/mice) at 12 hours prior to irradiation.

Results: By culturing mouse intestinal organoids and employing whole body irradiation of mice, we found that the radiation-induced damage on intestine was dramatically mitigated by pretreating with HSKT in vitro and in vivo, in which the structure of intestinal organoids and small intestine of the HKST-treated group was more integrated than of the radiation damage group, and HKST pretreatment remarkably promoted the proliferation of intestinal cells post IR exposure (Figure 1 and 2). Further study showed that the radio-protective effects of HKST was involved in DNA damage response (DDR) signaling. Moreover, the stimulation of DDR signaling by HKST upon radiation damage was mediated by Wnt signaling, in which the inhibition of Wnt signaling diminished the radio-protective effects of HKST.

Conclusion: Our study demonstrated that Heat Killed *Salmonella typhimurium* (HKST) could significantly alleviate the damage on intestine induced by IR, and the radio-protective effect of HKST was depended on DDR mediated by Wnt signaling pathway. These findings demonstrated that HKST is a potential bacterium using for the prevention of IR-induced GI toxicity in clinical practice.

Introduction

Patients received radiotherapy and victims from nuclear accident unavoidably suffer from the damage induced by ionizing radiation (IR) [1]. Acute radiation sickness, which is also known as acute radiation syndrome (ARS), often occurs after a sudden high dose of IR exposure. At a dose less than 6 Gy, the fatal injuries are primarily as a result of hematopoietic syndrome that can be prevented by the bone marrow shielding or cured by transplant of bone marrow [2, 3]. Gastrointestinal (GI) tract represents other susceptible organ to IR injury. GI toxicity (the GI syndrome) primarily induced by a higher dose of radiation, in which a mass of intestinal stem cells (ISCs) are irreparably killed, the regeneration of villi is severely impaired and the epithelial integrity along the entire GI tract is compromised [4]. Victims with GI syndrome usually bear the pain of fluid loss, malabsorption, and electrolyte imbalances [5]. Besides, the invasion of enteric pathogens and flora into the bloodstream due to the epithelial integrity damage could lead to sepsis and death [6]. Unfortunately, there are currently no medical countermeasures approved to

prevent or ameliorate GI syndrome [7]. Although a handful of Food and Drug Administration (FDA)–approved radioprotectors were reported to eliminate internally ingested radiation or scavenge free radical species, the unfavorable side effect profiles limit the treatment of patients on a large scale [8, 9]. There is a great need for agents to diminish radiation-induced injury on the intestine.

Toll-like receptors (TLRs) family agonists represent a series of effective agents in facilitating the nullification of the IR-induced injuries. Since the activation of TLR5 was demonstrated to counteract radiation-induced damage on mice and monkey [10], the activation of TLRs family, including TLR2/6, TLR4 and TLR9, have been reported to effectively alleviate irradiation-induced damage [11–13]. Nevertheless, as different TLRs distribute among various organs and initiate different signaling pathways, the radio-protective effects by co-activating multiple TLRs may be stronger than the stimulation of single TLRs. The evidence comes from that Escherichia coli O111:B4 LPS, the co-agonist of TLR4 and TLR2, exerts stronger immune stimulation effects than that TLR2 or TLR4 agonist used alone or the combined use of them [14].

Heat Killed *Salmonella typhimurium* (HKST) represents an potent co-agonist of TLR2 and TLR4 receptor, and immune system of mammalian cells can be stimulated as *Salmonella typhimurium* is recognized by multiple TLRs [15, 16]. Previous study have shown that the pretreatment with attenuated *Salmonwlla typhimurium* could induce protective cell-mediated immunity or facilitate the conversion of immunosuppressive myeloid-derived suppressor cells into TNF-α-secreting neutrophil-like myeloid cells thereby protecting mice against malaria or tumor [17, 18]. In our preliminary work, damages on bone marrow, spleen and testis which were induced by γ-irradiation could be significantly eased by the pretreatment of HKST [19]. However, the radio-protective effects of HKST on small intestine has remained unclear. In the current study, HKST was demonstrated to alleviate the radiation-induced injury on small intestine in vitro and in vivo, which was further confirmed to be in a Wnt signaling dependent manner.

Materials And Methods

Medium and reagentsHKST was purchased from InvivoGen Asia (Hong Kong, China) and suspended in phosphate buffered saline (PBS). PBS and RMPI1640 medium was obtained from Hyclone (Logan, UT). ICG-001 was obtained from Selleck Chemicals (Shanghai, China). IntestiCult Organoid Growth Medium (Mouse) was purchased from STEMCELL Technologies Inc. (Canada). Growth factor-reduced Matrigel was purchased from Corning Incorporated (USA) and stored in -20 °C.

Mouse intestinal organoids culture and treatment

Mouse intestinal crypts isolation and intestinal organoids culture were performed as previously described with modest modification [20]. Briefly, small intestine was obtained after mice was euthanized with CO_2 and then washed with PBS followed by being longitudinally opened. Intestinal crypts were dissociated with PBS plus EDTA (15 mM). 250 crypts/well were suspended in the mixture composed of 75% Growth factor-reduced Matrigel and 25% IntestiCult Organoid Growth Medium. Enough medium was supplement

after the Matrigel polymerized at cell incubator (37 ℃, 5% CO_2). For HKST treatment, HKST was dissolved in IntestiCult Organoid Growth Medium (10^7 cells/ml) and the medium was replaced every 3 days. Optical image was taken after 7 days of cultivation.

Cell treatment

Cells (Human intestinal epithelial cells (HIEC), ATCC) were maintained in 1640 medium with 1% antibiotics from Gibco and 10% fetal bovine serum (Gibco, Australia) at 37 ℃ in a 5% CO_2 cell incubator. Cells were pretreated with HKST (10^7 cells/ml) alone or in combined used with ICG-001 (25 μM) at 12 hours prior to IR.

Mice and treatment

Animals experiments were approved by the Ethic Committee of The Second Military Medical University according to the instruction about Care and Use of Laboratory Animals published by the US NIH (Publication No.96 − 01). Mice (C57BL/6J, Male, 6–8 weeks, Jihui Experimental Animal Breeding Co., Ltd) were housed in a room with a 12 h light/dark cycle, in which water and food were obtained ad libitum. HKST (Invivogen, US, Lot: HST-39-01) resuspended in PBS (10^7/mice) was administrated through gavage 12 hours prior to irradiation.

Irradiation

Mice (in transparent boxes) and HIEC cells were exposed to γ-radiation (^{60}Co source, Second Military Medical University, China). The dosage was set as following: whole body radiation of 8 Gy, a dose rate of 1 Gy/min. Mouse intestinal organoids were irradiated with 0 Gy, 4 Gy, and 6 Gy.

Histopathology and immunohistochemistry

Mice were randomly divided into Control group, ionizing radiation (IR) group, IR plus HKST (IR + HKST) group, IR plus ICG-001 (IR + ICG-001) group, and IR plus HKST in combined with ICG-001 (IR + HKST + ICG-001) group, each group contained at least 3 mice. Mice were anesthetized with pentobarbital sodium (60 mg/kg) through intraperitoneal injection and pretreated with HKST (10^7 cells/mice), ICG-001 (10 mg/kg), HKST (10^7 cells/mice) in combined with ICG-001 (10 mg/kg) or placebo at 12 hours before 8 Gy IR exposure. At 3 days post irradiation, mice were sacrificed; small intestine was then isolated and fixed in paraformaldehyde and subjected to histological and immunohistochemical examination. Hematoxylin and eosin (H&E) staining was employed for the pathological morphology evaluation, in which the tissue slices were 3 μm. To detect the apoptosis rate in the small intestine, TdT-mediated dUTP Nick-End Labeling (tunel) assay was employed according to the manufacture instructions of the tunel kit (Roche, Basel, Switzerland). For the immunohistochemistry examination, tissue slices were stained with Olfm4 antibody (Cell Signaling Technology, Inc), and then the biotinylated secondary antibodies and DAB substrate kit were employed for immunohistochemistry.

Immunofluorescence assay

Cell-Light EdU DNA cell proliferation kit (C103102, RiboBio) was employed for the EDU staining. Briefly, intestinal organoids were culture with EdU (25 mM) for 1.5 h at 37 ℃, after which the organoids were fixed with 4% paraformaldehyde and permeabilized with 0.5% Triton X-100. And then the organoids were reacted with 1 × Apollo cocktail (RiboBio). For immunofluorescence analysis, tissue slices were stained with antibody against ki67 (Abcam, USA) and phosphorylated P65 (Proteintech Group, China), and then the secondary antibodies were employed. The images of tissues were obtained using a fluorescent microscope (Olympus BX60, Center Valley, PA, USA) equipped with a digital camera (Retiga 2000R, Surrey, BC, Canada).

Western blot analysis

M-PER Mammalian Protein Extration Reagent was employed to extract proteins from irradiated cells at 8-hour post IR exposure. Proteins were separated by SDS-polyacrylamide gel electrophoresis and transferred to PVDF membranes. The antibodies used to probe the membranes were listed as following: GAPDH (1:1000) was purchased from Proteintech (China); phosphorylated-ATR (1:1000), ATR (1:1000), ATM (1:1000) and phosphorylated-ATM (1:1000) was purchased from Cell Signaling Technology (USA); CHK1 (1:1000), phosphorylated-CHK1 (1:1000), phosphorylated-CHK2 (1:1000), CHK2 (Abcam, 1:1000) and p21 (1:1000) was purchased from Abcam (USA). Levels of proteins were by an ECL western blotting detection system (Thermo Fisher Scientific, Waltham) after the specific secondary antibodies (Servicebio, China) were probed.

Statistical analysis

Data of each experiment are presented as means ± the standard error of mean (SEM). Student's t test was employed to evaluate the significant differences, in which P values less than 0.05 were considered significant. All the experiments were repeated for at least 3 independent times and the quantification were conducted in a blinded fashion.

Results

HKST protected radiation-induced intestinal injury in mice

When exposed to a high dose of radiation, gastrointestinal toxicity remains a critical clinical issue to be settled. Thus, the radio-protective of HKST on intestine in vivo was investigated using whole body irradiation of mice. As is shown in Fig. 1A, after radiation exposure, the structure of intestine was heavily damaged when compared with the control group, in which the villus became shorter and the villus cells of the small intestine became discontinuous. HKST pretreatment effectively preserved the structure of intestine upon IR exposure, in which the length, density and the distribution uniformity of villus were remarkably improved (Fig. 1A). Accordingly, high apoptosis rate in the small intestine was induced upon IR exposure, and HKST pretreatment remarkably decreased the apoptosis rate as compared to the IR group (Fig. 1B).

HKST promoted proliferation of intestinal cells in vitro and in vivo post irradiation

Intestinal organoids were primarily established to mimic the regeneration of intestinal crypts in vitro [20, 21], we therefore detected whether the pretreatment of HKST could protect the intestinal organoids against IR-induced injury. As our result showed that the structure of intestinal organoids was destroyed by IR, in which the surface area of organoids and the height of organoids' buds were dramatically decreased upon IR exposure as compared to the control group, and the destructive effect on organoids was elevated as the dosage of IR increased (Fig. 2A, B **and C**). Whereas, the structure of intestinal organoids was effectively preserved by HKST pretreatment upon IR damage, in which the budding number and surface area of organoids in the HKST-treated group were more striking than that of the IR group (Fig. 2A, B **and C**), indicating the protective effect of HKST on intestinal organoids against IR injury. With immunofluorescence staining, we found that the proliferation of cells in organoids and crypts of mice were inhibited in IR group, and the HKST pretreatment significantly promoted the proliferation of intestinal cells as compared to IR group (Fig. 2D, E **and F**).

Pretreatment with HKST stimulates DNA damage response upon IR exposure

DNA damage response (DDR) appears to be an essential mode for mammalian cells to maintain and modulate the genome integrity thereby nullifying IR-induced toxicity [22, 23], HIEC cells was employed to detect the effect of HKST on DDR upon radiation exposure. Our study showed that without IR exposure, there were minor differences of the levels of the kinases phosphorylated CHK2 (P-CHK2), phosphorylated ATM (P-ATM), phosphorylated ATR (P-ATR), phosphorylated CHK1 (P-CHK2) and p21 between the untreated group and the HKST-treated group (Fig. 3). Whereas, upon radiation damage, levels of P-ATR, P21, P-CHK1 were significantly increased in HKST-treated cells as compared to the IR damaged group (Fig. 3). With selective inhibitor of Wnt signaling named ICG-001 [24], the mechanism involved in the stimulation of DDR by HKST after IR exposure was determined. As is shown in Fig. 3, when compared with HKST-treated cells, there were minor changes on the levels of the kinases P-ATR, P-CHK1, P-ATM, P-CHK2 and p21 when combined used of HKST and ICG-001. However, after radiation exposure, the increased levels of these proteins by HKST were diminished with ICG-001, indicating the inhibition of DDR when the Wnt signaling was blocked.

Radioprotective effects of HKST on intestine was blocked by Wnt signaling inhibitor

The intestinal stem cells that are highly susceptive to IR damage represent the sources of intestine regeneration. By immunohistochemistry, we detected the levels of Olfm4 that is the marker of crypt base columnar (CBCs) stem cells in mice intestine [25]. In our result, level of Olfm4 in intestine was notably decreased upon IR injury as compared to the control group (Fig. 4), whereas the HKST pretreatment

preserved the levels of intestinal stem cells, which again confirmed the protective effect of HKST on intestine against radiation damage. However, after adding ICG-001 into the HKST-treated group, the radio-protective activity of HKST on intestinal stem cells was significantly blocked (Fig. 4), indicating the radio-protective of HKST on intestine was blocked by Wnt signaling inhibitor.

HKST activated target cells in liver and spleen besides intestine

Besides intestine, we wonder whether HKST activates other cells and whether there is any correlation between the whole body radioprotection and intestinal protection. As most TLRs activates NF-kB as a downstream signaling pathway. To identify target cells of HKST, we perform a p65 staining in liver, spleen, bone marrow, which are tissues with high level of TLRs. Our data showed that upon HKST treatment, p65 expression and translocation was strongly activated in liver and spleen (Fig. 5). These findings suggests that the protective effects of HKST on intestine is not just a local activation of DNA damage repair in intestine, but also a whole body protective effects.

Discussion

Since the intestinal epithelium is one of the fastest self-renewing tissue in mammals and therefore sensitive to IR damage, IR-induced GI toxicity is the limited factor in abdominal pelvic radiotherapy and a great threat to public health in the potential nuclear accident [26]. Nonetheless, there are no effective strategies approved to alleviate the pain of victims with GI syndrome, and the currently reported agents have unfavorable side effect profiles [8, 9]. The development of novel agents protecting against IR-induced intestinal damage is of great significance. Currently, the radio-protective effects of HKST on small intestine was investigated. As is shown in our results, the radiation-induced damage on intestine was dramatically mitigated by pretreating with HSKT in vitro and in vivo, in which the structure of intestinal organoids and small intestine of the HKST-treated group was more integrated than of the radiation damage group, and HKST pretreatment remarkably promoted the proliferation of intestinal cells post IR exposure (Figs. 1 and 2). HKST is the potent co-agonist of TLRs family which has been reported to alleviate the radiation-induced injury on bone marrow, spleen and testis in our previous study [19]. It has been demonstrated that the attenuated *Salmonwlla typhimurium* could be employed to stimulate immune system or developed into vaccine thereby protecting against malaria or tumor [17, 18]. Besides, the therapeutic use of bacteria in preventing IR-induced GI toxicity has begun to receive more attention [27–29]. Our study demonstrates that HKST is of great potential alleviating injury on intestine induced by radiation and facilitating the relief of victims with GI syndrome in clinical practice.

Generally, the critical target of IR is considered to be DNA [30]. Upon IR exposure, the irreparable DNA damage is induced and the unrepaired or error-repaired DNA damage subsequently leads to cellular damage or death [31]. DNA double-strand breaks (DSBs) is regarded as the most deleterious form of DNA damage, which can rise chromosomal aberrations, loss of genetic materials, the cell death, or other detrimental consequences [32]. Homologous recombination (HR) and non-homologous end joining

(NHEJ) and are the two main modes to repair DSBs in mammalian cells, in which ATR and ATM are confirmed to be the key components of DNA damage response (DDR) pathway [22, 23]. As DDR is critical for the maintenance and modulation of genome integrity in intestinal cells after radiation exposure [33, 34], we speculated that the radio-protective effects of HKST might be involved in DDR. As our results show that, after radiation exposure, levels of P-ATM, P-ATR, P-CHK1 and p21 were significantly increased in cells pretreated with HKST (Fig. 3). The dysfunction of genome integrity caused by radiation damage requires DDR to prevent the transmission of incompletely replicated or damaged chromosomes. DNA damage repair reactions in mammalian cells require the cell cycle arrest by the activation of a DNA damage checkpoint [35]. ATM and ATR are the main regulators of two major checkpoint pathways, in which ATR-CHK1 checkpoint signaling is essential for HR repair pathway while the conversion of ATM to monomers by autophosphorylation and then phosphorylates CHK2 appear to be essential for the checkpoint response [36, 37]. In some condition, the cell-cycle arrest is initiated by ATR-CHK1 signaling but maintained by ATM-CHK2 signaling [38]. Cell cycle arrest will activate the tumor suppressor p53 which then targets its transcriptional protein named cyclindependent kinase inhibitor (p21) [39]. As the elevated levels of P-ATR, P-CHK1 and p21 after radiation exposure in HKST-treated cells were observed, and there were minor changes on P-ATM and P-CHK2, between HKST treatment and IR group, the ATR pathway might be more crucial in facilitating the protective effects of HKST against IR.

The maintenance and self-renew of intestinal cells, especially the stem cells, in adult mammalian requires Wnt signaling pathway, whereas the inactivation of the essential components of Wnt signaling leads to the deficiency in intestine development and regeneration [40, 41]. Previous study have demonstrated that DDR activation was amplified by Wnt signaling in mammalian cells, whereas the inhibition of Wnt signaling fail to resolve DSBs after radiation [42–44]. As the protective activity of HKST against radiation injury was confirmed to be related to DDR, and the pretreatment of HKST effectively preserved intestinal stem cells when exposed to IR (Fig. 4), the radio-protective effect of HKST might be mediated by Wnt signaling pathway. As expected, the stimulation of ATR and ATM signaling upon IR in HKST-treated cells and the radio-protective effects of HKST on intestinal stem cells in mice were diminished by the selective inhibitor of Wnt signaling pathway (Figs. 3 and 4). Previous study have shown that the kinases ATM/ATR and CHK1/2 were involved in the inflammatory responses induced by TLRs signaling [45], and TLRs signaling was reported to modulate the DNA repair reaction in cells [46]. As HKST is a co-agonist of TLRs, the role of TLRs signaling in DDR mediated by Wnt signaling pathway upon IR damage remains further investigation. As HKST is the co-agonist of TLRs family, and TLRs family are expressed in different organs. We presumed that HKST might activates other cells and the protective effects of HKST against IR is a whole body protective effects. As the results showed that, besides small intestine, HKST pretreatment significantly activates NF-kB signaling pathway in liver and spleen.

Conclusion

Gastrointestinal (GI) toxicity caused by ionizing radiation (IR) appears to be a limited factor in radiotherapy and a great threat for soldiers on nuclear-related military missions. However, there are currently no approved medical strategies to effectively prevent or mitigate the damage on intestine

induced by IR. Our study demonstrated that Heat Killed *Salmonella typhimurium* (HKST) could significantly alleviate the damage on intestine induced by IR, and the radio-protective effect of HKST was depended on DDR mediated by Wnt signaling pathway. As HKST is a vaccine model employed to protect against facultative intracellular bacteria, our study provides a bacterium potentially used in clinical practice for IR-induced GI toxicity prevention.

Abbreviations

GI: Gastrointestinal；IR: ionizing radiation；HKST: Heat Killed Salmonella typhimurium; DDR: DNA damage response; ARS: acute radiation syndrome; ISCs: intestinal stem cells; TLRs: Toll-like receptors; HIEC: Human intestinal epithelial cells;

HR: Homologous recombination; NHEJ: non-homologous end joining.

Declarations

Ethics Approval and consent to participate

All the experiments were approved by the Second Military Medical University of China in accordance with the Guide for Care and Use of Laboratory Animals published by the US NIH (Publication No.96-01).

Consent for publication

Not applicable.

Availability of supporting data

Not applicable.

Competing interests

The data used to support the findings of this study are available from the corresponding author upon request. The authors declare that they have no conflicts of interest.

Funding

This study was supported in part by the grants from National Natural Science Foundation of China (**No. 11605289, No. 31700739, No. 81892559**), and Shanghai association for science and technology (**No. 20184Y0167**). The authors have no conflicts of interest to disclose.

Authors' contributions

Cai and Gao designed the study; Chen, Cao and Hu performed the experiment; Liu, Li and Cui analyzed data and edited the figures; Chen wrote the paper and Yang modified the article. All authors read and

approved the final manuscript.

Acknowledgements

Not applicable.

Authors' information

[1]Department of Radiation Medicine, Faculty of Naval Medicine, Second Military Medical University, Shanghai, P.R. China. [2]Department of Gastrointestinal Surgery, Second Military Medical University First Hospital, Shanghai, P. R. China.

References

1. Singh VK, Ducey EJ, Brown DS, Whitnall MH. A review of radiation countermeasure work ongoing at the Armed Forces Radiobiology Research Institute. Int J Radiat Biol. 2012;88:296–310.
2. Dorr H, Meineke V. Acute radiation syndrome caused by accidental radiation exposure - therapeutic principles. BMC Med. 2011;9:126.
3. Kirsch DG, Santiago PM, di Tomaso E, Sullivan JM, Hou WS, Dayton T, Jeffords LB, Sodha P, Mercer KL, Cohen R, et al. p53 controls radiation-induced gastrointestinal syndrome in mice independent of apoptosis. Science. 2010;327:593–6.
4. Koenig KL, Goans RE, Hatchett RJ, Mettler FA Jr, Schumacher TA, Noji EK, Jarrett DG. Medical treatment of radiological casualties: current concepts. Ann Emerg Med. 2005;45:643–52.
5. Gits J, Gerber GB. Electrolyte loss, the main cause of death from the gastrointestinal syndrome? Radiat Res. 1973;55:18–28.
6. Taniguchi CM, Miao YR, Diep AN, Wu C, Rankin EB, Atwood TF, Xing L, Giaccia AJ. PHD inhibition mitigates and protects against radiation-induced gastrointestinal toxicity via HIF2. Sci Transl Med. 2014;6:236ra264.
7. Hu B, Jin C, Li HB, Tong J, Ouyang X, Cetinbas NM, Zhu S, Strowig T, Lam FC, Zhao C, et al. The DNA-sensing AIM2 inflammasome controls radiation-induced cell death and tissue injury. Science. 2016;354:765–8.
8. Hospers GA, Eisenhauer EA, de Vries EG. The sulfhydryl containing compounds WR-2721 and glutathione as radio- and chemoprotective agents. A review, indications for use and prospects. Br J Cancer. 1999;80:629–38.
9. Waselenko JK, MacVittie TJ, Blakely WF, Pesik N, Wiley AL, Dickerson WE, Tsu H, Confer DL, Coleman CN, Seed T, et al. Medical management of the acute radiation syndrome: recommendations of the Strategic National Stockpile Radiation Working Group. Ann Intern Med. 2004;140:1037–51.
10. Burdelya LG, Krivokrysenko VI, Tallant TC, Strom E, Gleiberman AS, Gupta D, Kurnasov OV, Fort FL, Osterman AL, Didonato JA, et al. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. Science. 2008;320:226–30.

11. Holm KL, Syljuasen RG, Hasvold G, Alsoe L, Nilsen H, Ivanauskiene K, Collas P, Shaposhnikov S, Collins A, Indrevaer RL, et al: **TLR9 stimulation of B-cells induces transcription of p53 and prevents spontaneous and irradiation-induced cell death independent of DNA damage responses. Implications for Common variable immunodeficiency**. 2017, **12**:e0185708.
12. Liu C, Zhang C, Mitchel RE, Cui J, Lin J, Yang Y, Liu X, Cai J. A critical role of toll-like receptor 4 (TLR4) and its' in vivo ligands in basal radio-resistance. Cell Death Dis. 2013;4:e649.
13. Singh VK, Ducey EJ, Fatanmi OO, Singh PK, Brown DS, Purmal A, Shakhova VV, Gudkov AV, Feinstein E, Shakhov A. CBLB613: a TLR 2/6 agonist, natural lipopeptide of Mycoplasma arginini, as a novel radiation countermeasure. Radiat Res. 2012;177:628–42.
14. Xu WY, Wang L, Wang HM, Wang YQ, Liang YF, Zhao TT, Wu YZ. TLR2 and TLR4 agonists synergistically up-regulate SR-A in RAW264.7 through p38. Mol Immunol. 2007;44:2315–23.
15. Arpaia N, Godec J, Lau L, Sivick KE, McLaughlin LM, Jones MB, Dracheva T, Peterson SN, Monack DM, Barton GM. TLR signaling is required for Salmonella typhimurium virulence. Cell. 2011;144:675–88.
16. Lembo A, Kalis C, Kirschning CJ, Mitolo V, Jirillo E, Wagner H, Galanos C, Freudenberg MA. Differential contribution of Toll-like receptors 4 and 2 to the cytokine response to Salmonella enterica serovar Typhimurium and Staphylococcus aureus in mice. Infect Immun. 2003;71:6058–62.
17. Chang SY, Kim YJ, Ko HJ. Potential therapeutic anti-tumor effect of a Salmonella-based vaccine. Hum Vaccin Immunother. 2013;9:1654–60.
18. Sadoff JC, Ballou WR, Baron LS, Majarian WR, Brey RN, Hockmeyer WT, Young JF, Cryz SJ, Ou J, Lowell GH, et al. Oral Salmonella typhimurium vaccine expressing circumsporozoite protein protects against malaria. Science. 1988;240:336–8.
19. Xu Y, Chen Y, Liu H, Lei X, Guo J, Cao K, Liu C, Li B, Cai J, Ju J, et al. Heat-killed salmonella typhimurium (HKST) protects mice against radiation in TLR4-dependent manner. Oncotarget. 2017;8:67082–93.
20. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, Clevers H. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. Nature. 2009;459:262–5.
21. Gregorieff A, Liu Y, Inanlou MR, Khomchuk Y, Wrana JL. Yap-dependent reprogramming of Lgr5(+) stem cells drives intestinal regeneration and cancer. Nature. 2015;526:715–8.
22. Hartlerode AJ, Scully R. Mechanisms of double-strand break repair in somatic mammalian cells. Biochem J. 2009;423:157–68.
23. Pardo B, Gomez-Gonzalez B, Aguilera A. DNA repair in mammalian cells: DNA double-strand break repair: how to fix a broken relationship. Cell Mol Life Sci. 2009;66:1039–56.
24. Esposito M, Mondal N, Greco TM, Wei Y. **Bone vascular niche E-selectin induces mesenchymal-epithelial transition and Wnt activation in cancer cells to promote bone metastasis**. 2019, 21:627–639.

25. Barry ER, Morikawa T, Butler BL, Shrestha K, de la Rosa R, Yan KS, Fuchs CS, Magness ST, Smits R, Ogino S, et al. Restriction of intestinal stem cell expansion and the regenerative response by YAP. Nature. 2013;493:106–10.
26. van Es JH, Sato T, van de Wetering M, Lyubimova A, Yee Nee AN, Gregorieff A, Sasaki N, Zeinstra L, van den Born M, Korving J, et al. Dll1 + secretory progenitor cells revert to stem cells upon crypt damage. Nat Cell Biol. 2012;14:1099–104.
27. Ciorba MA, Riehl TE, Rao MS, Moon C, Ee X, Nava GM, Walker MR, Marinshaw JM, Stappenbeck TS, Stenson WF. Lactobacillus probiotic protects intestinal epithelium from radiation injury in a TLR-2/cyclo-oxygenase-2-dependent manner. Gut. 2012;61:829–38.
28. Ki Y, Kim W. **The effect of probiotics for preventing radiation-induced morphological changes in intestinal mucosa of rats**. 2014, 29:1372–1378.
29. Linn YH, Thu KK, Win NHH. Effect of Probiotics for the Prevention of Acute Radiation-Induced Diarrhoea Among Cervical Cancer Patients: a Randomized Double-Blind Placebo-Controlled Study. Probiotics Antimicrob Proteins. 2019;11:638–47.
30. Borgmann K, Roper B, El-Awady R, Brackrock S, Bigalke M, Dork T, Alberti W, Dikomey E, Dahm-Daphi J. Indicators of late normal tissue response after radiotherapy for head and neck cancer: fibroblasts, lymphocytes, genetics, DNA repair, and chromosome aberrations. Radiother Oncol. 2002;64:141–52.
31. Allison RR. Radiobiological modifiers in clinical radiation oncology: current reality and future potential. Future Oncol. 2014;10:2359–79.
32. Lou J, Chen H, Han J, He H, Huen MSY, Feng XH, Liu T, Huang J. **AUNIP/C1orf135 directs DNA double-strand breaks towards the homologous recombination repair pathway**. 2017, 8:985.
33. Chaves-Perez A, Yilmaz M. **URI is required to maintain intestinal architecture during ionizing radiation**. 2019, 364.
34. Gronke K, Hernandez PP, Zimmermann J, Klose CSN, Kofoed-Branzk M, Guendel F, Witkowski M, Tizian C, Amann L, Schumacher F, et al. Interleukin-22 protects intestinal stem cells against genotoxic stress. Nature. 2019;566:249–53.
35. Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem. 2004;73:39–85.
36. Bakkenist CJ, Kastan MB. DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. Nature. 2003;421:499–506.
37. Chen Y, Liu H, Zhang H, Sun C, Hu Z, Tian Q, Peng C, Jiang P, Hua H, Li X, Pei H. And-1 coordinates with CtIP for efficient homologous recombination and DNA damage checkpoint maintenance. Nucleic Acids Res. 2017;45:2516–30.
38. Abraham RT. Cell cycle checkpoint signaling through the ATM and ATR kinases. Genes Dev. 2001;15:2177–96.
39. Sperka T, Wang J, Rudolph KL. DNA damage checkpoints in stem cells, ageing and cancer. Nat Rev Mol Cell Biol. 2012;13:579–90.

40. Fevr T, Robine S, Louvard D, Huelsken J. Wnt/beta-catenin is essential for intestinal homeostasis and maintenance of intestinal stem cells. Mol Cell Biol. 2007;27:7551–9.
41. Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature. 2005;434:843–50.
42. Ayyanan A, Civenni G, Ciarloni L, Morel C, Mueller N, Lefort K, Mandinova A, Raffoul W, Fiche M, Dotto GP, Brisken C. Increased Wnt signaling triggers oncogenic conversion of human breast epithelial cells by a Notch-dependent mechanism. Proc Natl Acad Sci U S A. 2006;103:3799–804.
43. Lento W, Ito T, Zhao C, Harris JR, Huang W, Jiang C, Owzar K, Piryani S, Racioppi L, Chao N, Reya T. Loss of beta-catenin triggers oxidative stress and impairs hematopoietic regeneration. Genes Dev. 2014;28:995–1004.
44. Tao S, Tang D, Morita Y, Sperka T, Omrani O, Lechel A, Sakk V, Kraus J, Kestler HA, Kuhl M, Rudolph KL. Wnt activity and basal niche position sensitize intestinal stem and progenitor cells to DNA damage. Embo j. 2015;34:624–40.
45. Weintz G, Olsen JV, Fruhauf K, Niedzielska M, Amit I, Jantsch J, Mages J, Frech C, Dolken L, Mann M, Lang R. The phosphoproteome of toll-like receptor-activated macrophages. Mol Syst Biol. 2010;6:371.
46. Wang Z, Yan J, Lin H, Hua F, Wang X, Liu H, Lv X, Yu J, Mi S, Wang J, Hu ZW. Toll-like receptor 4 activity protects against hepatocellular tumorigenesis and progression by regulating expression of DNA repair protein Ku70 in mice. Hepatology. 2013;57:1869–81.

Figures

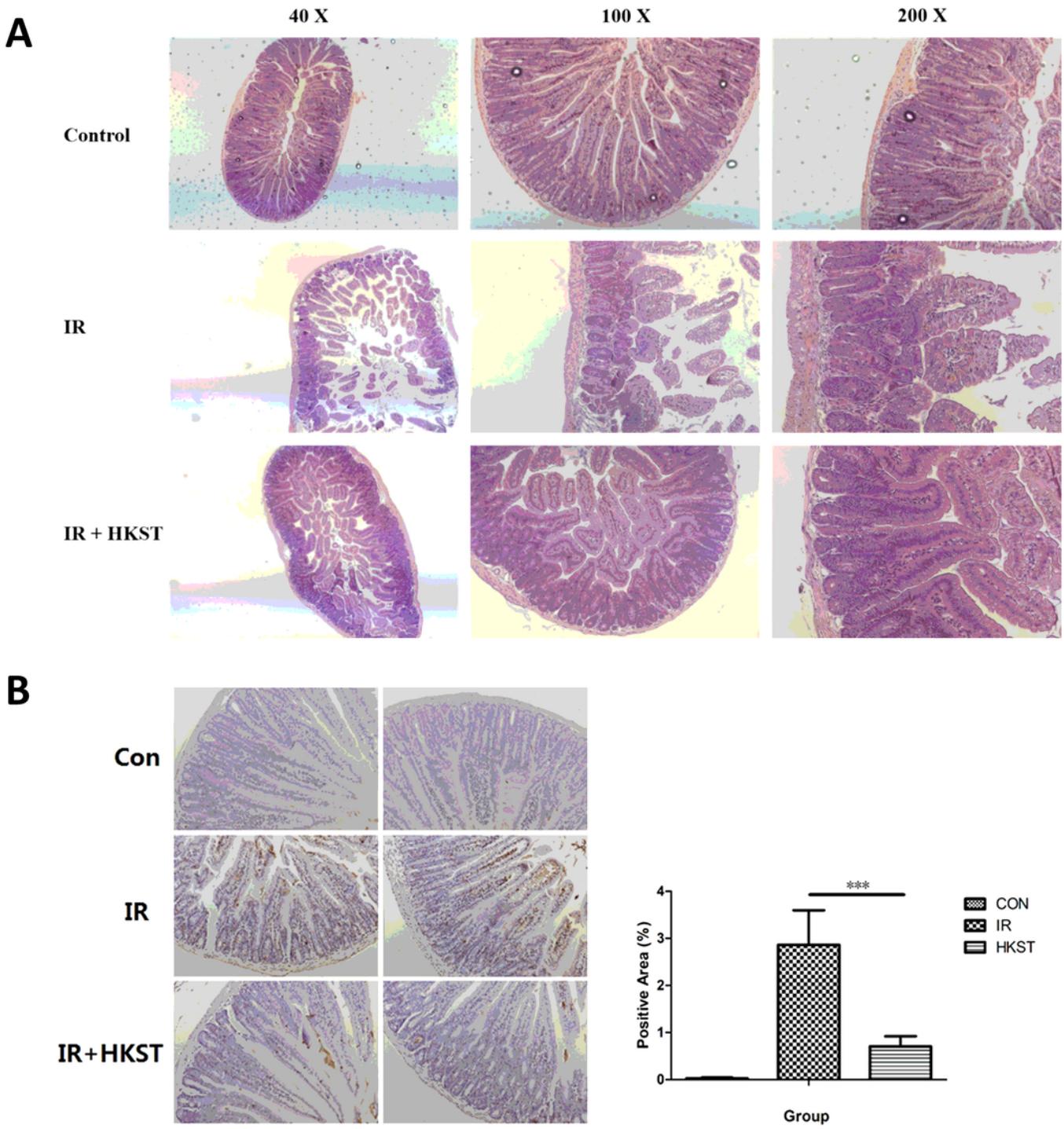

Figure 1

Mice intestine damage induced by radiation exposure was alleviated by HKST pretreatment. Mice receiving IR exposure were pretreated with PBS or HKST. At 3 days post irradiation, mice were sacrificed, and the protective effect of HKST on intestine were test by Hematoxylin-Eosin (HE) staining (A) and TdT-mediated dUTP Nick-End Labeling (tunel) assay (B). Value are given as mean ±SEM (n=6), *** P<0.001 represents the HKST+IR group versus IR group.

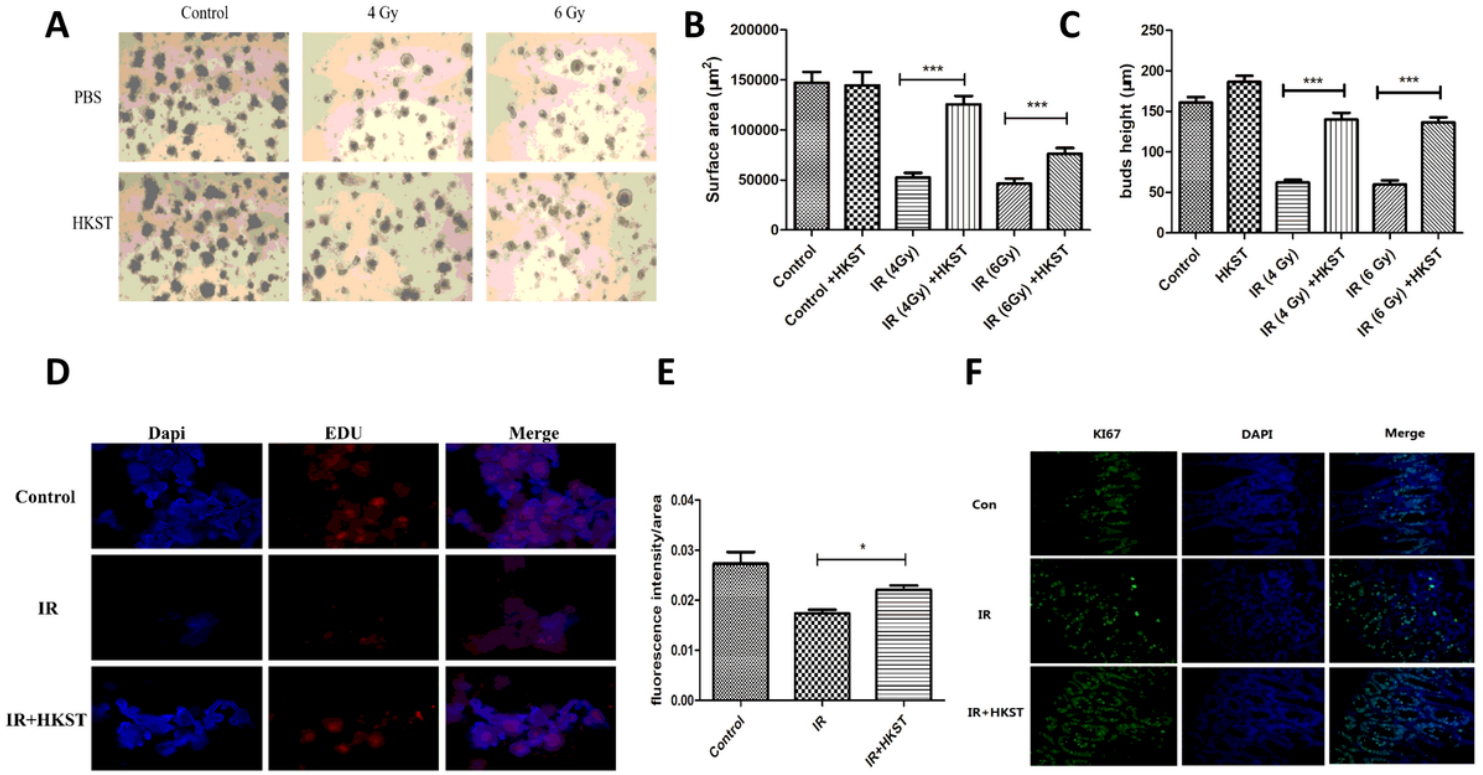

Figure 2

HKST promoted proliferation in intestinal organoids and in vivo post-irradiation. Intestinal crypts were isolated from mice small intestine and then seeded into Matrigel. Upon IR exposure, photos of intestinal organoids were taken after intestinal crypts were cultured after 7 days (A). Surface area and bud height of intestinal organoids were measured with Image J software and analyzed with graphpad prism 5, in which 30 organoids were calculated for each group (B, C). Value are given as mean ±SEM (n=30), *** P<0.001 represents the HKST+IR group versus IR group. The proliferation of inteatinal organoids and crypts was detected by immunofluorescence staining of EDU (D) and ki67 (F), respectively. The fluorescence intensity of EDU in organoids was measured by Image J software and analyzed with graphpad prism 5 (E). Value are given as mean ±SEM (n=6), *P<0.05 represents the HKST+IR group versus IR group.

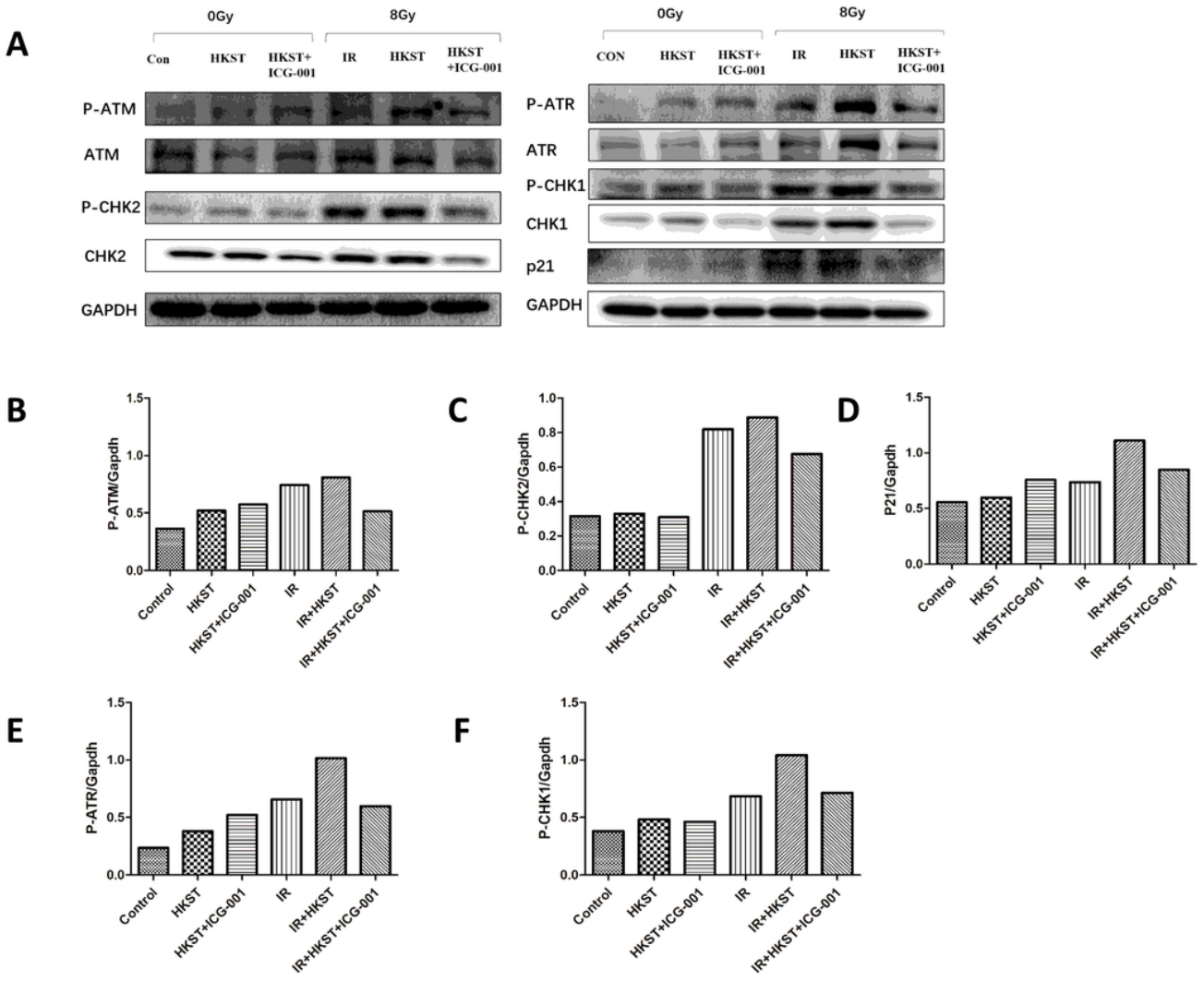

Figure 3

HKST treatment promoted the activation of DDR pathway and the stimulated activity was diminished by ICG-001. HIEC cells were pretreated with HKST alone or in combined with ICG-001 for 12 h prior to radiation exposure (8Gy). Proteins were extracted at 8 hours post radiation and then the levels of phosphorylated-ATR (P-ATR), ATR, phosphorylated-CHK1 (P-CHK1), CHK1, phosphorylated-ATM (P-ATM), ATM, phosphorylated-CHK2 (P-CHK2), CHK2 and p21 were detected with Western blot (A). The levels of P-ATM (B), P-ATR(C), P21(D), P-CHK1(E) and P-CHK2 (F) were measured by Image J software and analyzed with graphad prism 5.

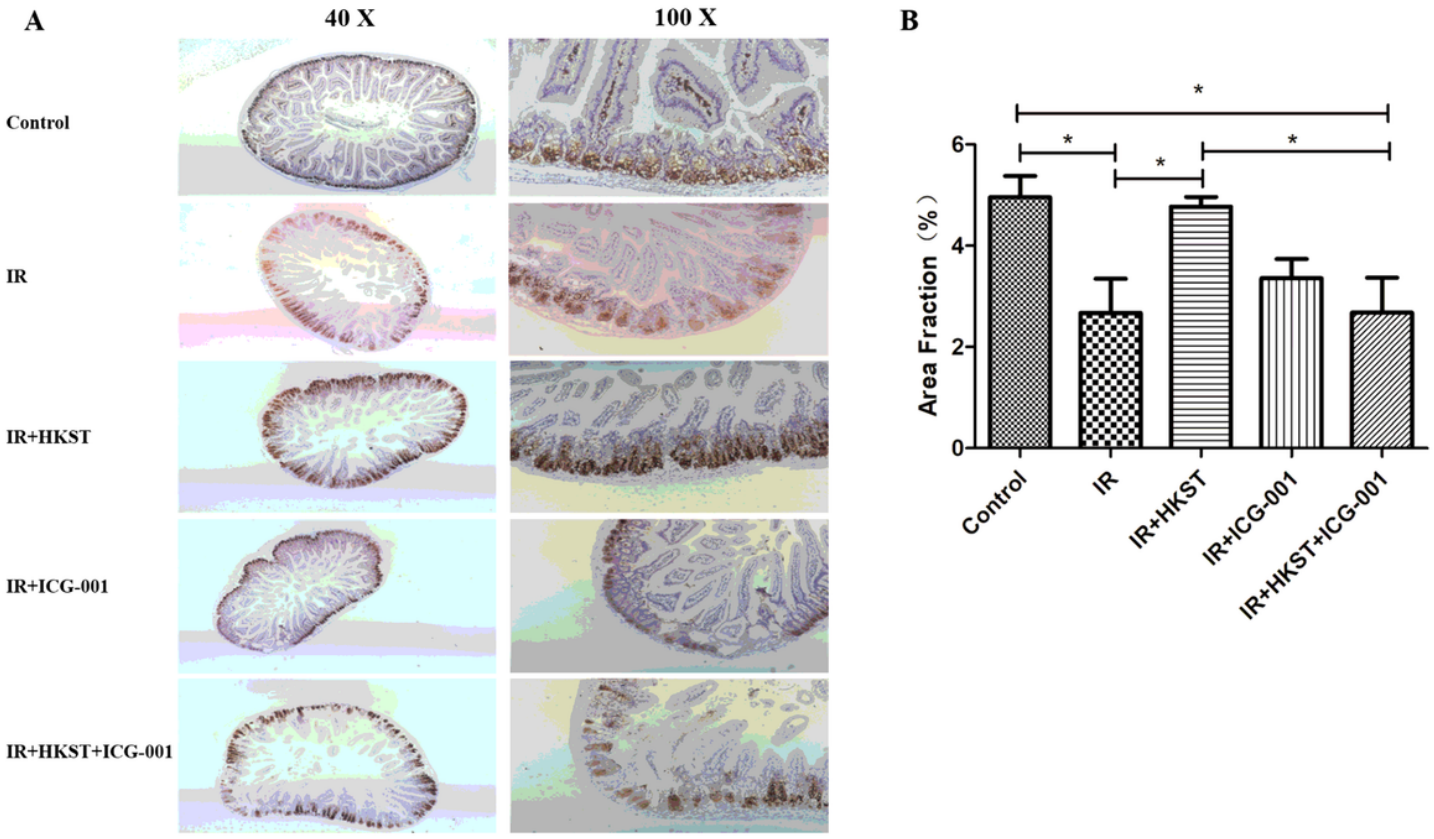

Figure 4

IR-induced damage on intestinal stem cells was alleviated by HKST and the preserved effect was diminished by ICG-001. Small intestine isolated from mice untreated or receiving IR exposure plus agents were sectioned into 3μm, and immunohistochemistry was employed to detect the levels of Olfm4. The positive area of Olfm4 staining cells were measured by Image J software and analyzed with graphad prism 5. Value are given as mean ±SEM (n=6), *P<0.05

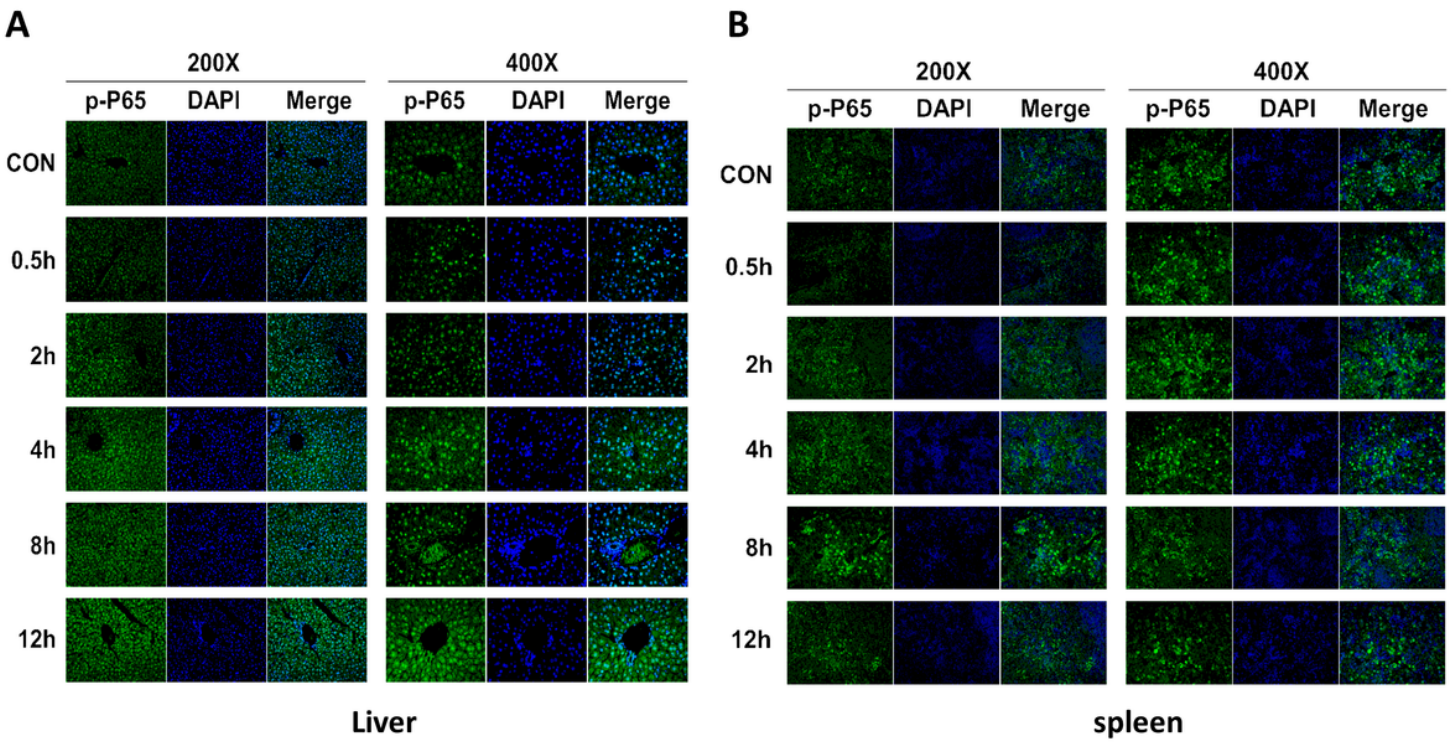

Figure 5

NF-kB signaling in liver and spleen were activated by HKST treatment. Mice untreated or treated with HKST were sacrificed, and the liver and spleen were collected. The activation of NF-kB signaling in liver (A) and spleen (B) was detected by immunofluorescence staining of phosphorylated P65 (P-P65).