Original Article

Effect and mechanism of the miR-1284/EIF4A1 axis on the cGAS-STING pathway under radiotherapy

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Background: Gastric cancer (GC) remains a major global health concern, with limited treatment options, especially in advanced stages. Radiotherapy (RT) plays a vital role in GC management, but resistance to DNA damage impedes its effectiveness. MicroRNA-1284 (miR-1284), a tumor suppressor, regulates eukaryotic translation initiation factor 4A1 (EIF4A1), which is involved in DNA damage repair through homologous recombination (HR). This axis has been implicated in enhancing GC cell survival following RT. Additionally, the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway, activated by DNA damage, plays a key role in triggering an anti-tumor immune response. However, the interaction between the miR-1284/EIF4A1 axis, DNA repair, and the cGAS-STING pathway in GC under RT conditions remains unclear. This study aims to investigate how the miR-1284/EIF4A1 axis influences DNA repair and its role in activating the cGAS-STING pathway to enhance RT efficacy in GC.

Methods: A stably expressed messenger miR-1284 cell line was established. Quantitative reverse transcription and western blot were used to examine the expression of miR-1284 and EIF4A1, and the effect of blocking the miR-1284/EIF4A1 axis on the cGAS-STING pathway and interferon-β (IFN-β) in GC cells after RT; cytotoxicity experiments were conducted to explore the mechanism of the miR-1284/EIF4A1 axis in radiation-induced DNA damage repair; animal experiments were conducted to explore the translational application of rocaglamide (RocA) combined with the programmed cell death-ligand 1 (PD-L1) antibody in RT.

Results: The miR-1284/EIF4A1 axis in the GC cells promoted the repair of radiation-induced DNA damage and was associated with the prognosis of GC patients. Blocking this axis delayed the C-terminal binding protein interacting protein (CtIP)-mediated DNA repair, enhanced RT effectiveness, and activated the cGAS-STING pathway, while increasing the rate of apoptosis. *In vivo* experiments based on RocA binding to PD-L1 antibodies under RT had good biological safety, and thus provide a potential therapeutic strategy for the treatment of GC.

Conclusions: The miR-1284/EIF4A1 axis promotes the repair of DNA damage caused by RT, promotes the activation of the cGAS-STING pathway in GC, and has good biological safety. Our findings provide an important experimental basis for enhancing the anti-tumor immune effect of RT in the treatment of GC.

Keywords: Gastric cancer (GC); radiotherapy (RT); microRNA-1284/eukaryotic translation initiation factor 4A1 axis (miR-1284/EIF4A1 axis); cyclic GMP-AMP synthase-stimulator of interferon genes pathway (cGAS-STING pathway)

Submitted Mar 17, 2025. Accepted for publication Apr 10, 2025. Published online Apr 18, 2025.
doi: 10.21037/tcr-2025-603
View this article at: https://dx.doi.org/10.21037/tcr-2025-603

Introduction

Gastric cancer (GC) is a common malignant tumor worldwide. In 2022, there were more than 968,000 new GC cases, and 660,000 GC-related deaths worldwide (1). China is a country with a high incidence of CG, and its incidence ranks first in the world, which seriously endangers the physical and mental health of Chinese residents (2,3).

Radiotherapy (RT), also known as radiation therapy, is gradually becoming an important treatment for GC. RT mainly acts on tumor cells through ionizing radiation, causing single-stranded DNA or double-stranded DNA (dsDNA) breaks, thus inducing tumor cells to enter different death pathways, and activating the anti-tumor immune response mediated by the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway, thereby controlling tumor growth and prolonging patient survival (4). With the wide application of D2 for radical resection of GC, and both the ARTIST and ARTIST-2 clinical studies have reported negative results. GC cells repair radiation-induced DNA damage through an efficient and stable homologous recombination (HR) pathway; however, under the influence of RT dose on the gastric wall, gastric mucosa and surrounding organs (such as pancreas, small intestine, etc.), thus, the clinical value of RT in the treatment of GC is still controversial (5,6).

MicroRNA-1284 (miR-1284) is a non-coding microRNA (miRNA), and its expression in tumor cells plays an important role in the occurrence and development of tumors. Previous studies have shown that miR-1284 effectively inhibits the proliferation, migration, and invasion capabilities of GC cells by targeting eukaryotic translation initiation factor 4A1 (EIF4A1) (7,8). Mejías-Navarro *et al.* (9) found that the overexpression of EIF4A1 promotes the translation of C-terminal binding protein interacting protein (CtIP), a key protein for DNA repair, thereby activating the HR repair pathway and promoting the DNA damage repair process. This discovery showed the important role of EIF4A1 in regulating CtIP translation and HR pathway DNA damage repair.

Thus, if the radiation dose is strictly controlled, GC cells have strong repair and proliferation capabilities, which may be an important reason for RT resistance in GC. Based on the findings of this study, we speculated that miR-1284 reduces the translation level of CtIP by inhibiting the expression of EIF4A1, thereby weakening the DNA repair ability of the HR pathway, making it impossible for cancer cells to effectively repair DNA damage caused by radiation during RT, leading to the accumulation of more DNA damage in cells, which in turn activates the cGAS-STING pathway. This mechanism could not only help improve the efficacy of RT, but could also provide a potential target for the treatment of GC, which has important practical significance in enhancing the sensitivity of GC to RT. We present this article in accordance with the ARRIVE and MDAR reporting checklists (available at https://tcr.amegroups.com/article/view/10.21037/tcr-2025-603/rc).

Highlight box

Key findings

- This study explored the mechanism of microRNA-1284 (miR-1284)/eukaryotic translation initiation factor 4A1 (EIF4A1) axis in the radiotherapy (RT) response in gastric cancer (GC), including how it regulates the expression of C-terminal binding protein interacting protein (CtIP), and its effect on the DNA repair process to activate the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway and trigger the anti-tumor immune response.

What is known, and what is new?

- The cGAS-STING signaling pathway plays a key role in tumor immune surveillance, and reveals the importance of common anti-tumor means such as RT, chemotherapy, targeted therapy and immunotherapy. The question of how to activate the cGAS-STING pathway is a current research hot spot.
- This study not only provided a new perspective for understanding the mechanism of action of GC RT, but also laid a theoretical foundation for the development of new tumor treatment strategies by revealing the interaction between the miR-1284/EIF4A1 axis and the cGAS-STING pathway.

What is the implication, and what should change now?

- This study was the first to reveal the important role of the miR-1284/EIF4A1 axis in regulating the process of CtIP-mediated DNA repair. This axis could serve as a potential target for promoting the activation of the cGAS-STING pathway. We intend to explore whether the miR-1284/EIF4A1 axis via the cGAS-STING pathway can mediate anti-tumor immune effects *in vivo* and *in vitro*. Our findings have the potential to provide a scientific strategy for the therapeutic effect and prognosis of GC patients.

Methods

Selection of GC cell lines, exploration of RT conditions

Selection of cell lines

The human GC cell line MGC-803 and the mouse GC cell line mouse forestomach carcinoma (MFC) cells were obtained from the Shanghai Institute of the Cell Biology

Cell Bank Chinese Academy of Sciences (Shanghai, China). A miR-1284 overexpressing lentivirus with the green fluorescent protein (GFP) and puromycin resistance was built. The cells were stored in Roswell Park Memorial Institute Medium 1640 at 37 ℃ in 5% carbon dioxide (CO_2; Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Thermo Fisher Scientific), 100 μg/mL of streptomycin, and 100 units/mL of penicillin.

Exploration of RT conditions

In the early stage of this study, the RT conditions were explored. The DNA damage marker γ-H2AX was detected by treating the GC cells with MGC-803 at different time points (0, 1, 6, and 12 h).

Experimental groups

The following groups were established: the negative control group, which received the empty lentiviral vector (LV); the miR-1284 overexpressing group, which received the overexpression LV-miR-1284-miR-1284; the RT + negative control group, which received RT (10 Gy) and the empty LV; and the RT + miR-1284 overexpressing group, which received RT (10 Gy) and the overexpression LV-miR-1284.

To verify the regulatory effect of the miR-1284/EIF4A1 axis on CtIP-mediated DNA repair under RT, cells were collected at different time points (0, 1, 6, and 12 h) for follow-up research.

Study methods

Establishment of stable miR-1284 expressing cell lines

Transfection of cell lines: the miR-1284 overexpression LV-miR-1284-GFP and the null LV-GFP were provided by GeneChem (Shanghai, China). After being seeded into 6-well plates for 36 h, the cells were infected with the LV at 100 plaque-forming units (PFU)/cell [multiplicity of infection (MOI) =100] without penicillin or streptomycin. To acquire stably transfected cells, the cells were cultured in 600 mg/mL of G418 (Invitrogen Corporation, Carlsbad, CA, USA) for 14–21 days. The following three groups of cells were identified: GC cells in the LV-miR-1284-GFP group, which were transfected with the recombinant LV-miR-1284-GFP; GC cells in the LV-GFP group, which were transfected with the negative control LV-GFP; and GC cells in the control group, which did not receive any treatment.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated from cultured cells using the TRIzol reagent (Invitrogen) following the standard protocol. Subsequently, 1 μg of RNA was reverse transcribed into complementary DNA (cDNA) using the PrimeScript™ RT kit (Takara Bio, Tokyo, Japan). Quantitative expression of messenger RNA (mRNA) and miRNA was normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6, respectively. qRT-PCR was conducted using SYBR® Premix Ex Taq™ II (Tli RNaseH Plus) combined with the ROX reference dye (Takara Bio), according to the manufacturer's guidelines. Relative gene expression was determined using the comparative threshold cycle (Ct) ($2^{-\Delta\Delta Ct}$) method.

Western blot analysis

Total protein was extracted from cells using lysis buffer (Solarbio, Beijing, China). Protein samples were resolved by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and subsequently transferred onto nitrocellulose membranes. After blocking, the membranes were incubated overnight at 4 ℃ with primary antibodies diluted at 1:1,000. Following incubation, membranes were rinsed with TBST buffer (Beyotime, Shanghai, China) and then exposed to an infrared-conjugated secondary antibody (1:10,000 dilution) for 1 hour at room temperature. Protein bands were visualized and quantified using the Odyssey imaging system (LI-COR Biosciences, Lincoln, NE, USA). GAPDH served as an internal loading control. The following antibodies were used: γ-H2AX, EIF4A1, CtIP, STING, pSTING, TBK1, pTBK1, IRF3, pIRF3, and GAPDH, which were purchased from Abcam (Cambridge, UK).

Cytotoxicity assay

The cells were implanted in 96-well plates at a density of 2.0×10^3 cells/well. After 24 hours of cell culture, vincristine was prepared at six gradient concentrations (0, 0.2, 0.4, 0.8, 1.6, and 3.2 mg/mL) and added to the culture medium. Cells were then incubated for an additional 48 hours. Subsequently, 10 μL of Cell Counting Kit-8 (CCK-8) reagent (Dojindo Laboratories, Tokyo, Japan) was added to each well, and the plates were incubated at 37.8 ℃ in a humidified incubator containing 5% CO_2 for 1 hour. The absorbance at 450 nm was measured using a microplate reader, and the half-maximal inhibitory concentration (IC_{50})

value was determined based on the cell viability curve. All assays were conducted in quadruplicate.

Dual luciferase assay

The 3'-untranslated region of the EIF4A1 mRNA containing the miR-1284 binding site was amplified by PCR, and wild-type and mutant plasmids were constructed. After transfection for 48 h, the luciferase activity of firefly and renilla was measured, and the interaction between miR-1284 and EIF4A1 was evaluated by comparing the luciferase activity of the wild-type and mutant experimental groups; and three replicate experiments were performed to ensure the reliability of the results.

Human Protein Atlas

Human Protein Atlas (https://www.proteinatlas.org/) is a comprehensive online resource designed to map the expression and localization of proteins in human tissues and cells. This study was used to analyze the expression and prognosis of miR-1284 and EIF4A1 in GC.

Co-immunoprecipitation (Co-IP)

An EIF4A1 overexpression vector with GST-tagged EIF4A1 was constructed by Co-IP and transfected into the GC cell line MGC-803 to allow the cells to express the GST-EIF4A1 fusion protein. Subsequently, protein interactions were analyzed by Co-IP experiments. During this process, the cells were lysed to release proteins, and after a pre-clearance step to remove the non-specifically bound proteins, the GST antibodies were used for Co-IP.

Apoptosis assay

Apoptosis was tested using the apoptosis detection kit (BD Biosciences, San Jose, CA, USA) in accordance with the manufacturer's instructions. The cells were hatched in a solution containing 5 μL/mL of Annexin V-phycoerythrin (PE) and 5 μL/mL of 7-amino-actinomycin D at 4 ℃ in the dark (MultiSciences Biotech, Hangzhou, China). The results were tested by flow cytometry (BD Accuri C6 Plus; BD Biosciences).

Animal experiments

Mouse strain selection: male, 615 strain mice, weighing 18–22 g, were selected as the experimental model for the subcutaneous transplanted tumor experiments. The animals used were sourced from Guangxi Medical University Animal Center. Establishment of mouse subcutaneous transplanted tumor models: the 615 strain mice were injected in the axilla with 2.5×10^5 of the mouse GC cell line (MFC). RT group received a radiation dose of 10 Gy on day 7 after modeling when the longest diameter of the transplanted tumor reached 3–5 mm. The programmed cell death-ligand 1 (PD-L1) group received intraperitoneal injections of PD-L1 antibody (10 mg/kg) every 3 days for a total of five administrations, starting on day 7 after modeling. The rocaglamide (RocA) group received intraperitoneal injections of RocA at a dose of 1 mg/kg every 2 days, starting from day 7 after modeling, for a total of seven treatments (five mice in each group). The white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), platelet (PLT), blood urea nitrogen (BUN), alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) of the blood samples were determined, and damage to important organs was observed on the 15 and 30 days. Animal experiments were performed under a project license (No. 2024-D125-01) granted by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, in compliance with Animal (Scientific Procedures) Act 1986 guidelines for the care and use of animals. A protocol was prepared before the study without registration.

Statistical analysis

SPSS (version 13.0; SPSS Inc., Chicago, IL, USA) was used to conduct the data analysis. All the data are expressed as the mean ± standard error (SE). Statistical analysis for comparing multiple groups was conducted using the χ^2 test or the rank sum test, as appropriate. For survival analysis, the Kaplan-Meier method was employed, with significance determined by the log-rank test. Comparisons between two groups were carried out using the Student's *t*-test. All results are presented as mean ± standard deviation (n=3), with a P value of less than 0.05 considered to indicate statistical significance.

Results

The intrinsic miR-1284/EIF4A1 axis in GC cells promotes radiation-induced DNA damage repair and is associated with GC prognosis

In view of the biological function of miR-1284 in GC and its regulatory effect on EIF4A1, we further explored the effect of the miR-1284/EIF4A1 axis on the radiation-induced DNA damage repair process in GC cells. To explore the effect of the RT dose on the GC cells,

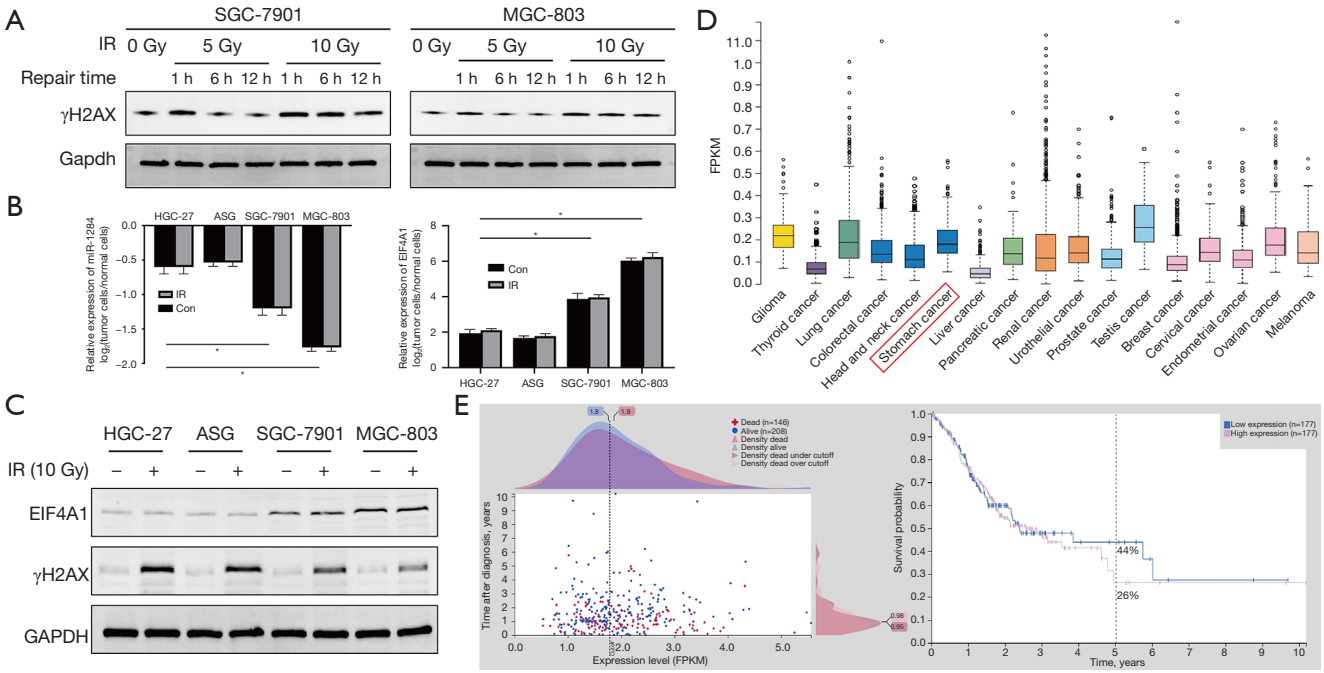

Figure 1 The miR-1284/EIF4A1 axis promotes the repair of radiation-induced DNA damage and is related to the prognosis of GC. (A) Exploration of RT conditions for GC cells. (B) Expression of the miR-1284/EIF4A1 axis after RT. (C) Effect of EIF4A1 expression in GC cells on radiation-induced DNA damage repair. (D) Expression of EIF4A1 in tumors. The red box is used to highlight the expression of EIF4A1 in gastric cancer. (E) Effect of EIF4A1 expression in GC on patient survival. *, P<0.05. Con, control; EIF4A1, eukaryotic translation initiation factor 4A1; FPKM, fragments per kilobase of transcript per million; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GC, gastric cancer; IR, ionizing radiation; miR-1284, microRNA-1284; RT, radiotherapy.

different RT doses were used (0, 5, and 10 Gy) at different time points (0, 1, 6, and 12 h). The expression of the DNA damage marker γ-H2AX in the GC cells after RT was determined. The results showed that when the RT dose was 5 Gy, the DNA damage to the GC cells was efficiently repaired at 6 and 12 h after RT, while when the RT dose was 10 Gy, DNA damage could be observed in the GC cells at 1, 6, and 12 h after receiving RT. Therefore, a RT dose of 10 Gy was used for the subsequent studies (P<0.05, *Figure 1A*).

We then detected the expression of the miR-1284/EIF4A1 axis in different GC cells at 12 h after RT and its effect on radiation-induced DNA damage repair. The results showed that RT had no effect on the expression of miR-1284 and EIF4A1 in the GC cells, and there was a negative correlation between the expression of miR-1284 and EIF4A1 in the GC cell lines (P<0.05, *Figure 1B*). The expression of EIF4A1 in the GC cells promoted radiation-induced DNA damage repair (P<0.05, *Figure 1C*). This suggests that the miR-1284/EIF4A1 axis plays a key role in the repair of radiation-induced DNA damage.

The expression and prognosis of EIF4A1 in GC were further analyzed by human protein mapping. The results showed that EIF4A1 was expressed in GC, and its fragments per kilobase of transcript per million (FPKM) value of 1.8 was second only to testicular cancer and glioma (*Figure 1D*); compared with the GC patients with high expression of EIF4A1, those with low expression of EIF4A1 had a higher 5-year overall survival rate (P<0.05, *Figure 1E*).

The above results showed that the miR-1284/EIF4A1 axis in the GC cells promotes the repair of radiation-induced DNA damage and is closely related to the prognosis of GC. However, its specific mechanism is not yet clear, and further in-depth research needs to be conducted to clarify its mechanism of action.

Blocking the miR-1284/EIF4A1 axis in GC cells delays the CtIP-mediated DNA repair process under RT

First, to investigate the mechanism of the miR-1284/

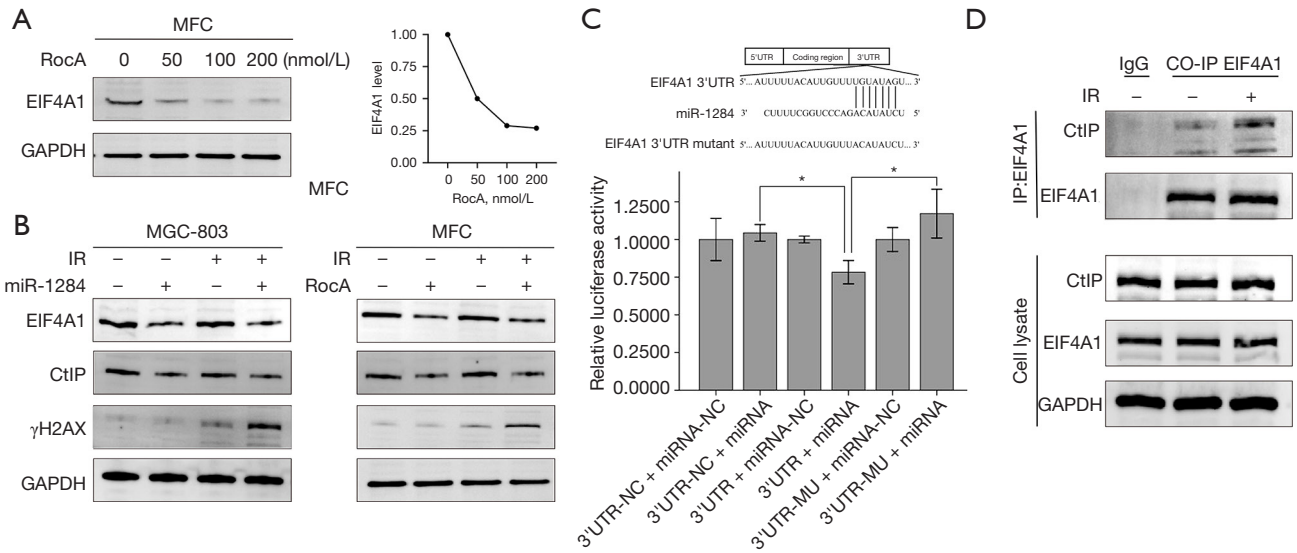

Figure 2 Blocking the miR-1284/EIF4A1 axis of GC cells delayed the CtIP-mediated DNA repair process under RT. (A) Exploration of RocA concentration in GC cells. (B) Blocking the effect of the miR-1284/EIF4A1 axis on CtIP and DNA repair. (C) Double luciferase verified the targeting relationship between miR-1284 and EIF4A1. (D) Co-IP verified the interaction between EIF4A1 and CtIP protein. *, P<0.05. Co-IP, co-immunoprecipitation; CtIP, C-terminal binding protein interacting protein; EIF4A1, eukaryotic translation initiation factor 4A1; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GC, gastric cancer; IgG, immunoglobulin G; IP, immunoprecipitation; IR, ionizing radiation; MFC, mouse forestomach carcinoma; miR-1284, microRNA-1284; miRNA, microRNA; MU mutant; NC, negative control; RocA, rocaglamide; RT, radiotherapy; UTR, untranslated region.

EIF4A1 axis in radiation-induced DNA damage repair, the concentration of RocA (the EIF4A1 inhibitor purchased from MCE, Monmouth Junction, NJ, USA) in the MFC cells was explored. The results showed that the inhibition rate of EIF4A1 reached 73% when the concentration of RocA was 100 nmol/L, and the inhibition rate of EIF4A1 reached 75% when the concentration of RocA was 200 nmol/L. There was no difference in the inhibition rate between the RocA concentration of 100 nmol/L and the RocA concentration of 200 nmol/L (P>0.05, *Figure 2A*); thus, we selected 100 nmol/L of RocA as the experimental concentration for the MFC cells. Second, we used lentivirus technology and RocA to block the miR-1284/EIF4A1 axis, and tested the effects of the miR-1284/EIF4A1 axis on CtIP and γ-H2AX after RT 12 hours in GC cells. The results showed that blocking the miR-1284/EIF4A1 axis of the GC cells down-regulated CtIP expression and delayed DNA repair (P<0.05, *Figure 2B*). Finally, the targeting relationship between miR-1284 and EIF4A1 was verified via dual luciferase, and the results showed that EIF4A1 was the direct target gene of miR-1284 (P<0.05, *Figure 2C*). Co-IP was used to verify the targeting relationship between EIF4A1 and CtIP, and the results showed that there was an interaction between EIF4A1 and the CtIP protein (P<0.05, *Figure 2D*).

Blocking the miR-1284/EIF4A1 axis in GC cells promotes the RT activation of the cGAS-STING pathway and increases the apoptosis rate

We then sought to investigate whether blocking the miR-1284/EIF4A1 axis in the GC cells promoted the RT activation of the cGAS-STING pathway. First, we used lentivirus technology and RocA to block the miR-1284/EIF4A1 axis, and measured the axis effect on the free dsDNA content in the culture supernatant of the GC cells after RT (10 Gy, 12 h) through trace DNA. The results showed that blocking the miR-1284/EIF4A1 axis of the GC cells increased dsDNA induced by RT (P<0.05, *Figure 3A*), which laid an experimental foundation for activating the cGAS-STING pathway. Second, western blot and fluorescence qRT-PCR were used to detect the effect of blocking the miR-1284/EIF4A1 axis on the cGAS-STING pathway and interferon-β (IFN-β) in the GC cells after RT. The results showed that blocking the miR-1284/EIF4A1 axis under RT significantly down-regulated the protein

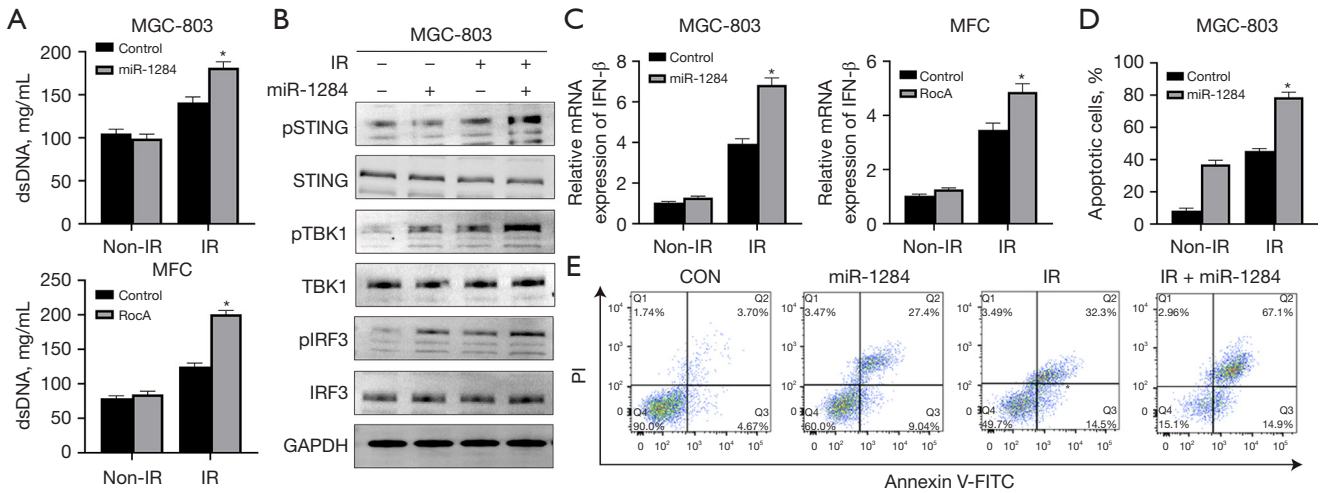

Figure 3 Blocking the miR-1284/EIF4A1 axis in GC cells promoted the RT activation of the cGAS-STING pathway and increased the apoptosis rate. (A) Effect of blocking the miR-1284/EIF4A1 axis on radiation-induced dsDNA. (B) Effect of blocking the miR-1284/EIF4A1 axis on the radiation-induced cGAS-STING pathway. (C) Effect of blocking the miR-1284/EIF4A1 axis on radiation-induced IFN-β. (D,E) Effect of blocking the miR-1284/EIF4A1 axis on radiation-induced apoptosis. *, P<0.05. cGAS-STING, cyclic GMP-AMP synthase-stimulator of interferon genes; CON, control; dsDNA, double-stranded DNA; EIF4A1, eukaryotic translation initiation factor 4A1; FITC, fluorescein isothiocyanate; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GC, gastric cancer; IFN-β, interferon-β; IR, ionizing radiation; MFC, mouse forestomach carcinoma; miR-1284, microRNA-1284; mRNA, messenger RNA; PI, propidium iodide; RocA, rocaglamide; RT, radiotherapy.

expression levels of pSTING, pTBK1, and pIRF3 (P<0.05, *Figure 3B*), and upregulated the expression level of IFN-β mRNA (P<0.05, *Figure 3C*). Finally, flow cytometry was used to detect GC cells apoptosis rate. The results showed that blocking the miR-1284/EIF4A1 axis increased the apoptosis of GC cells induced by RT (P<0.05, *Figure 3D,3E*).

Exploring the biological safety of RocA-binding PD-L1 antibodies in vivo experimental research based on RT

To explore the possibility of transforming the application of RocA-binding PD-L1 antibodies in RT, we further explored its biological safety. First, for the *in vivo* experiments, the 615 strain mice were intervened according to the grouping protocol (*Figure 4A*). Blood samples were taken to detect the WBC, RBC, HB, PLT, BUN, ALT, AST, and ALP indicators on days 15 and 30 after the intervention. The test results revealed no hematological toxicity (*Figure 4B*). Subsequently, the heart, liver, spleen, lung, and kidney tissues of the mice were extracted and stained with hematoxylin and eosin. The test results showed no significant damage to important organs (*Figure 4C*). These results suggest that *in vivo* experimental studies based on RocA binding to PD-L1 antibodies under RT have good biological safety, and further *in vivo* experiments can be carried out.

Discussion

China has the highest incidence and mortality rates of GC worldwide, accounting for 37.0% and 39.5% of all new GC cases and GC-related deaths worldwide, respectively (2). In China, GC is the third leading cause of cancer-related death, second only to lung cancer and liver cancer (2,3). RT is an important treatment method for GC, and uses high-energy radiation to induce DNA damage in tumor cells, including single-stranded DNA or dsDNA breaks, causing tumor cells to lose their ability to proliferate and eventually go to apoptosis, and activating the anti-tumor immune response in the body (4,10-12). However, tumor cells can activate the repair pathway through the DNA damage response (DDR) mechanism and reduce the accumulation of cytoplasmic DNA, inhibiting the activation of the cGAS-STING pathway and hindering the generation of specific anti-tumor immune responses, thereby reducing the effect of tumor RT (13-16). Thus, hindering or delaying the

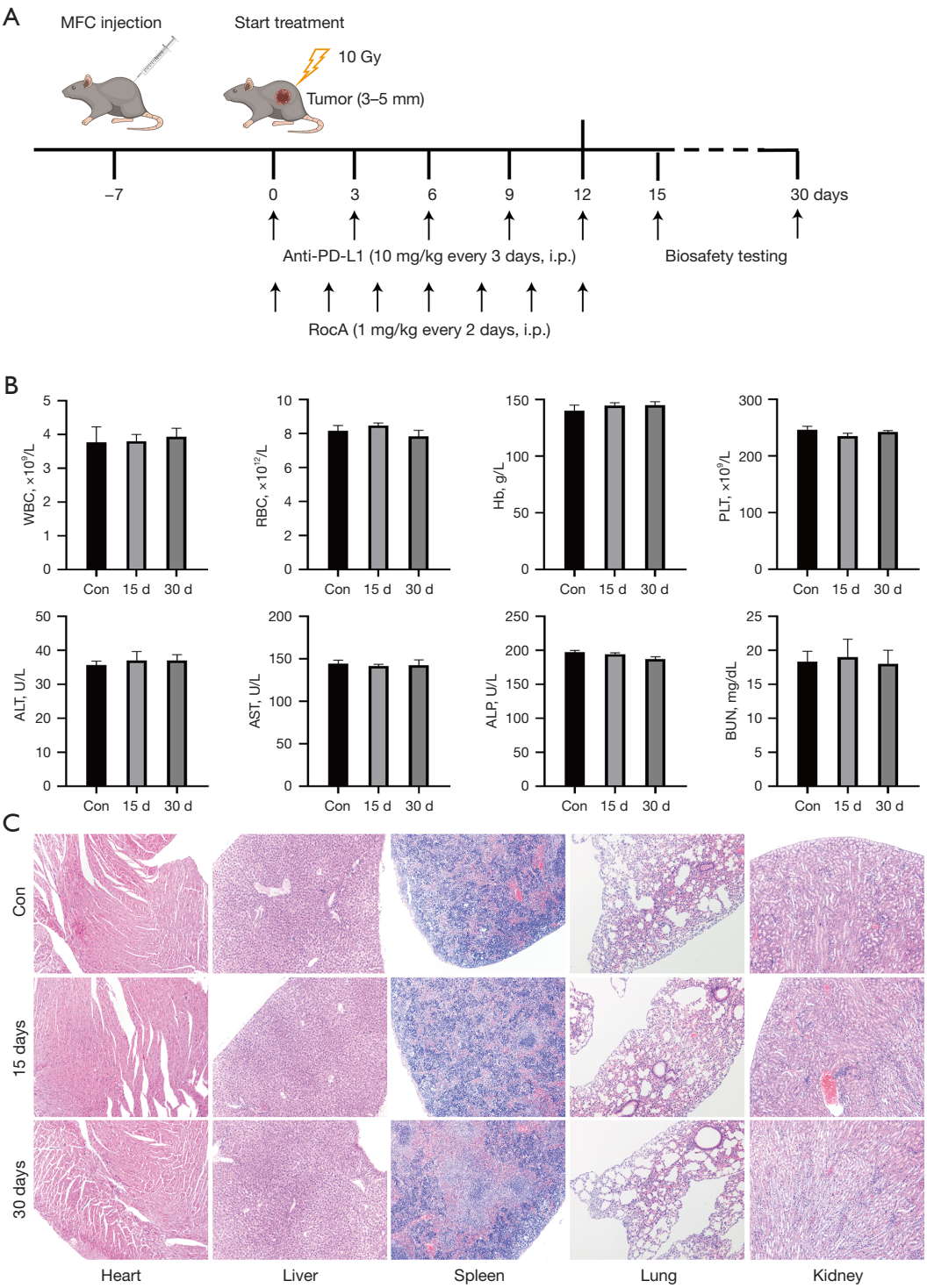

Figure 4 Biological safety verification of the *in vivo* experiments. (A) Intervention schematic diagram. (B) Blood toxic reaction testing. (C) Important organ safety testing (hematoxylin and eosin staining, ×100). ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Con, control; Hb, hemoglobin; i.p., intraperitoneal injection; MFC, mouse forestomach carcinoma; PD-L1, programmed cell death-ligand 1; PLT, platelet; RBC, red blood cell; RocA, rocaglamide; WBC, white blood cell.

repair of radiation-induced DNA damage can effectively enhance the efficacy of RT.

Recent studies have shown that in various tumor treatments, RT can activate the cyclic cGAS-STING pathway by inducing DNA damage, thereby triggering a strong specific anti-tumor immune effect (4,10,17,18). However, during RT for GC, the radiation dose is strictly limited to ensure the safety of the surrounding normal tissues (19,20). The excessive repair ability of the GC cells directly limits the activation of the cGAS-STING pathway by RT and it becomes one of the key factors affecting the efficacy of RT for GC (13,14).

Specifically, the DDR mechanism mainly includes two repair pathways: the HR and non-homologous end-joining pathways. The HR pathway is advantageous in maintaining DNA integrity because of its high accuracy, and thus it is the main DDR pathway of radiation-resistant cells (5). The main pathological type of GC is adenocarcinoma, and adenocarcinoma cells are more likely to radiation-induced DNA damage through HR, a more stable repair pathway, which in turn leads to RT resistance in GC (6). Resistance to RT in GC is a complex and multi-dimensional process involving multiple mechanisms, including DDR, immunosuppression, tumor stem cell characteristics, cell cycle arrest, and metabolic adaptation to a hypoxic environment. These mechanisms are intertwined, leading to RT resistance in GC and limiting RT efficacy (21).

Research has found that during the initial stage of HR pathway repair in tumor cells, various protein molecules, such as CtIP, the major histocompatibility (MHC) complex, and Exo1, are recruited to DNA damage sites; These proteins aggregate at the site of DNA damage, laying an important foundation for subsequent DNA repair initiation (22). CtIP plays a particularly critical role as a core regulator of the HR pathway. It can bind to the ends of dsDNA and effectively promote DNA end excision and ligation, which in turn promotes the DNA damage repair process of the HR pathway (22-25). Therefore, the targeted regulation of CtIP may become an efficient and precise strategy for regulating the HR pathway. Notably, Mejías-Navarro *et al.* (9) revealed the important role of EIF4A1 as a key eukaryotic translation initiation factor in the targeted regulation of CtIP. Specifically, EIF4A1 can unlock the G4 four-strand structure of the 5'-untranslated region of CtIP mRNA, thereby promoting the translation of CtIP, which in turn promotes the DNA damage repair process of the HR pathway. They also found that the miR-1284/EIF4A1 axis in GC cells plays an important role in promoting radiation-induced DNA damage repair. Additionally, by blocking the miR-1284/EIF4A1 axis, the axis can target and inhibit CtIP expression and delay the process of radiation-induced DNA damage repair. In addition, another study clearly showed that directly inhibiting the expression of EIF4A1 significantly delayed the DNA repair process of cervical cancer cells and increased the radiation sensitivity of tumor cells (26), which is consistent with our research results.

Another important factor affecting the efficacy of RT is whether radiation-induced DNA damage effectively activates the cGAS-STING signaling pathway, thereby triggering a strong and effective anti-tumor immune effect. The cGAS-STING pathway, as an important component of the innate immune system, can sense the dangerous signal of the accumulation of cytoplasmic free dsDNA caused by radiation-induced DNA damage, and establish a natural immune response by inducing the expression and secretion of type I interferons (IFN-I) and interferon-stimulated genes. It is one of the most important mechanisms for maintaining the immune defense of the organism's host (27,28).

In view of the core position of the GAS-STING signaling pathway in anti-tumor immunity, recent research has explored its potential value in mediating anti-tumor immunity. Yan *et al.* showed that as a targeted inhibitor of EIF4A1, RocA effectively promotes the infiltration of natural killer cells by activating the cGAS-STING signaling pathway, and significantly enhances the anti-tumor immune response (29). In addition, Liu *et al.* also confirmed that activating the cGAS-STING pathway through RT in GC enhances the body's anti-tumor immune effect, thereby significantly improving the sensitivity of GC patients to RT (30). Based on these findings, promoting the activation of the cGAS-STING pathway could become an effective strategy for enhancing the anti-tumor immune effect of RT for GC.

Based on the above existing literature reports and previous research related to this project, we showed the potential role of the miR-1284/EIF4A1 axis in the process of DNA damage repair (9,22-26), and the core role of the cGAS-STING pathway in anti-tumor immunity (29,30). However, it is unclear whether the regulation of the DNA damage repair process via the miR-1284/EIF4A1 axis effectively stimulates the anti-tumor immune response mediated by the cGAS-STING pathway during RT. Our research found that blocking the miR-1284/EIF4A1 axis of GC cells significantly promotes the activation of the cGAS-STING pathway by RT, and the activation of this pathway

provides an important experimental basis for enhancing the anti-tumor immune effect of RT in GC. In this study, we observed a significant increase in cytoplasmic dsDNA and downstream signaling following RT, which suggests a functional connection. However, to further confirm the causative upstream-downstream relationship, additional experiments such as cGAS knockdown or overexpression will be conducted in future work.

Clinically, the miR-1284/EIF4A1 axis represents a promising therapeutic target. High EIF4A1 expression is associated with poor prognosis, while upregulation of miR-1284 correlates with increased radiosensitivity. Targeting this axis may enhance RT efficacy and simultaneously activate tumor immunity via the cGAS-STING pathway, suggesting dual therapeutic benefits. Further investigation into its predictive value for patient stratification and therapeutic response is warranted.

To sum up, traditional RT has limited effects in the treatment of GC. The reason is that because of the RT dose limit, which is set to protect normal tissues, the rapid DDR of GC cells promotes the process of radiation-induced DNA damage repair, which in turn limits the direct killing and indirect anti-tumor immune activation of RT, which is a key factor leading to RT resistance in GC. Based on the findings from this study, we speculate that blocking the miR-1284/EIF4A1 axis to regulate the CtIP-mediated DNA damage repair process in GC cells and promoting the RT activation of the cGAS-STING pathway may be an effective strategy for increasing the sensitivity of GC to RT. However, in tumor immune regulation, the cGAS-STING pathway has complex dual effects. This pathway can effectively induce anti-tumor immune effects; however, it may also upregulate the expression of PD-L1 in tumor cells, further weakening its anti-tumor immune efficacy (31).

The use of RocA as a targeted inhibitor of EIF4A1 in conjunction with PD-L1 antibodies can further enhance radiation-induced anti-tumor immune responses, providing transformation possibilities for our results. Specifically, RocA inhibits the expression of EIF4A1, delays the repair of radiation-induced DNA damage and promotes radiation-induced activation of the cGAS-STING pathway, while PD-L1 antibodies relieve the tumor suppression of the immune system. This joint strategy could greatly improve the anti-tumor effect of RT. However, in the exploration of this combination therapy, biological safety is a key factor in clinical application. The potential toxic effects of RocA cannot be ignored, including bone marrow suppression, WBC, and thrombocytopenia, which can affect immune function, and even cause side effects such as gastrointestinal discomfort and hair loss. These toxic side effects indicate that its anti-cancer effect and its effects on normal cells need to be carefully weighed in clinical applications to ensure the safety of the treatment (32,33). To explore the possibility of the translational application of RocA-binding PD-L1 antibodies in RT, we further explored its biological safety. The results of the *in vivo* experiments based on RocA binding to PD-L1 antibodies under RT showed that it has good biological safety.

However, this study has several limitations. First, the translational relevance of findings from cell lines and mouse models may be constrained by inherent biological differences from human tumors. Second, the focus on the miR-1284/EIF4A1 axis did not account for interactions with broader regulatory networks that may also influence the DDR. Integrative transcriptomic and proteomic analyses could reveal additional modulators involved in RT sensitivity and immune activation.

Future studies will evaluate the therapeutic potential of targeting the miR-1284/EIF4A1 axis in patient-derived xenograft (PDX) models and investigate its predictive value for clinical outcomes. We also plan to explore the combinatorial efficacy of EIF4A1 inhibition and immune checkpoint blockade in larger animal models. Importantly, we will assess antitumor immune responses, including T cell infiltration and cytokine profiles, to better understand the immunomodulatory effects of this strategy and its potential for clinical translation.

Conclusions

The miR-1284/EIF4A1 axis plays a crucial role in facilitating the repair of DNA damage induced by RT in GC cells. Furthermore, it actively promotes the activation of the cGAS-STING pathway, which is essential for enhancing the body's anti-tumor immune response. Importantly, this axis exhibits good biological safety, making it a promising therapeutic target. Our findings not only deepen our understanding of the mechanisms underlying RT's anti-tumor effects but also provide a solid experimental basis for developing novel strategies to enhance the efficacy of RT in the treatment of GC by modulating the miR-1284/EIF4A1 axis.

Acknowledgments

We would like to thank those at the Guangxi Key

Laboratory of Enhanced Recovery after Surgery for Gastrointestinal Cancer and the Clinical Medicine Experimental Center of The First Affiliated Hospital of Guangxi Medical University for their support.

Footnote

Reporting Checklist: The authors have completed the ARRIVE and MDAR reporting checklists. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-2025-603/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-2025-603/dss

Peer Review File: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-2025-603/prf

Funding: The study was supported by grants from the National Natural Science Foundation of China (No. 82460569) and the Natural Science Foundation of Guangxi (No. 2024GXNSFAA010224). The study received the "Medical Excellence Award", which was funded by the Creative Research Development Grant, from The First Affiliated Hospital of Guangxi Medical University.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-2025-603/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Animal experiments were performed under a project license (No. 2024-D125-01) granted by the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University, in compliance with Animal (Scientific Procedures) Act 1986 guidelines for the care and use of animals.

References

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2024;74:229-63.
2. Yao YF, Sun KX, Zheng RS. Interpretation and analysis of the Global Cancer Statistics Report 2022: a comparison between China and the world. Chinese Journal of Bases and Clinics in General Surgery 2024;31:769-80.
3. Li ZY, Liu K, Zhang WH, et al. Epidemiological characteristics and trends of gastric cancer in the world and in China: Interpretation of the 2018 - 2022 Global Cancer Statistical Report. Chinese Journal of Bases and Clinics in General Surgery 2024;31:1236-45.
4. Petroni G, Cantley LC, Santambrogio L, et al. Radiotherapy as a tool to elicit clinically actionable signalling pathways in cancer. Nat Rev Clin Oncol 2022;19:114-31.
5. Setton J, Hadi K, Choo ZN, et al. Long-molecule scars of backup DNA repair in BRCA1- and BRCA2-deficient cancers. Nature 2023;621:129-37.
6. Guo S, Yao Y, Tang Y, et al. Radiation-induced tumor immune microenvironments and potential targets for combination therapy. Signal Transduct Target Ther 2023;8:205.
7. Cao W, Wei W, Zhan Z, et al. MiR-1284 modulates multidrug resistance of gastric cancer cells by targeting EIF4A1. Oncol Rep 2016;35:2583-91.
8. Wei W, Cao W, Zhan Z, et al. MiR-1284 suppresses gastric cancer progression by targeting EIF4A1. Onco Targets Ther 2019;12:3965-76.
9. Mejías-Navarro F, Rodríguez-Real G, Ramón J, et al. ALC1/eIF4A1-mediated regulation of CtIP mRNA stability controls DNA end resection. PLoS Genet 2020;16:e1008787.
10. Samson N, Ablasser A. The cGAS-STING pathway and cancer. Nat Cancer 2022;3:1452-63.
11. Chen G, Yu Z, Zhang Y, et al. Radiation-induced gastric injury during radiotherapy: molecular mechanisms and clinical treatment. J Radiat Res 2023;64:870-9.
12. Xhemalçe B, Miller KM, Gromak N. Epitranscriptome in action: RNA modifications in the DNA damage response. Mol Cell 2024;84:3610-26.
13. Wang L, Li S, Wang K, et al. DNA mechanical flexibility

controls DNA potential to activate cGAS-mediated immune surveillance. Nat Commun 2022;13:7107.
14. Song JX, Villagomes D, Zhao H, et al. cGAS in nucleus: The link between immune response and DNA damage repair. Front Immunol 2022;13:1076784.
15. Dvorkin S, Cambier S, Volkman HE, et al. New frontiers in the cGAS-STING intracellular DNA-sensing pathway. Immunity 2024;57:718-30.
16. Federica G, Michela C, Giovanna D. Targeting the DNA damage response in cancer. MedComm (2020) 2024;5:e788.
17. Yu L, Liu P. cGAS/STING signalling pathway in senescence and oncogenesis. Semin Cancer Biol 2024;106-107:87-102.
18. Song X, Ma F, Herrup K. Accumulation of Cytoplasmic DNA Due to ATM Deficiency Activates the Microglial Viral Response System with Neurotoxic Consequences. J Neurosci 2019;39:6378-94.
19. Polanowski P, Wydmański J, Tukiendorf A, et al. The analysis of absorbed dose by pancreas during gastric cancer radiotherapy. Radiother Oncol 2020;151:20-3.
20. Gao L, Zhang A. Low-dose radiotherapy effects the progression of anti-tumor response. Transl Oncol 2023;35:101710.
21. Usman M, Beilerli A, Sufianov A, et al. Investigations into the impact of non-coding RNA on the sensitivity of gastric cancer to radiotherapy. Front Physiol 2023;14:1149821.
22. Qiu Z, Hao S, Song S, et al. PLK1-mediated phosphorylation of PPIL2 regulates HR via CtIP. Front Cell Dev Biol 2022;10:902403.
23. Genetic Tumor Markers Collaboration Group, Tumor Biomarker Committee; China Anti-cancer Association, Molecular Pathology Group, Chinese Society of Pathology. Expert consensus on clinical detection and application of homologous recombination to repair defects (2021 edition). Chinese Journal of Oncology Prevention and Treatment 2021;13:329-38.
24. Yue W, Zhang HY, Schatten H, et al. CtIP regulates G2/M transition and bipolar spindle assembly during mouse oocyte meiosis. J Genet Genomics 2024;51:1435-46.
25. Zhang Z, Samsa WE, Gong Z. NUDT16 regulates CtIP PARylation to dictate homologous recombination repair. Nucleic Acids Res 2024;52:3761-77.
26. Liang S, Ju X, Zhou Y, et al. Downregulation of eukaryotic initiation factor 4A1 improves radiosensitivity by delaying DNA double strand break repair in cervical cancer. Oncol Lett 2017;14:6976-82.
27. Storozynsky Q, Hitt MM. The Impact of Radiation-Induced DNA Damage on cGAS-STING-Mediated Immune Responses to Cancer. Int J Mol Sci 2020;21:8877.
28. Li Q, Wu P, Du Q, et al. cGAS-STING, an important signaling pathway in diseases and their therapy. MedComm (2020) 2024;5:e511.
29. Yan X, Yao C, Fang C, et al. Rocaglamide promotes the infiltration and antitumor immunity of NK cells by activating cGAS-STING signaling in non-small cell lung cancer. Int J Biol Sci 2022;18:585-98.
30. Liu B, Liu H, Ren F, et al. cGAS regulates the DNA damage response to maintain proliferative signaling in gastric cancer cells. Oncol Res 2021;29:87-103.
31. Fan Y, Gao Y, Nie L, et al. Targeting LYPLAL1-mediated cGAS depalmitoylation enhances the response to anti-tumor immunotherapy. Mol Cell 2023;83:3520-3532.e7.
32. Zhu X, Zuo Q, Xie X, et al. Rocaglamide regulates iron homeostasis by suppressing hepcidin expression. Free Radic Biol Med 2024;219:153-62.
33. DiVita DJ, Kearse MG. A double on the Rocs with a twist: Rocaglamide A targets multiple DEAD-box helicases to inhibit translation initiation. Cell Chem Biol 2021;28:431-3.

(English Language Editor: L. Huleatt)

Cite this article as: Cao W, Su K, Lu C, Li J, Gui X. Effect and mechanism of the miR-1284/EIF4A1 axis on the cGAS-STING pathway under radiotherapy. Transl Cancer Res 2025;14(4):2483-2494. doi: 10.21037/tcr-2025-603