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Long Noncoding RNA Solute Carrier Family 25 Member 21 Antisense RNA 1 Inhibits Cell Malignant Behaviors and Enhances Radiosensitivity of Gastric Cancer Cells by Upregulating Synuclein Gamma Expression

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Long noncoding RNAs (LncRNAs) were reported to be implicated in the progression of gastric cancer (GC). This study aimed to explore the role of solute carrier family 25 member 21 antisense RNA 1 (SLC25A21-AS1) in radiosensitivity of GC cells. In the present study, reverse transcription quantitative polymerase chain reaction (RT-qPCR) showed that the expression of SLC25A21-AS1 and synuclein gamma (SNCG) was downregulated in GC tissues and cells, while the expression of microRNA-15a-5p (miR-15a-5p) was upregulated in GC tissues and cells. The expression of SLC25A21-AS1 was elevated in GC cells after radiation treatment. SLC25A21-AS1 overexpression enhanced GC cell radiosensitivity, inhibited cell proliferation and promoted apoptosis. SLC25A21-AS1 overexpression also facilitated the DNA damage caused by radiation in GC cells. Mechanically, SLC25A21-AS1 interacted with miR-15a-5p and negatively regulated miR-15a-5p expression in GC cells. SNCG was directly targeted by miR-15a-5p at the 3' untranslated region (3'UTR). In GC tissues, the expression of SNCG was negatively correlated with that of miR-15a-5p, but was positively correlated with that of SLC25A21-AS1. Rescue assays revealed that SNCG silencing rescued the tumor-suppressive effect of overexpressed SLC25A21-AS1 on GC cells. The enhanced radiosensitivity caused by SLC25A21-AS1 overexpression was also reduced by SNCG knockdown. In conclusion, lncRNA SLC25A21-AS1 inhibits cell malignant behaviors and enhances cell radiosensitivity in GC by elevating SNCG expression.

Keywords: gastric cancer; long noncoding RNA; radiosensitivity; solute carrier family 25 member 21 antisense RNA 1; synuclein gamma
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Introduction

Gastric cancer (GC) is the fifth most prevalent malignancy and the third leading cause of cancer mortality (Smyth et al. 2020). Over a million new cases of GC were reported in 2018, which accounts for 5.7% of all cancer cases (Li and Gao 2019). With a death number of 782,685, the mortality rate (8.2%) is also high (Bray et al. 2018). The primary risk factor for GC is *Helicobacter pylori* infection, which is implicated in 90% of non-cardia GC cases (Plummer et al. 2015). Other risk factors such as age, diet, alcohol consumption and smoking are also critical for the incidence of GC (Kumar et al. 2020; Larsson et al. 2020; Suzuki et al. 2020). There are multiple ways for GC treatment, such as clinical surgery, radiotherapy, chemotherapy, and molecular targeted therapy (Sexton et al. 2020). Preoperative radiotherapy is effective and indicates better clinical outcomes in GC (Lim et al. 2005). Although the incidence is reducing, GC at high grade is characterized by a poor prognosis. The 5-year survival in GC patients is approximately 4% (Thrumurthy et al. 2015). Obscurity in the underlying hinders the clinical improvements in GC therapy (Song et al. 2017). Thus, it is imperative to explore the underlying mechanism in GC for the improvement of

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clinical outcome.

Long noncoding RNAs (LncRNAs) are transcripts more than 200 nucleotides long without protein coding capacity (Spokoini-Stern et al. 2020). Increasing studies suggest that many lncRNAs are aberrantly expressed in multiple human cancers and serve as critical regulators of proteins or RNA molecules as well as diverse biological functions (Tano and Akimitsu 2012; Beermann et al. 2016). Moreover, many investigations reveal that lncRNAs can regulate radioresistance in various aspects, such as DNA damage repair, epithelial-mesenchymal transition (EMT), apoptosis and cancer cell stemness (Zhang et al. 2020b). For example, lncRNA nuclear paraspeckle assembly transcript 1 (NEAT1) knockdown promotes the inhibitory effects induced by radiation on cell survival and tumor growth in GC via the negative regulation of miR-27b-3p (Jiang et al. 2020). LincRNA-p21 enhances the radiosensitivity of GC cells with decreased cell proliferation and cell cycle by modulating the *β*-catenin signaling pathway (Chen et al. 2019). LncRNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1) improves the inhibition caused by radiation on GC cell viability (Xiao et al. 2019). In our study, whether solute carrier family 25 member 21 antisense RNA 1 (SLC25A21-AS1) modulates cell radioresistance in GC was under discussion.

In our study, we intended to investigate the molecular mechanism underlying SLC25A21-AS1 in GC, and its potential function on GC cell radioresistance. We hypothesized that SLC25A21-AS1 might enhance the radiosensitivity and inhibit the malignant behaviors of GC cells by regulating the miR-15a-5p/synuclein gamma (SNCG) axis. Then, a series of gain-of-function assays investigated the effects of SLC25A21-AS1 overexpression on the proliferation, DNA damage and apoptosis of radiation-treated GC cells. Our study might provide theoretical basis for improving radiotherapy of GC.

Materials and Methods

Bioinformatic analysis

Gene Expression Profiling Interactive Analysis (GEPIA) database (Tang et al. 2017) was used to analyze SLC25A21-AS1 and SNCG expression in GC tissues and normal gastric tissues based on The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) datasets. The Encyclopedia of RNA Interactomes (ENCORI) database (Li et al. 2014) was applied to detect miR-15a-5p and SNCG expression in GC tissues and normal gastric tissues based on TCGA dataset, the binding region of miR-15a-5p on SLC25A21-AS1 (or SNCG), and the correlation among SLC25A21-AS1, miR-15a-5p and SNCG expression in GC tissues.

Cell culture and radiation treatment

Human GC cell lines (HGC-27, SNU-1, and KATO III) were purchased from National Collection of Authenticated Cell Cultures (Shanghai, China) and human gastric epithelium cell GES-1 was supplied by Procell (Wuhan, Hubei, China). Then the cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM; Thermo Fisher Scientific, Shanghai, China) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific) and 1% penicillin/streptomycin (Solarbio, Beijing, China) in a humidified incubator at 37°C in 5% CO_2. Next, the GC cells were treated with radiation using a RS-2000 Pro X-ray irradiator (Rad Source Technologies, Inc., Suwanee, GA, USA) for 24 h. A single radiation dose of 4 Gy was used or combined with overexpressed SLC25A21-AS1 (Shen et al. 2018; Zhang et al. 2020a).

Cell transfection

Short hairpin RNA (sh-RNA) against SNCG (sh-SNCG#1/2), miR-15a-5p mimics and pcDNA3.1/SLC25A21-AS1 and their negative control vectors (sh-NC, NC mimics, pcDNA3.1) were synthesized by GenePharma (Shanghai, China) and transfected into HGC-27 and SNU-1 cell lines with Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) before exposing to 4 Gy of radiation.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA from GC cells were collected using a TRIzol kit (Invitrogen). The extracted RNAs were reverse transcribed into cDNAs using a Reverse Transcription kit (Thermo Fisher Scientific). RT-qPCR was performed using the SYBR Green PCR Master Mix (TOYOBO, Osaka, Japan) and the Bio-Rad CFX-96 detection system (Bio-Rad, Hercules, CA, USA). The relative gene expression was calculated using the $2^{-\Delta\Delta Ct}$ method. The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was the internal control for lncRNAs and mRNAs, and U6 for miRNAs. The primer sequences are presented in Table 1.

Cell Counting Kit-8 (CCK-8) assay

GC cell viability was detected with CCK-8 assay. After indicated treatment or transfection, HGC-27 and SNU-1 cells were plated into 96-well plates and incubated for 48 h. Then, each well was supplemented with 10 μL of CCK-8 solution (Dojindo, Tokyo, Japan) and incubated for another 4 h at ambient temperature. A microplate reader (Thermo Fisher Scientific) was used to determine the absorbance at 450 nm.

Colony formation assay

The proliferation capacity of GC cells was accessed by colony formation assay. The transfected GC cells were grown into the 6-well plates containing DMEM and cultured for 14 days. Subsequently, the colonies were fixed with paraformaldehyde for 10 min, and then dyed with crystal violet solution (Dingguo Biotech, Shanghai, China) for 30 min. Forty-eight h later, the colony number was manually counted in five randomly chosen visual fields using a microscope (Olympus, Tokyo, Japan).

Table 1. Primer sequences used for RT-qPCR.

Gene	Primer sequences
SLC25A21-AS1	Forward: 5′-GACCTAAACGGAATCAGAATCTC-3′ Reverse: 5′-ACAATCAACAGAGAACTCGT-3′
SNCG	Forward: 5′-TGTATGTGGGAGCCAAGAC-3′ Reverse: 5′-CAGATGGCCTCAAGTCCTC-3′
LITAF	Forward: 5′-TAACAATCCAATTACCGTGCAG-3′ Reverse: 5′-GGACAACACATTTGGATAGGG-3′
JARID2	Forward: 5′-GTGCATCTATAAGGGAAGGTC-3′ Reverse: 5′-CTTGCTGAAACACATGCTC-3′
SRPK1	Forward: 5′-CAAAGGAATCAGTACTGCTCC-3′ Reverse: 5′-CTAGTAATTCTGCCTGGCG-3′
ZBTB34	Forward: 5′-AGATGTTGACTCTGTTACCGT-3′ Reverse: 5′-TTTCACTCCGTTTCGACTC-3′
ITPR1	Forward: 5′-GCAATGAGGTCAATTCCGT-3′ Reverse: 5′-TGTCGTCTTTGTTATCACTCC-3′
PLPP6	Forward: 5′-CCCAGACGAATTGGAATCC-3′ Reverse: 5′-AAACAGTTGAGTTGGTGCT-3′
SPRYD3	Forward: 5′-GATGGCAAGCTGTACAATGG-3′ Reverse: 5′-ACATCAAAGGACACAGGCT-3′
GAPDH	Forward: 5′-TCATTTCCTGGTATGACAACGA-3′ Reverse: 5′-GTCTTACTCCTTGGAGGCC-3′
miR-15a-5p	Forward: 5′-TAGCAGCACATAATGGTTTGTGC-3′ Reverse: 5′-CTCTACAGCTATATTGCCAGCCAC-3′
miR-497-5p	Forward: 5′-AACAGTGCAAACCACACTGTG-3′ Reverse: 5′-GTCGTATCCAGTGCAGGGT-3′
miR-670-3p	Forward: 5′-CTGATCGTGAGGAGAGTGT-3′ Reverse: 5′-GGTCTTCGACATCGGGGCGG-3′
miR-16-5p	Forward: 5′-GGAAGATGAGGAGGTCGCTG-3′ Reverse: 5′-GACTTGACTGGAAGGGTGGG-3′
miR-15b-5p	Forward: 5′-TCGGGTAGCACACATAATGG-3′ Reverse: 5′-GTGCAGGGTCCGAGGT-3′
miR-424-5p	Forward: 5′-CAGCAGCAATTCATGT-3′ Reverse: 5′-TGGTGTCGTGGAGTCG-3′
miR-195-5p	Forward: 5′-AACCGGTAGCAGCACAGAAATG-3′ Reverse: 5′-CAGTGCAGGGTCCGAGGT-3′
miR-6838-5p	Forward: 5′-GCACTCCTGGATGCCAATCT-3′ Forward: 5′-CTCTACAGCTATATTGCCAGCCAC-3′
U6	Reverse: 5′-ATACAGAGAAAGTTAGCACGG-3′ Forward: 5′- GGAATGCTTCAAAGAGTTGTG-3′

Western blot analysis

The proteins in GC cells were collected using radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China). The protein concentration in lysis buffer was identified by bicinchoninic acid assay. Next, the extracted proteins were isolated with 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and electro-transferred to polyvinyl difluoride (PVDF) membranes (Millipore Sigma, Burlington, MA, USA). Subsequently, these membranes were blocked with 5% non-fat milk and cultured at 4°C overnight with primary antibodies, including anti-SNCG (#ab55424, 1:1,000), anti-p-γ-H2AX (#ab81299, 1:5,000), anti-γ-H2AX (#ab124781, 1:1,000), anti-p-RAD51 (#ab61111, 1:1,000), anti-RAD51 (#ab133534, 1:10,000), anti-p-CHK1 (#ab79758, 1:1,000), anti-CHK1 (#ab40866, 1:10,000), anti-Cleaved caspase 3 (#ab32042, 1:500), anti-Bax (#ab182734, 1:1,000), anti-Bcl-2 (#ab182858, 1:2,000), anti-PARP1 (#ab191217, 1:1,000) with GAPDH as the internal control. All the primary antibodies were purchased from Abcam company (Shanghai, China). Then the membranes were cultured with the second antibody at room temperature for 2 h in dark. The enhanced chemiluminescence solution (Millipore, Plano, TX, USA) was used to visualize protein signals.

Luciferase reporter assay

Wild-type (Wt) or mutant (Mut) sequences of miR-15a-5p containing the binding site with SLC25A21-AS1 were inserted into pmirGLO vectors (Promega, Madison, WI, USA) to construct miR-15a-5p-Wt and miR-15a-5p-Mut plasmids. Both plasmids were co-transfected with pcDNA3.1 or pcDNA3.1/SLC25A21-AS1 into GC cells using Lipofectamine 3000 (Invitrogen). Wild-type or

mutant fragments of SNCG 3′-untranslated region (3'UTR) with binding sites to miR-15a-5p were subcloned into the PGL3 vector (Promega) to construct SNCG-Wt and SNCG-Mut plasmids. Next, the plasmids were co-transfected into GC cells with pcDNA3.1, pcDNA3.1/SLC25A21-AS1, pcDNA3.1/SLC25A21-AS1+miR-15a-5p mimics by Lipofectamine 3000 (Invitrogen). Forty-eight h after transfection, a Dual-Luciferase Reporter Assay System (Promega) was applied to measure the relative luciferase activity.

RNA Immunoprecipitation (RIP) assay

RIP assay was conducted to investigate the interaction among genes. The Magna RIP Kit (Millipore, Billerica, MA, USA) was used to conduct the RIP assay. HGC-27 and SNU-1 cells were lysed in RIP lysis buffer. Then the cell lysate was co-cultured with A/G-plus agarose beads at 4°C for 4 h. The beads were washed with wash buffer, and then RNAs were isolated with protease K and quantified by the RT-qPCR analysis.

Statistical analysis

The SPSS 17.0 (IBM, Armonk, NY, USA) was utilized to analyze the statistical significance with Student's *t*-test for difference evaluation between two groups, and one-way analysis of variance for differences among multiple groups. All data are expressed as the means ± standard deviation. $p < 0.05$ was regarded as statistical significance.

Results

SLC25A21-AS1 overexpression enhances the radiosensitivity and inhibits the malignant behaviors of GC cells

Based on GEPIA database, SLC25A21-AS1 was significantly downregulated in GC tissues compared to normal tissues (Fig. 1A). SLC25A21-AS1 was also expressed at a lower level in GC cells compared with normal human gastric epithelium GES-1 cell, as shown by RT-qPCR (Fig. 1B). To figure out the influence of SLC25A21-AS1 on radioresistance in GC, SLC25A21-AS1 expression in GC cells treated with 4 Gy of radiation for 24 h was measured using RT-qPCR. The results indicated that SLC25A21-AS1 expression was elevated in the 4 Gy group compared to the control 0 Gy group (Fig. 1C). The overexpression efficiency of SLC25A21-AS1 in GC cells was then verified by RT-qPCR, which indicated that SLC25A21-AS1 expression was upregulated after overexpressing SLC25A21-AS1 in GC cells, regardless of whether the cells were treated with radiation (Fig. 1D). Next, a series of gain-of-function assays were performed to investigate the impacts of SLC25A21-AS1 overexpression on the malignant behaviors of radiation-treated GC cells. CCK-8 assay showed that GC cell viability was suppressed by SLC25A21-AS1 overexpression or radiation treatment (4 Gy). The combination of radiation and transfection of pcDNA3.1 showed most significant inhibitory effect on cell viability in GC (Fig. 2A). GC cell proliferation was accessed using colony formation assay, which indicated that cell proliferation was suppressed after either radiation treatment (4 Gy) or SLC25A21-AS1 overexpression, and the simultaneous radiation treatment and SLC25A21-AS1 overexpression resulted in the weakest colony formation ability (Fig. 2B). The levels of key factors related to DNA damage and repair were examined using western blot, and the protein levels of p-γ-H2AX, p-RAD51 and p-CHK1 were elevated by radiation treatment (4 Gy), while overexpressing SLC25A21-AS1 alone exerted no evident effects on their levels. However, compared with the 4 Gy group, the 4 Gy+pcDNA3.1-SLC25A21-AS1 group displayed increased protein levels of p-γ-H2AX, p-RAD51 and p-CHK1, which indicated that SLC25A21-AS1 overexpression further facilitated the DNA damage caused by radiation in GC cells (Fig. 3A). Moreover, the levels of apoptosis-associated proteins were measured by western blot analysis to evaluate how SLC25A21-AS1 influences GC cell apoptosis. The results demonstrated that the protein levels of Cleaved caspase 3, Bax and PARP1 were increased by SLC25A21-AS1 overexpression or radiation treatment (4 Gy), and further elevated by the combination of radiation and transfection of pcDNA3.1-SLC25A21-AS1, while the Bcl-2 expression showed contrary changes in GC cells (Fig. 3B). It suggested that overexpressing SLC25A21-AS1 further stimulated radiation-induced apoptosis in GC cells.

SLC25A21-AS1 binds with miR-15a-5p in GC cells

As shown in Fig. 4A, subcellular fractionation assay demonstrated that SLC25A21-AS1 was mainly localized in the cytoplasm of GC cells. Based on ENCORI database, eight miRNAs were sorted out under the screening condition of CLIP-Data ≥ 2 (Table 2). Among all candidate miRNAs, only miR-15a-5p was significantly downregulated by SLC25A21-AS1 overexpression (Fig. 4B). MiR-15a-5p expression was discovered to be significantly higher in GC tissues and cells than in normal gastric tissues and normal gastric epithelium GES-1 cell (Fig. 4C, D). After the treatment of radiation for 24 h, GC cells exhibited a lower level of miR-15a-5p compared with the control group (Fig. 4E). ENCORI database showed that the expression of miR-15a-5p was negatively corelated to that of SLC25A21-AS1 in GC tissues (Fig. 4F). The potential binding site of miR-15a-5p on SLC25A21-AS1 is predicted by the ENCORI database (Fig. 4G). RIP assay was used to explore the interaction between SLC25A21-AS1 and miR-15a-5p, and the results showed the enrichment of both SLC25A21-AS1 and miR-15a-5p in the anti-Ago2 precipitates in GC cells (Fig. 4H). According to luciferase reporter assay, the luciferase activity of wild-type miR-15a-5p was significantly decreased by the SLC25A21-AS1 overexpression in GC cells, while that of the mutant miR-15a-5p showed no evident change (Fig. 4I).

MiR-15a-5p directly targets on SNCG in GC cells

The miR-15a-5p target genes were searched on the

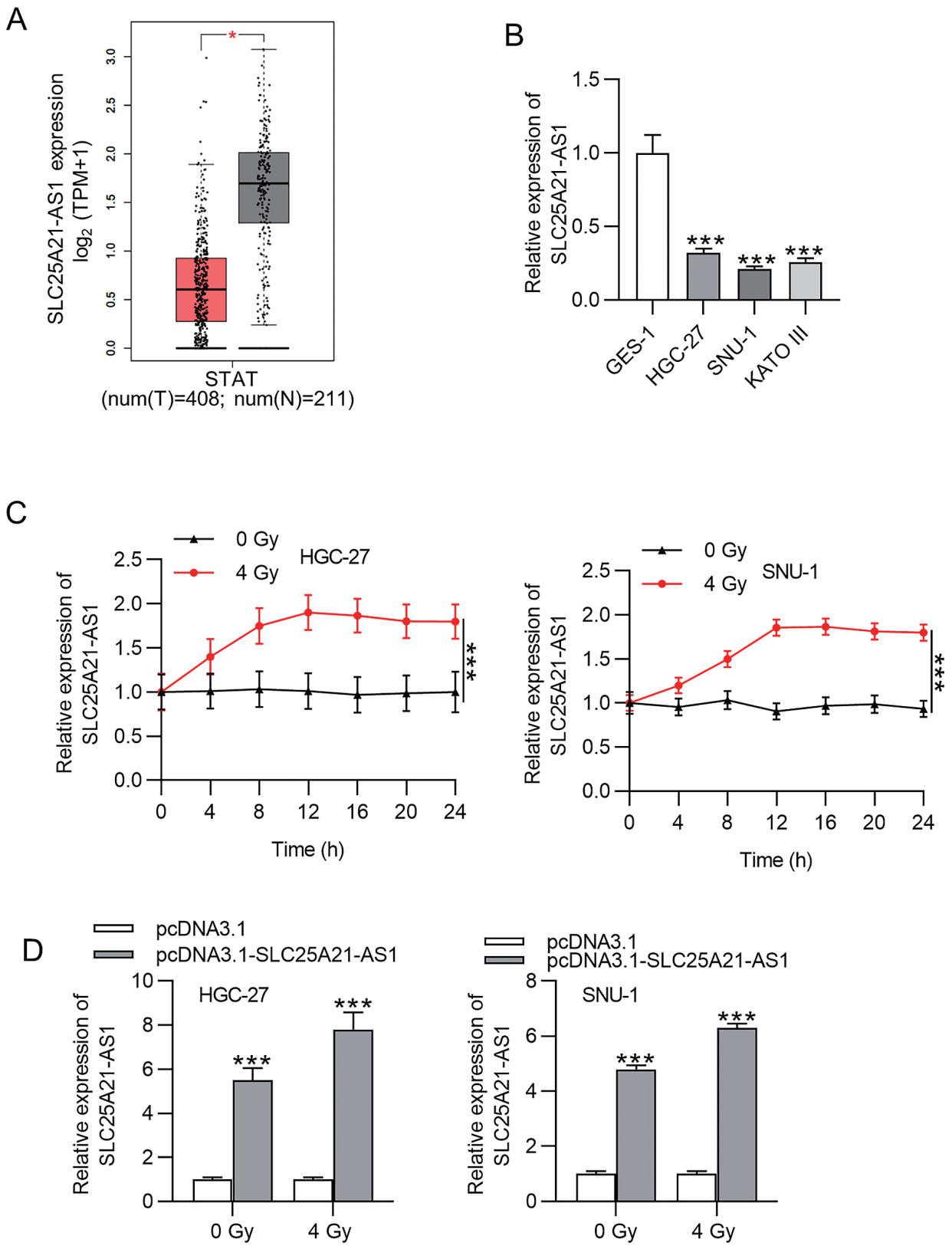

Fig. 1. SLC25A21-AS1 is downregulated in gastric cancer (GC) tissues and cells.
(A) The expression of SLC25A21-AS1 in GC tissues compared to normal gastric tissues was predicted by GEPIA database. (B) SLC25A21-AS1 expression was measured using RT-qPCR in GC cell lines (HGC-27, SNU-1 and KATO III) and human normal gastric epithelium cell GES-1. (C) The expression of SLC25A21-AS1 in GC cells exposed to 4 Gy of radiation for 24 h was detected by RT-qPCR. (D) The overexpression efficiency of SLC25A21-AS1 in GC cells treated with or without 4 Gy of radiation was accessed by RT-qPCR. *$p<0.05$, ***$p<0.001$.

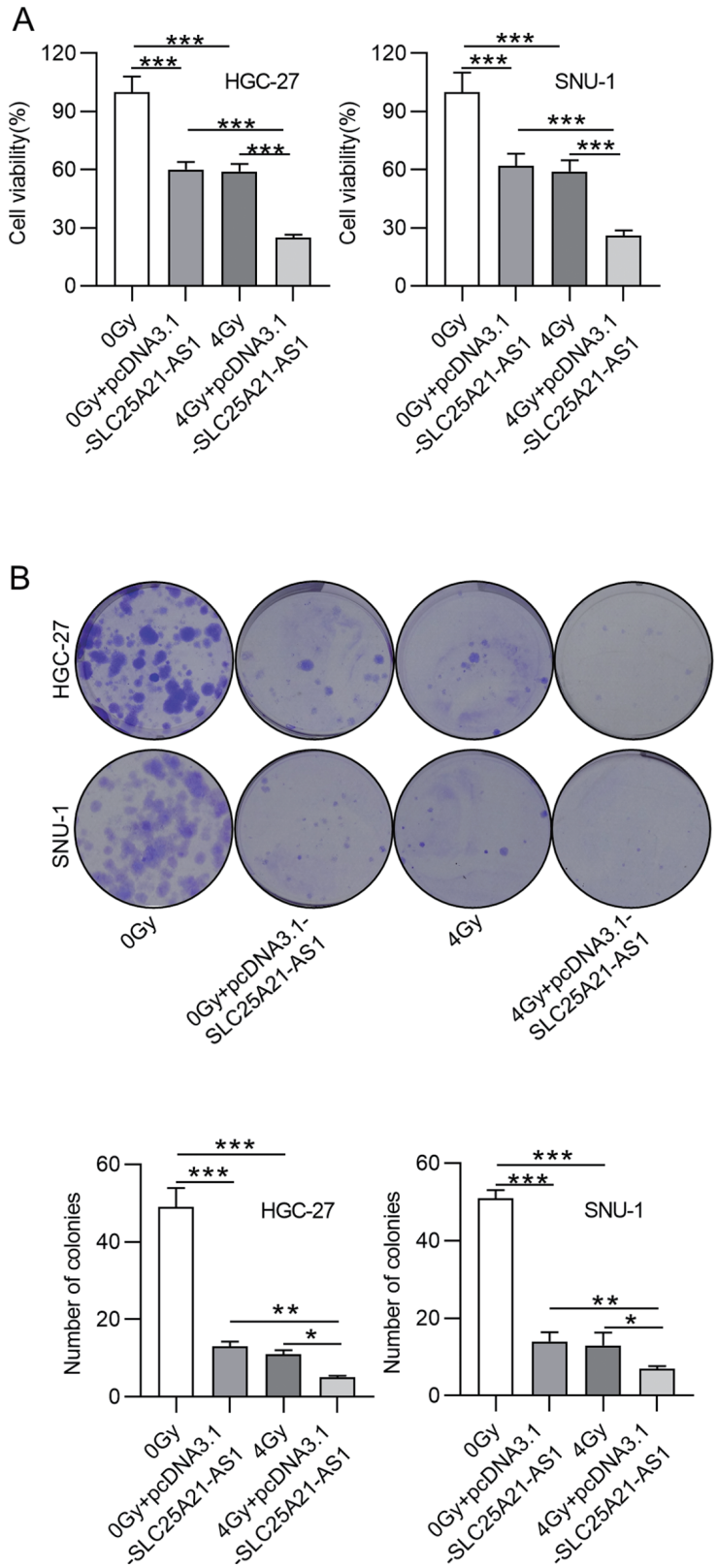

Fig. 2. SLC25A21-AS1 overexpression deepens the inhibitory effects of radiation treatment on gastric cancer (GC) cell proliferation.

(A) CCK-8 assay was conducted to detect the influence of radiation treatment, SLC25A21-AS1 overexpression, or the combination of both on GC cell viability. (B) Colony formation assay was performed to evaluate GC cell proliferation after above treatment or transfection. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

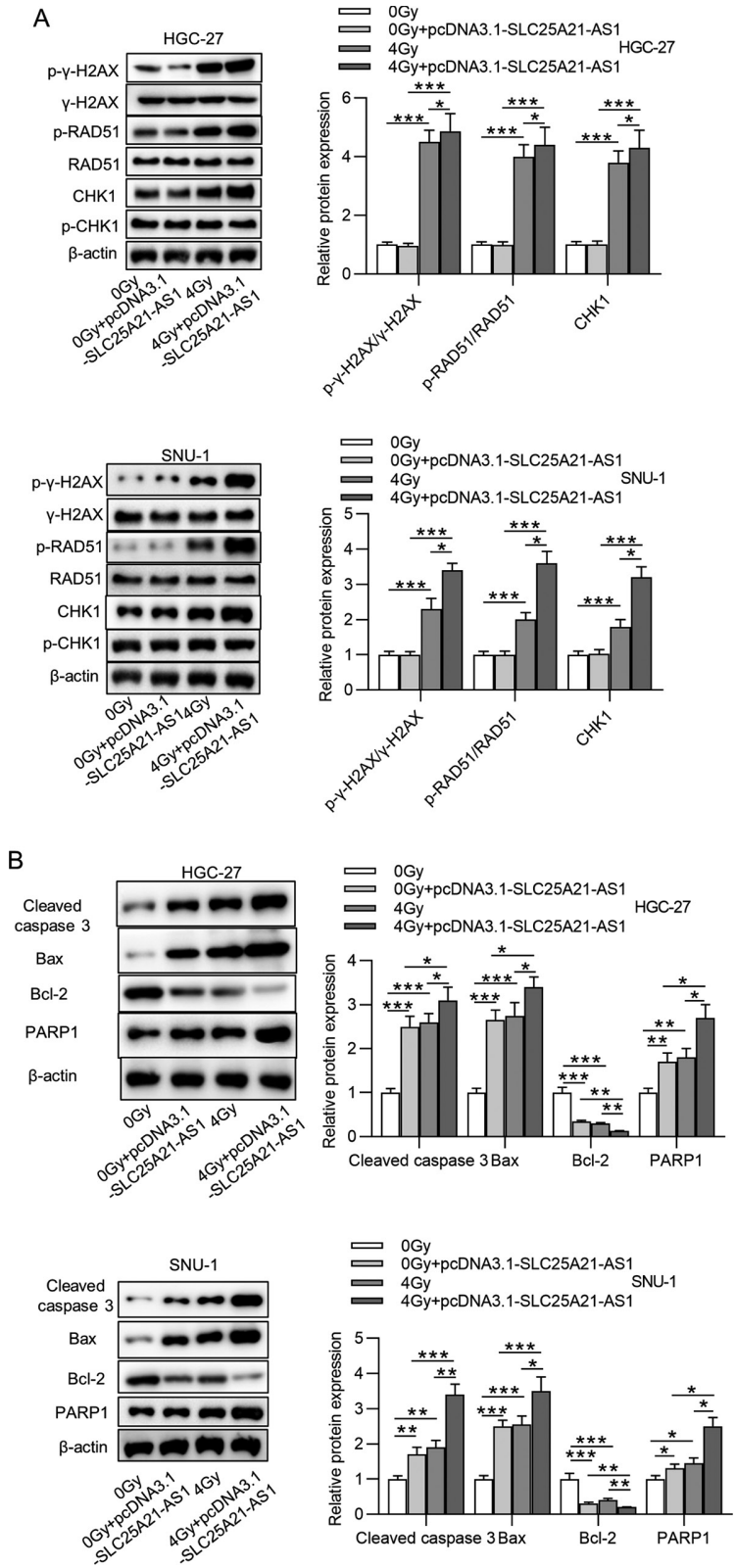

Fig. 3. SLC25A21-AS1 overexpression further intensifies DNA damage and cell apoptosis induced by radiation treatment in gastric cancer (GC) cells.

(A) The impacts of radiation treatment, SLC25A21-AS1 overexpression, or the combination of both on the protein levels of key factors related to DNA damage and repair (p-γ-H2AX, p-RAD51 and p-CHK1) in GC cells were measured by western blot. (B) The effects of the above treatment or transfection on the protein levels of cell apoptosis markers (Cleaved caspase 3, Bax, Bcl-2 and PARP1) in GC cells were examined by western blot. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

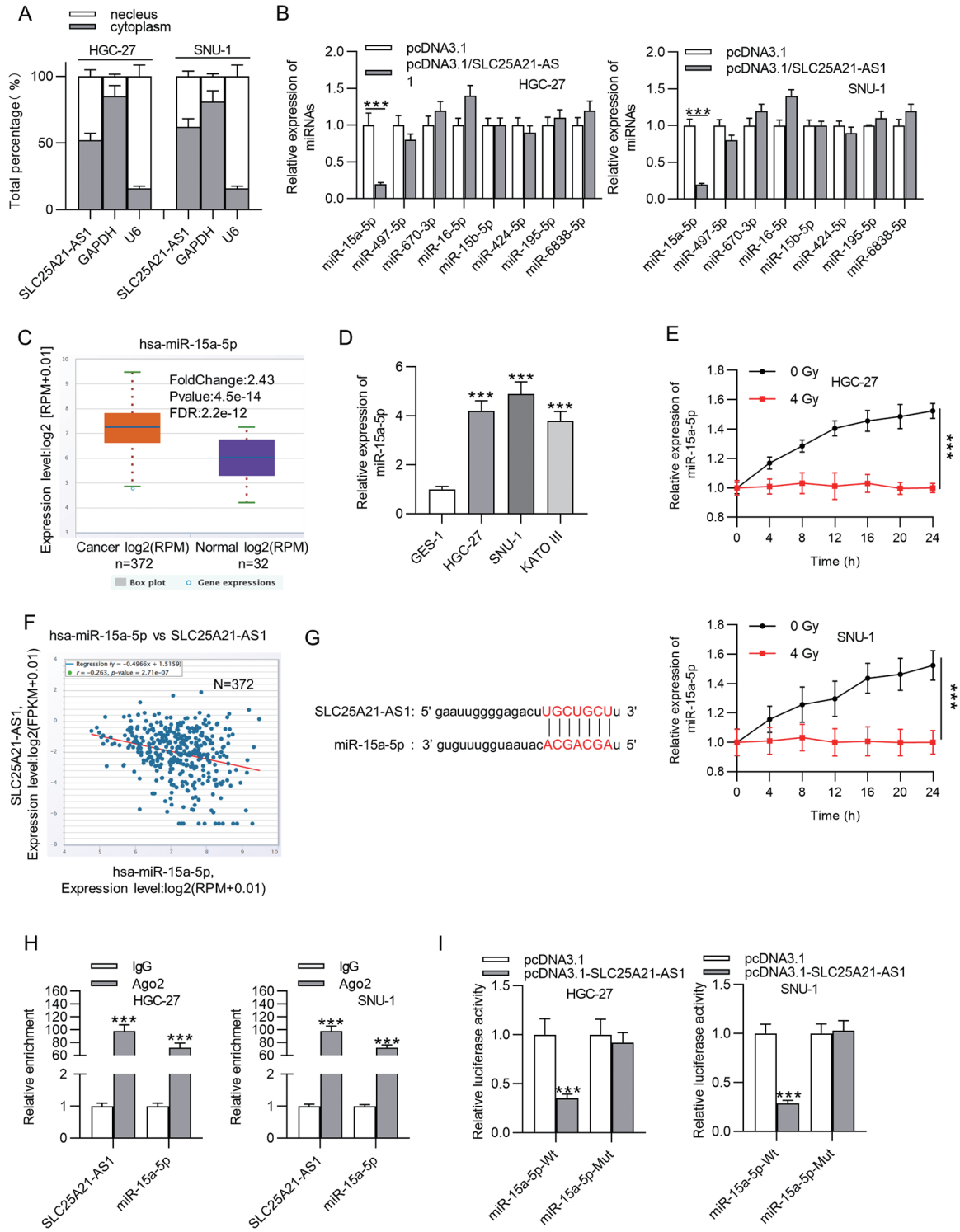

Fig. 4. SLC25A21-AS1 binds with miR-15a-5p in gastric cancer (GC) cells.
(A) Subcellular fractionation assay was used to access the localization of SLC25A21-AS1 in GC cells. (B) The levels of candidate miRNAs were measured using RT-qPCR in HGC-27 and SNU-1 cells after transfection of pcDNA3.1-SLC25A21-AS1. (C) The expression of miR-15a-5p in GC tissues compared to normal gastric tissues was predicted by ENCORI database. (D) MiR-15a-5p expression in GC cell lines (HGC-27, SNU-1 and KATO III) and human normal gastric epithelium cell GES-1 was measured using RT-qPCR. (E) The levels of miR-15a-5p in GC cells treated with 4 Gy of radiation for 24 h were detected by RT-qPCR. (F) The correlation between miR-15a-5p expression and SLC25A21-AS1 expression in GC tissues was predicted by ENCORI database. (G) The binding site of miR-15a-5p on SLC25A21-AS1 was shown according to ENCORI database. (H) RIP assay was conducted to show the enrichment of SLC25A21-AS1 and miR-15a-5p in Ago2 group of GC cells. (I) Luciferase reporter assay was used to verify the binding capacity between SLC25A21-AS1 and miR-15a-5p in GC cells. ***$p < 0.001$.

Table 2. Eight candidate miRNAs under the screening condition (CLIP-Data ≥ 2) based on the Encyclopedia of RNA Interactomes (ENCORI) database.

miRNA ID	miRNA name	Gene ID	Gene name	Gene type
MIMAT0026640	hsa-miR-670-3p	ENSG00000258708	SLC25A21-AS1	antisense
MIMAT0002820	hsa-miR-497-5p	ENSG00000258708	SLC25A21-AS1	antisense
MIMAT0000068	hsa-miR-15a-5p	ENSG00000258708	SLC25A21-AS1	antisense
MIMAT0000069	hsa-miR-16-5p	ENSG00000258708	SLC25A21-AS1	antisense
MIMAT0000417	hsa-miR-15b-5p	ENSG00000258708	SLC25A21-AS1	antisense
MIMAT0001341	hsa-miR-424-5p	ENSG00000258708	SLC25A21-AS1	antisense
MIMAT0027578	hsa-miR-6838-5p	ENSG00000258708	SLC25A21-AS1	antisense
MIMAT0000461	hsa-miR-195-5p	ENSG00000258708	SLC25A21-AS1	antisense

Table 3. Eight candidate mRNAs under the screening condition (CLIP-Data ≥ 1, Pan-Cancer ≥ 4, Program Number ≥ 1, Predicted Program: microT, miRanda, miRmap, PITA, RNA22) based on ENCORI database.

miRNA ID	miRNA name	Gene ID	Gene name	Gene type
MIMAT0000068	hsa-miR-15a-5p	ENSG00000173267	SNCG	protein_coding
MIMAT0000068	hsa-miR-15a-5p	ENSG00000167778	SPRYD3	protein_coding
MIMAT0000068	hsa-miR-15a-5p	ENSG00000189067	LITAF	protein_coding
MIMAT0000068	hsa-miR-15a-5p	ENSG00000150995	ITPR1	protein_coding
MIMAT0000068	hsa-miR-15a-5p	ENSG00000096063	SRPK1	protein_coding
MIMAT0000068	hsa-miR-15a-5p	ENSG00000008083	JARID2	protein_coding
MIMAT0000068	hsa-miR-15a-5p	ENSG00000205808	PLPP6	protein_coding
MIMAT0000068	hsa-miR-15a-5p	ENSG00000177125	ZBTB34	protein_coding

ENCORI database under specific screening conditions, and eight candidate mRNAs were screened out (Table 3). RT-qPCR showed that only SNCG was significantly downregulated in GC cells, while another seven mRNAs were upregulated in GC cells (Fig. 5A). Based on GEPIA and ENCORI database, SNCG was lowly expressed in GC tissues compared with normal gastric tissues (Fig. 5B, C). Then we measured the expression of SNCG in GC cells treated with 4 Gy of radiation for 24 h. The results revealed that SNCG was highly expressed in the radiation group compared with the control group (Fig. 5D). The influence of SLC25A21-AS1 upregulation or miR-15a-5p overexpression on the mRNA and protein levels of SNCG in GC cells was accessed using RT-qPCR and western blot. The results demonstrated that SNCG mRNA and protein levels were positively regulated by SLC25A21-AS1 and negatively regulated by miR-15a-5p in GC cells (Fig. 5E, F). Moreover, according to ENCORI database, the expression of SNCG was negatively correlated to that of miR-15a-5p but was positively correlated with that of SLC25A21-AS1 in GC tissues (Fig. 5G, H). The putative binding site of miR-15a-5p on SNCG was shown in Fig. 5I. The interaction among SLC25A21-AS1, miR-15a-5p and SNCG in GC cells was investigated by a RIP assay. The abundant enrichment of SLC25A21-AS1, miR-15a-5p and SNCG was found in the precipitates of anti-Ago2, which suggested that SLC25A21-AS1, miR-15a-5p and SNCG coexisted in the RNA induced silencing complex (Fig. 5J). Luciferase reporter assay indicated that SLC25A21-AS1 overexpression elevated the luciferase activity of SNCG-Wt, which was reversed by miR-15a-5p overexpression (Fig. 5K). No significant change was observed on the luciferase activity of SNCG-Mut.

SLC25A21-AS1 enhances the radiosensitivity and represses the malignant behaviors of GC cells by upregulating SNCG

The interfering efficiency of SNCG in GC cells was confirmed using RT-qPCR (Fig. 6A). Based on CCK-8 and colony formation assays, the reduction in cell viability and colony numbers caused by SLC25A21-AS1 overexpression or treatment of radiation or the combination of radiation and pcDNA3.1/SLC25A21-AS1 transfection was rescued by SNCG silencing in GC cells (Fig. 6B, C). The proteins related to DNA damage or repair showed no significant change after the transfection of pcDNA3.1-SLC25A21-AS1 or pcDNA3.1-SLC25A21-AS1+sh-SNCG#2, which suggested that SLC25A21-AS1 and SNCG were not directly implicated in the modulation of DNA damage or repair in GC cells. However, after the GC cells were treated with 4 Gy of radiation, the protein levels of p-γ-H2AX, p-RAD51 and p-CHK1 were increased and further elevated by SLC25A21-AS1 overexpression, while the knockdown of SNCG reversed the increase caused by SLC25A21-AS1 upregulation (Fig. 7). The levels of proteins associated

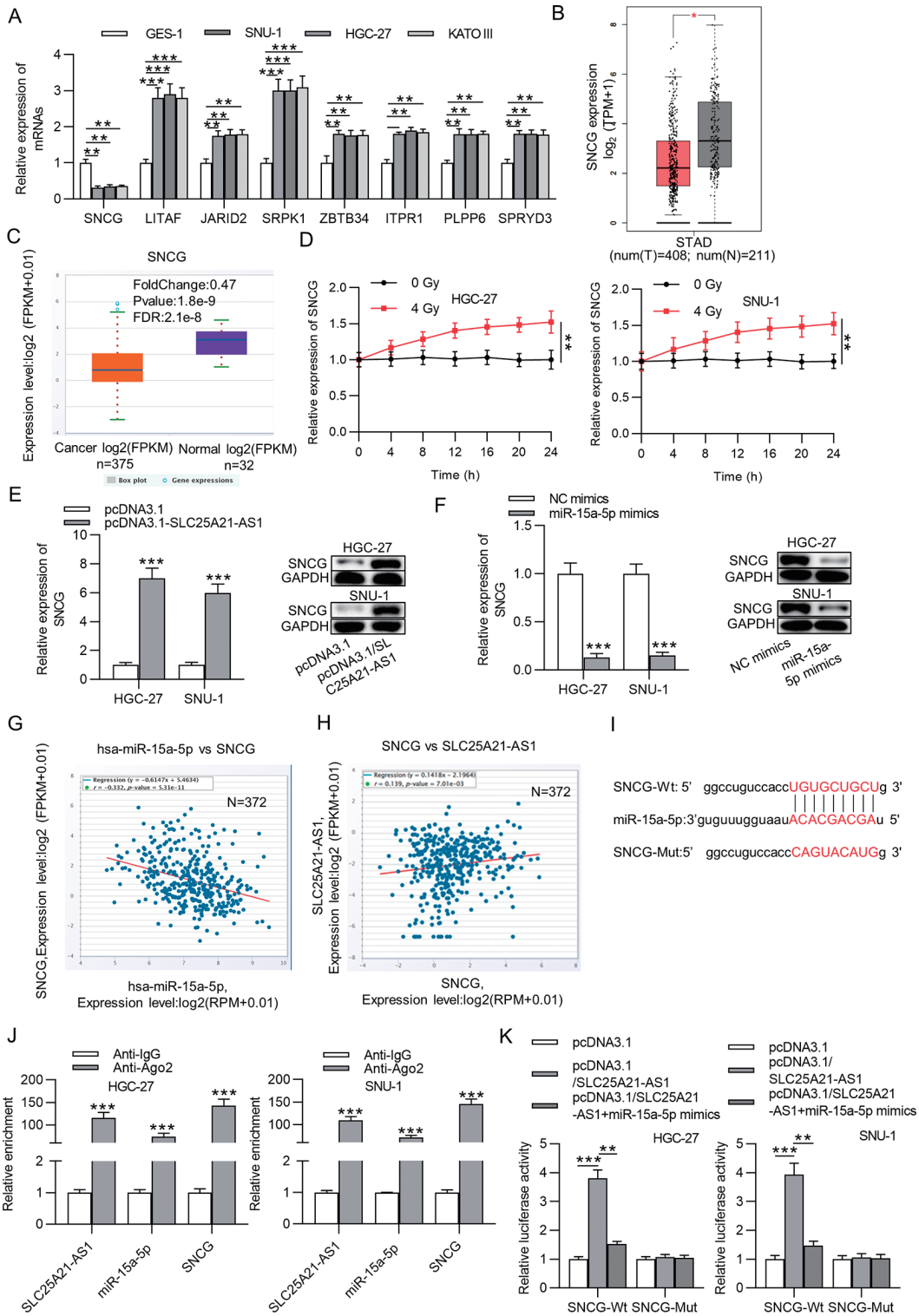

Fig. 5. MiR-15a-5p directly targets on SNCG in gastric cancer (GC) cells.
(A) The levels of eight candidate targets of miR-15a-5p in GC cells compared to normal GES-1 cell were examined using RT-qPCR. (B, C) SNCG expression in GC tissues compared with normal tissues was predicted by GEPIA and ENCORI database. (D) The expression of SNCG in GC cells exposed to 4 Gy or 0 Gy of radiation for 24 h was tested by RT-qPCR. (E, F) The SNCG mRNA and protein levels in GC cells after overexpressing SLC25A21-AS1 or miR-15a-5p were accessed using RT-qPCR and western blot. (G, H) The correlation between the expression of SNCG and miR-15a-5p (or SLC25A21-AS1) in GC tissues was predicted by ENCORI database. (I) The putative binding site of SNCG on miR-15a-5p was shown according to ENCORI database. (J) The interaction among SLC25A21-AS1, miR-15a-5p and SNCG was explored using RIP assay. (K) The binding among SLC25A21-AS1, miR-15a-5p and SNCG in GC cells was validated by luciferase reporter assay. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

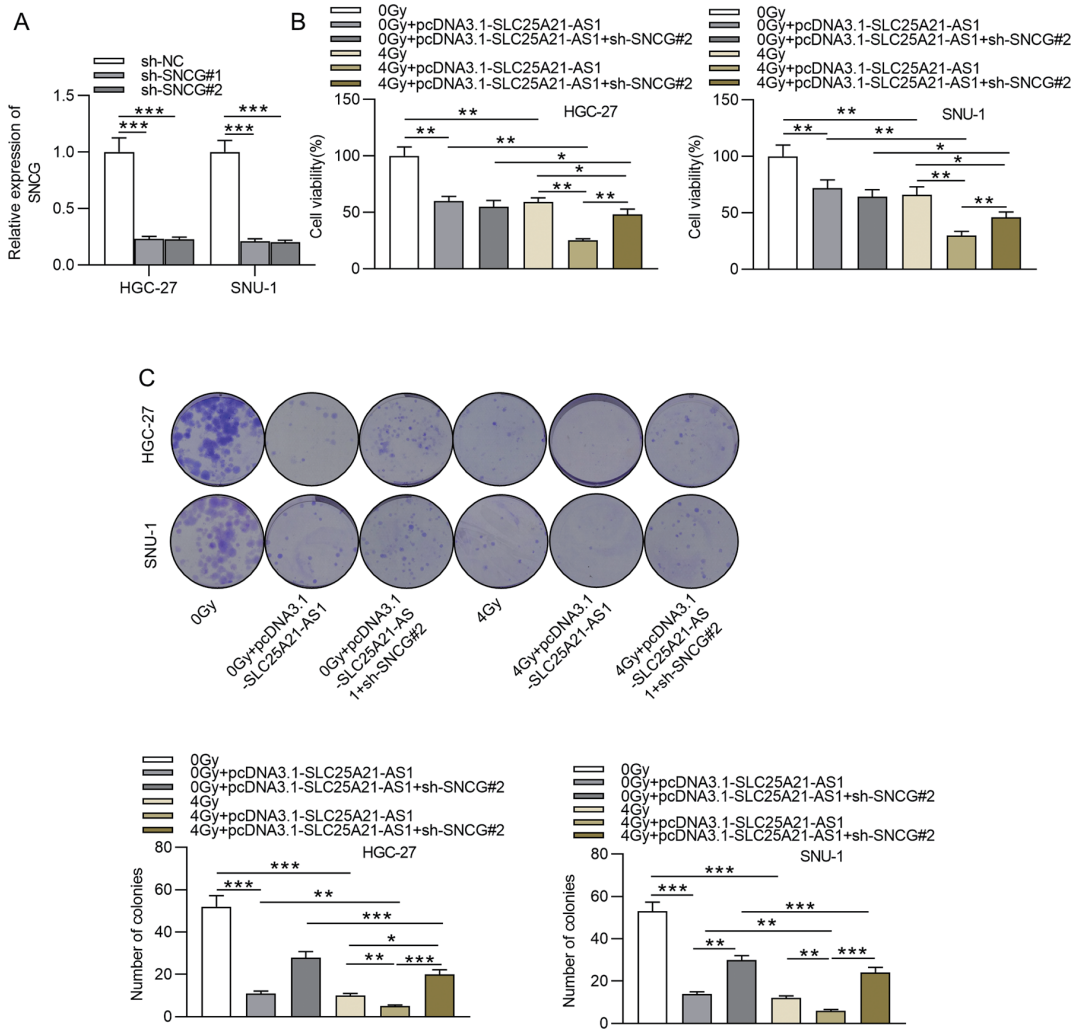

Fig. 6. SLC25A21-AS1 overexpression deepens the inhibitory effects of radiation treatment on gastric cancer (GC) cell proliferation by upregulating SNCG.
(A) The knockdown efficiency of SNCG in GC cells was examined using RT-qPCR. (B, C) CCK-8 and colony formation assays showed the effects of SNCG silencing on the viability and proliferation of GC cells treated with both radiation and SLC25A21-AS1 overexpression. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

with cell apoptosis were accessed by western blot. The increase in expression of cleaved caspase 3, Bax and PARP1, and the decrease in expression of Bcl-2 induced by SLC25A21-AS1 overexpression was reversed by silencing SNCG. Furthermore, radiation treatment (4 Gy) also caused the elevation of cleaved caspase 3, Bax and PARP1 levels as well as the reduction of Bcl-2 level, and SLC25A21-AS1 overexpression further deepened the effects of radiation on the levels of these proteins, which was finally reversed by SNCG knockdown (Fig. 8).

Discussion

Radiotherapy was reported to play important roles for the treatment of GC (Zhou et al. 2017). Radiation induces DNA damage via double-stranded DNA breaks (DSBs) which can lead to cell death by apoptosis (Mouw et al. 2017). Studies also indicate that lncRNAs can guide the organismal DNA damage response (Schmitt et al. 2016). However, in the process of treatment, the cancer cells can gain radioresistance which impairs the effect of therapy. The molecule targeted therapies provide an alternative approach to radiosensitization (Ratnayake et al. 2018). Several lncRNAs were demonstrated to attenuate the radioresistance of GC cells, thereby alleviating the development of GC (Lu et al. 2020; Xiao et al. 2020; Qin et al. 2022). Previously, SLC25A21-AS1 was discovered to enhance drug resistance in nasopharyngeal carcinoma via modulating the miR-324-3p/interleukin 6 (IL-6) axis (Wang et al. 2020). In our study, the role of SLC25A21-AS1 in radiotherapy of GC was under discussion. First, SLC25A21-AS1 was found to be downregulated in GC cell lines and tissues. In the GC cells treated with 4 Gy of radiation, SLC25A21-AS1 presented a higher level compared with the cells without radiation treatment. SLC25A21-AS1 overexpression suppressed the viability and proliferation, and facilitated apoptosis of GC cells, while no significant

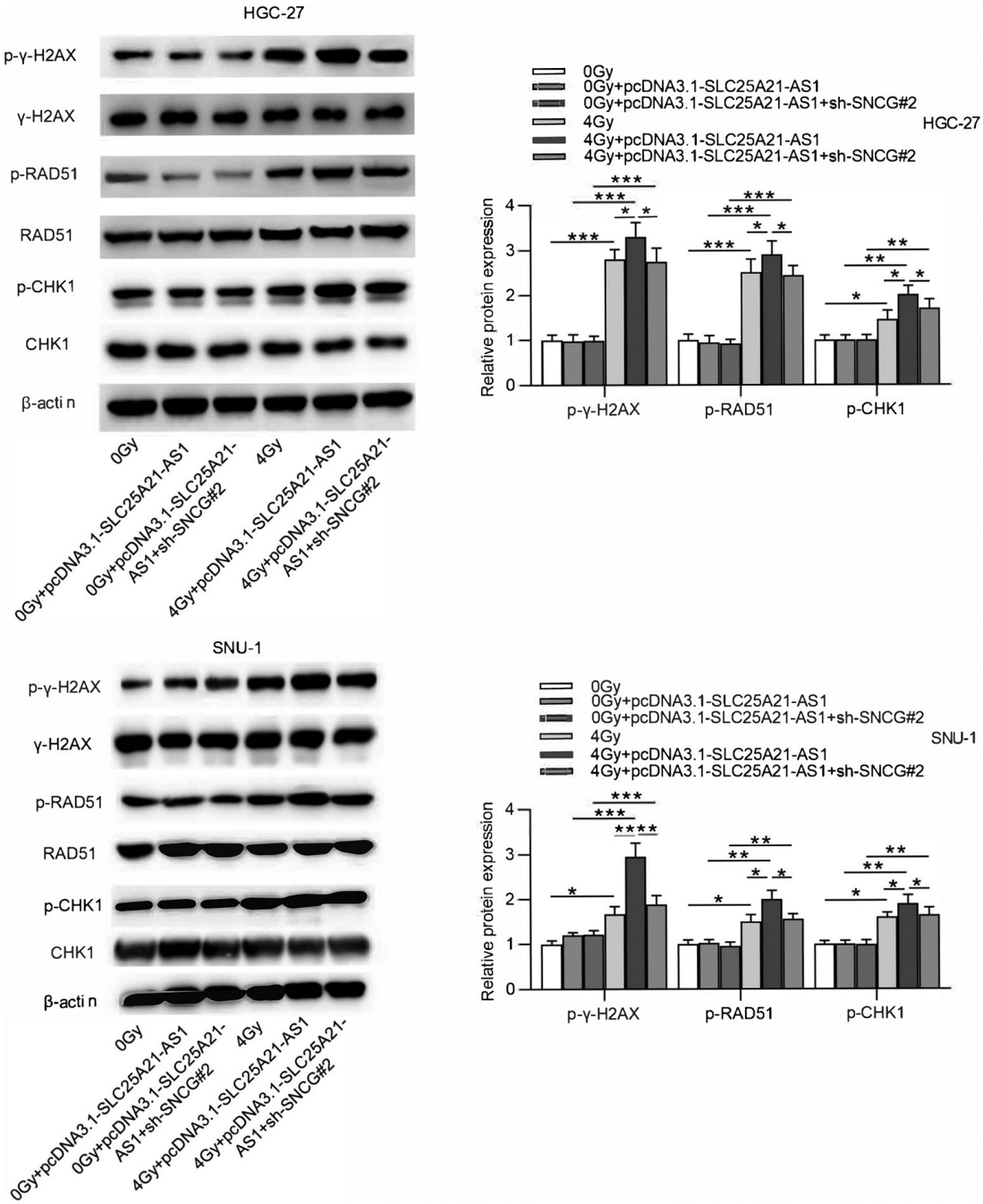

Fig. 7. SLC25A21-AS1 overexpression further intensifies DNA damage induced by radiation treatment in gastric cancer (GC) cells by upregulating SNCG.
Western blot was used to analyze the impacts of SNCG silencing on the protein levels of key factors related to DNA damage and repair (p-γ-H2AX, p-RAD51 and p-CHK1) in GC cells treated with both radiation and SLC25A21-AS1 overexpression. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

influence on cell DNA damage was detected. Moreover, radiation also inhibits the malignant phenotypes of GC cells as similarly as SLC25A21-AS1 did, except that radiation also caused the DNA damage of GC cells. Further, the combination of SLC25A21-AS1 overexpression and radiation treatment showed the best anti-tumor effect in GC cells, which also indicated that overexpressed SLC25A21-AS1 enhances radiosensitivity of GC cells.

Previous studies indicate that lncRNAs exert their functions in cancer progression via diverse mechanisms and competing endogenous RNA (ceRNA) is one of them that regulate the lncRNA functions (Tay et al. 2014; Qi et al. 2015). For example, LINC02381 serves as a ceRNA for miR-21, miR-590 and miR-27a, and suppresses cell cycle and proliferation while elevating cell apoptosis in GC via the Wnt signaling pathway (Jafarzadeh and Soltani 2020).

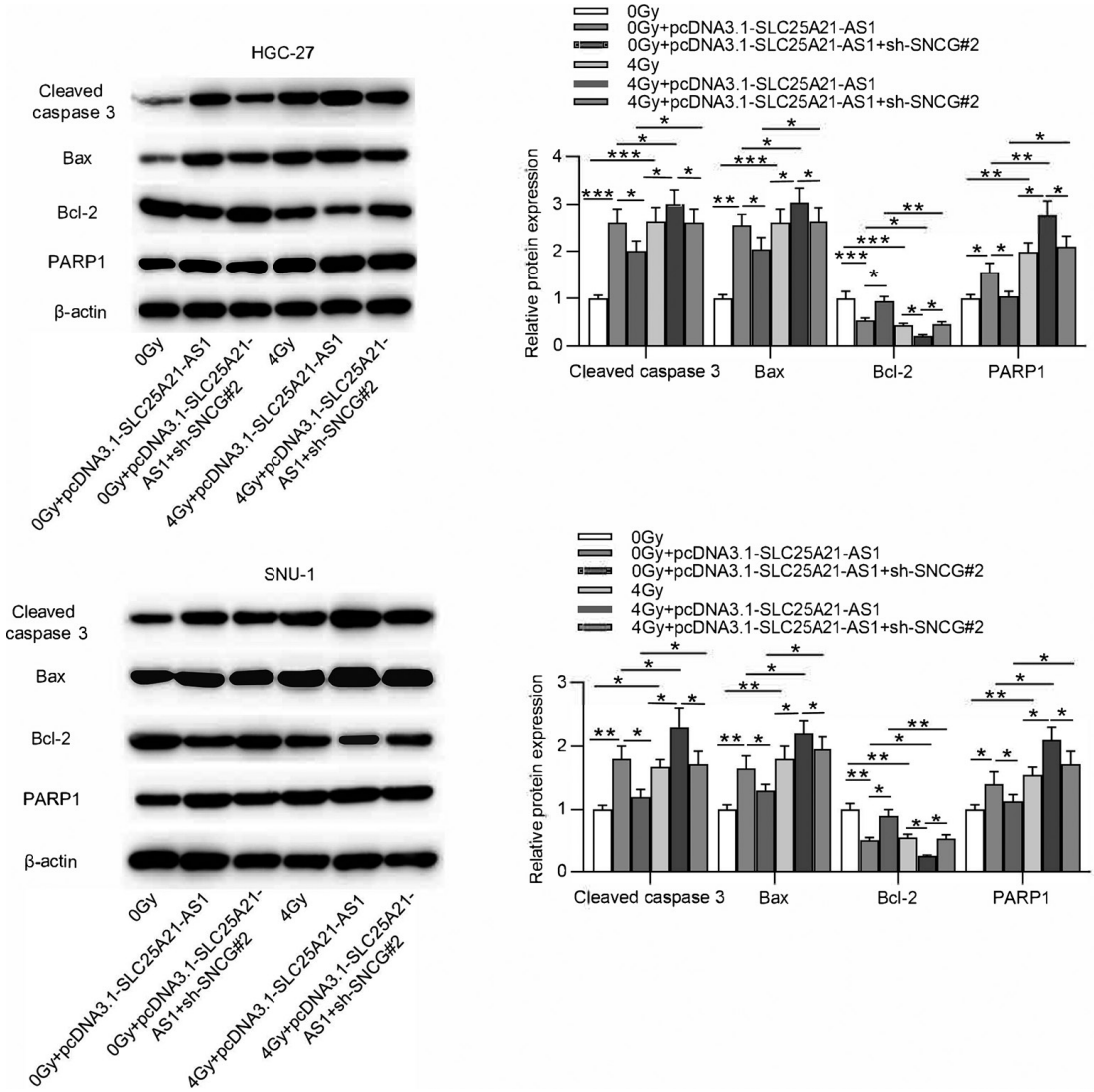

Fig. 8 SLC25A21-AS1 overexpression further promotes cell apoptosis induced by radiation treatment in gastric cancer (GC) cells by upregulating SNCG.
Western blot was applied to evaluate the influence of SNCG downregulation on the protein levels of cell apoptosis markers (Cleaved caspase 3, Bax, Bcl-2 and PARP1) in GC cells treated with both radiation and SLC25A21-AS1 overexpression. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

LncRNA CCDC144NL antisense RNA 1 (CCDC144NL-AS1) acts as a ceRNA sponging miR-143-3p to regulate mitogen-activated protein kinase kinase kinase 7 (MAP3K7), promoting tumorigenesis and lymph node metastasis in GC (Fan et al. 2020). LncRNA Pvt1 oncogene (PVT1) functions as a ceRNA for miR-30a to elevate Snail expression, which promotes the migration, EMT process and tumor metastasis in GC (Wang et al. 2021). SLC25A21-AS1 binds with miR-324-3p to upregulate the expression of IL-6, facilitating the cell growth and multidrug resistance in nasopharyngeal carcinoma (Wang et al. 2020). The underlying downstream mechanism of SLC25A21-AS1 was further investigated in this study. Through ENCORI database, miR-15a-5p was found to harbor potential binding sites on SLC25A21-AS1. MiR-15a-5p has been reported by numerous studies to be implicated in the cancer progression. For example, miR-15a-5p upregulation enhances glioma cell growth and attenuated cell apoptosis by targeting AE binding protein 1 (AEBP1) (Liu et al. 2021). MiR-15a-5p contributes to platinum resistance in GC cells by targeting PH domain and leucine rich repeat protein phosphatase 2 (PHLPP2), and its low expression in serum is correlated with enhanced cell response to oxaliplatin (Pang et al. 2020). MiR-15a-5p knockdown suppresses cell growth and facilitates cell apoptosis in cervical cancer by targeting tumor protein p53 inducible nuclear protein 1 (TP53INP1) (Zhao et al. 2019). The interaction between SLC25A21-AS1 and miR-15a-5p was verified. MiR-15a-5p was highly expressed in GC tissues and cells based on bioinformatics analysis and RT-qPCR. MiR-15a-5p expression was lower in GC cells treated with 4 Gy of radiation than 0 Gy. Moreover, miR-15a-5p expression was negatively modu-

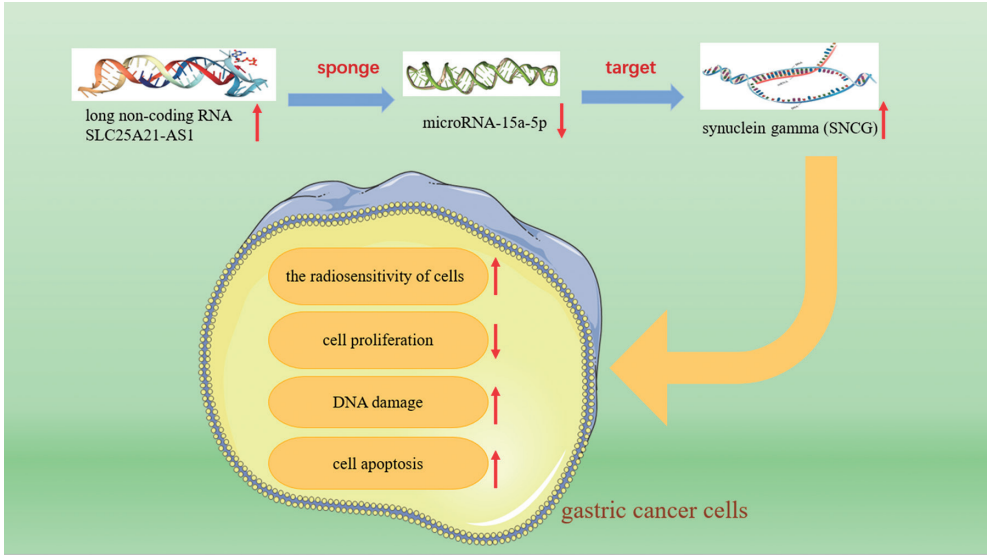

Fig. 9. A schematic diagram of the role of the SLC25A21-AS1/miR-15a-5p/SNCG axis in regulating the radiosensitivity and the malignant phenotypes of GC cell.

lated by SLC25A21-AS1 in GC cells.

SNCG was reported to be implicated in the development of multiple cancers, such as breast cancer (Guo et al. 2007), endometrial cancer (Mhawech-Fauceglia et al. 2012) and ovarian cancer (Zhang et al. 2020a). In the current study, SNCG was revealed as a direct target of miR-15a-5p at the 3'UTR region. SNCG was upregulated at mRNA and protein levels in GC cells. The bioinformatics analysis also indicated that SNCG is upregulated in GC tissues. A relatively high expression of SNCG was observed in GC cells after treatment of 4 Gy radiation. The SNCG mRNA and protein expression was positively regulated by SLC25A21-AS1 and negatively modulated by miR-15a-5p. A negative correlation between the expression of SNCG and miR-15a-5p as well as a positive correlation between the expression of SNCG and SLC25A21-AS1 were validated in GC tissues based on ENCORI database. The interaction among SLC25A21-AS1, miR-15a-5p and SNCG was verified in the study. Rescue assays revealed that SNCG silencing reversed the anti-tumor effect caused by SLC25A21-AS1 overexpression, radiation and the combination of radiation and SLC25A21-AS1 overexpression.

There also exist several limitations in our study. First, the data about the expression pattern of SLC25A21-AS1, miR-15a-5p and SNCG as well as their correlation in GC tissues and normal gastric tissues were all obtained from database. Clinical data are missed. Second, although our study suggested the role of the SLC25A21-AS1/miR-15a-5p/SNCG axis in suppressing the radioresistance of GC cells, the detailed downstream signaling mechanisms need to be fully explored in future studies. Third, through a series of *in vitro* studies, we discovered that SLC25A21-AS1 can enhance GC cell radiosensitivity. *In vivo* animal experiments are also required to further validate the role of SLC25A21-AS1 in GC.

Nevertheless, our research displays the first evidence on confirming the role of SLC25A21-AS1 in GC cell radiosensitivity. SLC25A21-AS1 was demonstrated to enhance the anti-tumor effects induced by radiation by binding with miR-15a-5p to upregulate SNCG in GC. These findings highlight the potential of SLC25A21-AS1 as a novel therapeutic target for GC (Fig. 9).

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Conflict of Interest

The authors declare no conflict of interest.

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