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ORIGINAL PAPER

LncRNA OGFRP1 promotes cell proliferation and suppresses cell radiosensitivity in gastric cancer by targeting the miR-149-5p/MAP3K3 axis

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Abstract

Gastric cancer (GC) is an aggressive malignancy with high incidence and mortality. Radiotherapy is a common treatment for patients with advanced GC. Many long noncoding RNAs (lncRNAs) have been verified to affect the radiosensitivity of multiple cancers in previous studies. Nevertheless, whether lncRNA opioid growth factor receptor pseudogene 1 (OGFRP1) affects the radiosensitivity of GC has not been determined. We hypothesized that OGFRP1 might affect cellular processes in GC development. The present study aims to explore the role of OGFRP1 in GC development. First, high expression of OGFRP1 in GC tissues and cells was determined through RT-qPCR. Subsequently, functional assays including colony formation assays, 5-Ethynyl-2′-deoxyuridine assays and flow cytometry analyses were performed to probe the biological functions of OGFRP1 in GC. Specifically, the effect of OGFRP1 on the radiosensitivity of GC cells was detected. Subsequently, with the help of the starBase tool, we found that miR-149-5p might bind to OGFRP1, which was confirmed through a luciferase reporter assay. Furthermore, we identified that MAP3K3 was targeted by miR-149-5p in GC cells. Finally, the results from rescue experiments validated that enhanced MAP3K3 expression counteracted the effect of OGFRP1 silencing on GC cell proliferation, apoptosis and radiosensitivity. Overall, OGFRP1 was determined to promote GC cell proliferation while suppressing cell apoptosis and radiosensitivity by regulating the miR-149-5p/MAP3K3 axis.

Keywords Gastric cancer · lncRNA OGFRP1 · miR-149-5p · MAP3K3 · Radiosensitivity

Introduction

Gastric cancer (GC) is a prevalent malignancy originating from the gastric mucosa, and the incidence rate of GC ranks first among all types of malignant tumors in China (Chen et al. 2015; Kamangar et al. 2006; Xu et al. 2016). Gastric cancer is the fifth most common malignancy worldwide with high mortality rate (Eusebi et al. 2020). Helicobacter pylori infection is a well-known risk factor for gastric cancer, and other factors including drug use, lifestyle and diet also contribute to the carcinogenesis of GC (Eusebi et al. 2020; Sexton et al. 2020). The five-year postoperative survival rate of patients diagnosed with GC at an early stage can reach 90.9% to 100%, but there is a lack of effective treatments available for patients diagnosed with advanced GC (Correa 2013; Kamangar et al. 2006). The five-year survival rate of patients with advanced GC is lower than 30%, even with the advancement of neoadjuvant chemoradiotherapy, immunotherapy and molecular-targeted therapy (Kamangar et al. 2006; Roviello et al. 2013; Song et al. 2017). More studies are required on the theoretical basis of GC to achieve a better prognosis for GC patients. Radiotherapy has been shown by meta-analysis to have a significant influence on the five-year survival rate in patients with resectable GC. The overall five-year risk ratio was 1.31 utilizing per-protocol analysis (Valentini et al. 2009). Moreover, approximately half of cancer patients will receive radiotherapy during their illness, and it is estimated that radiation therapy represents nearly 40% of

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curative treatments (Baskar et al. 2012). Although chemoradiotherapy is inferior when compared to chemotherapy (Cats et al. 2018; Zhu et al. 2020) and radiotherapy for cancer treatment is associated with side effects, such as toxicity (Brown et al. 2018), the improvement of radiation planning will enable more accurate and precise radiation treatment in the future (Lordick et al. 2021). Radiotherapy was shown in previous studies to be effective for metastatic GC when combined with immune checkpoint inhibitors (Qian et al. 2020; Sasaki et al. 2020). Previous studies revealed that numerous genes are associated with the radiosensitivity of many different tumors (Wang et al. 2020b; Wu et al. 2017; Zheng et al. 2016). Hence, it is important to search for more effective biological markers associated with the radiosensitivity of GC to better guide radiotherapy for patients with GC.

Recently, numerous studies have demonstrated that long noncoding RNAs (lncRNAs) are key regulators of the development of many different cancers, including GC. For example, lncRNA lung cancer associated transcript 1 (LUCAT1) (Chi et al. 2019), lncRNA LINC00858 (Du et al. 2020) and lncRNA LINC01314 (Tang et al. 2019b) were reported to affect GC cellular processes. The expression of lncRNA opioid growth factor receptor pseudogene 1 (OGFRP1) was reported to be upregulated in hepatocellular carcinoma, lung cancer, cervical carcinoma and prostate cancer, in which it acts as an oncogene (Chen et al. 2018; Tang et al. 2018; Yan et al. 2020; Zou et al. 2019). Herein, we explored the effect of OGFRP1 expression on the development of GC. The competing endogenous RNA (ceRNA) network mediated by lncRNAs was widely reported in GC development (Zhang et al. 2020b; Zheng et al. 2020). Following the lncRNA-miRNA-mRNA pattern, lncRNAs positively regulated the expression of messenger RNAs (mRNAs) by binding to microRNAs (miRNAs), and the mRNAs are target genes of the miRNAs (Li et al. 2016; Qi et al. 2020). For example, lncRNA DEAD/H-box helicase 11 antisense RNA 1 (DDX11-AS1) promotes GC cell proliferation by functioning as a ceRNA against miR-873-5p to increase the expression of signal peptidase complex 18 (SPC18) (Ren et al. 2020). LncRNA LINC00511 promotes the growth of GC cells by serving as a ceRNA for miR-124-3p, thereby upregulating the expression of pyruvate dehydrogenase kinase 4 (PDK4) (Sun et al. 2020). LncRNA LINC00163 suppresses the invasive capacity of GC cells by binding to miR-183 as a ceRNA to decrease the expression of A Kinase Anchoring protein 12 (AKAP12) (Zhang et al. 2020a). Hence, we hypothesized that OGFRP1 might function as a ceRNA to affect GC cell proliferation, apoptosis and radiosensitivity.

The main purpose of the study was to probe the functions and mechanisms of OGFRP1 in the development of GC. For this purpose, we first examined OGFRP1 expression in GC tissues and cells and then investigated the biological functions of OGFRP1 in GC cells. In addition, we explored the underlying regulatory mechanism mediated by OGFRP1 in GC cells. The study may provide novel insight into lncRNA investigation in GC development.

Materials and methods

Tissue samples

GC tissues (n = 30) and normal tissues (n = 30) were collected from 30 patients with primary and high-grade GC at Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine (Jiangsu, China). Informed consent was provided by the patients. Before the surgery, no patients had received chemotherapy or radiotherapy. All samples were maintained at -80 °C for further study. This study was approved by the Medical Ethics Committee of Affiliated Hospital of Integrated Traditional Chinese and Western Medicine, Nanjing University of Chinese Medicine (2019–035).

Cell culture

Four GC cell lines (AGS, HGC-27, ACP01 and ALF) and normal human gastric mucosal cells (GES-1) were obtained from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Radiation-resistant (RR) GC cell lines (HGC-27-R and AGS-R) were obtained through sublethal dose of irradiation utilizing the Small Animal Radiation Research Platform (PXI X-RAD 225Cx, CT, USA) as previously described (Yang et al. 2019). Cells were cultured in RPMI 1640 medium (Thermo Fisher, Waltham, MA, USA) containing 10% fetal bovine serum (FBS; Thermo Fisher) in a humidified atmosphere of 5% CO_2 at 37 °C.

Establishment and validation of radioresistant GC cell lines

The irradiation treatment was conducted based on the protocol outlined in a previous study (Yang et al. 2019). To establish radioresistant GC cell lines, HGC-27 cells and AGS cells were grown in 6-well plates (600 cells/well) and irradiated with 2 Gy X-rays at a dose rate of 300 cGy/min using a medical linear accelerator (Elekta Precise, Stockholm, Sweden). The cells were exposed to irradiation for 6 months, and the total exposure dose was 60 Gy.

Then, cells were plated and exposed to indicated radiation doses (0, 2, 4, 6, 8 Gy) for 2 weeks to allow the formation of colonies. Finally, 0.1% crystal violet was utilized to stain the colonies. The number of cell colonies was counted to calculate the surviving fraction.

Cell transfection

After the cells reached 40–50% confluence, they were transfected with short hairpin RNAs (sh-RNAs) against OGFRP1 (sh-OGFRP1#1/2), miR-149-5p mimics, pcDNA3.1 OGFRP1 plasmids, pcDNA3.1/MAP3K3 plasmids, or their corresponding nonspecific controls (sh-NC, NC mimics and pcDNA3.1 plasmids). The plasmids were purchased from GenePharma (Shanghai, China). Transfection was conducted utilizing Lipofectamine™ 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The silencing or overexpression efficiency was determined utilizing RT-qPCR after 48 h of transfection. The plasmid showing the most significant knockdown efficiency was used to conduct functional investigations.

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

TRIzol reagent (Invitrogen) was applied to extract RNA from GC tissues and cells according to the manufacturer's instructions. The cDNAs were reverse transcribed from total RNA utilizing a reverse transcription kit (Invitrogen) on an ABI Veriti96 gradient PCR instrument (Thermo Fisher) following the manufacturer's recommendations. Next, qPCR was employed to evaluate OGFRP1 expression and its downstream genes utilizing SYBR Premix Ex Taq II (TaKaRa, Tokyo, Japan) on an ABI 7500 system following the manufacturer's instructions. The primer sequences utilized in the study are listed in Supplementary table 1. qPCR included an initial denaturation step at 95 °C for 30 s, 40 cycles of 95 °C for 5 s, and 60 °C for 34 s. The expression fold changes were calculated utilizing the $2^{-\Delta\Delta Ct}$ method. GAPDH was an internal control for OGFRP1 and mRNAs, while U6 was a control for miRNAs.

Fluorescence in situ hybridization (FISH)

OGFRP1, miR-149-5p and MAP3K3 probes were synthesized by RiboBio (Guangzhou, China). A FISH kit (RiboBio) was used to detect probe signals following the manufacturer's recommendations. In brief, the probe was labeled with FITC. Tissue samples were fixed with 4% paraformaldehyde solution, rinsed in phosphate buffered saline (PBS), and permeabilized in PBS with 0.5% Triton X-100. After that, samples were washed with 0.1% Tween-20 for 1 min, followed by incubation with premade hybridization buffer at 37 °C overnight. Finally, cells were stained with DAPI stain solution and images were captured using immunofluorescence microscope (Olympus, Tokyo, Japan).

Western blot analysis

Western blot analysis was employed to evaluate gene expression at the protein level. Total protein was extracted from HGC-27 and AGS cells using an RIPA lysis buffer (Beyotime, Haimen, Nantong, China) 48 h after transfection, and a bicinchoninic acid kit (Beyotime) was applied to quantify the protein concentration. Next, the proteins were separated with 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA, USA). The membrane was blocked with 5% skim milk and then was incubated with primary antibodies specifically against MAP3K3 (ab154362; 1:1000), cleaved caspase-3 (ab32042; 1:500), Bax (ab53154; 1:500), Bcl-2 (ab182858; 1:2000), γ-H2AX (ab81299; 1: 5000), β-actin (ab8227; 1:1000) and GAPDH (ab8245; 1:2000) (all from Abcam, Cambridge, UK) at 4 °C overnight. GAPDH or β-actin was utilized as a control. Afterwards, the membrane was then washed with Tris-buffered saline and further incubated with secondary antibodies of goat anti-Rabbit IgG H&L (ab216773; 1:10,000) and goat anti-Mouse IgG H&L (ab216776; 1:10,000) at room temperature for 1 h. Finally, the protein bands were visualized by enhanced chemiluminescence detection kits (BB-3501) and a Bio-Rad image analysis system (Bio-Rad, Hercules, CA, USA).

Subcellular fractionation assay

A DNA/RNA Isolation Kit (Tiangen Biotech, Beijing, China) was utilized to extract RNA from HGC-27 and AGS cells. NE-PER Nuclear and Cytoplasmic Extraction Reagent (Thermo Fisher) was applied to separate the nuclear and cytoplasmic fractions. Total RNA was extracted from the nuclear and cytoplasmic fractions with TRIzol (Invitrogen). The expression of OGFRP1, GAPDH and U6 in the nuclear and cytoplasmic fractions is presented as percentages. U6 was used as a nuclear control and GAPDH was a cytoplasmic control.

Colony formation assay

The cells were plated in 6-well plates (500 cells/well) with or without 2 Gy radiation. After two weeks of incubation, 6% paraformaldehyde (Sigma-Aldrich) was applied to fix the cells. Next, the fixed cells were stained with 0.1% crystal violet solution. Finally, the cell colonies were counted in five visual views selected at random. Three replicates were performed per experiment.

5-Ethynyl-2′-deoxyuridine (EdU) assay

An EdU labeling/detecting kit (RiboBio, Guangzhou, Guangdong, China) was applied to evaluate GC cell proliferation. The cells were seeded into 96-well plates (5×10^3 cells/well) and incubated for 24 h with 0.1% DMSO or 5, 10 or 20 μM anlotinib (a tyrosine kinase inhibitor). Next, 50 μM EdU labeling solution was added for another 2 h of incubation at 37 °C under 5% CO_2. Afterwards, the cells were treated with twice 5-min PBS washing. The cells were then fixed with 4% paraformaldehyde and washed with PBS and 2 mg/ml glycine. Next, the cells were permeabilized with 0.5% Triton X-100 for 10 min and then treated with PBS washing. Subsequently, the cells were stained with anti-EdU solution, and DAPI was employed to label the nuclei of the cells. After staining, the cells were preserved at room temperature and protected from light. The number of EdU-positive cells was determined under a fluorescence microscope (Leica, Wetzlar, Germany). Five visual fields were identified at random to observe cell apoptosis in each treatment group.

Flow cytometry analysis

After transfection, the cells were treated with 2 Gy radiation as above described. The apoptosis of HGC-27-R and AGS-R cells was detected utilizing an Annexin V-fluorescein isothiocyanate/propidium iodide (PI) kit (BD Biosciences, Franklin Lakes, NJ, USA). The status of the cells revealed that they were divided into groups of viable, dead, early apoptotic, and late apoptotic cells. PI was employed to fix and stain the cells utilizing a Cycletest™ Plus DNA Reagent kit (BD Biosciences) based on the manufacturer's recommendations. FACS analysis was employed to examine the cell apoptosis rate.

RNA pulldown assay

An assay was conducted to explore the interaction between OGFRP1 and candidate miRNAs in HGC-27 and AGS cells according to the method described before (Liu et al. 2019). Biotinylated OGFRP1 (Bio-OGFRP1) and the negative control (Bio-NC) were obtained from Sangon (Shanghai, China) and transfected into HGC-27 and AGS cells. After 24 h of transfection, GC cells were lysed in RIPA buffer for 30 min. Next, the lysates were incubated with streptavidin agarose beads (Life Technologies, Gaithersburg, MD, USA) at room temperature for 1 h. RT-qPCR was used to examine the purified RNAs.

Luciferase reporter assay

The binding site between miR-149-5p and OGFRP1 (or MAP3K3) were predicted with the starBase website. The wild-type (Wt) or mutated (Mut) sequence of OGFRP1 or MAP3K3 3' untranslated region (UTR) containing the predicted binding site of miR-149-5p was inserted into pmirGLO vectors (Promega, Madison, WI, USA) to construct OGFRP1-Wt/Mut or MAP3K3-Wt/Mut. A Phusion Site-Directed Mutagenesis Kit (Thermo Fisher) were used to mutate the predicted binding area. MiR-149-5p mimics or NC mimics were cotransfected with above vectors into HGC-27 and AGS cells. Lipofectamine™ 3000 (Invitrogen) was employed for 48 h of cell transfection. The luciferase activities in HGC-27 and AGS cells were examined by the dual-luciferase luciferase reporter assay system (Thermo Fisher) according to the manufacturer's recommendations. Relative firefly luciferase activity was normalized to Renilla luciferase activity.

Statistical analysis

SPSS 20.0 software (Chicago, IL, USA) was applied to analyze statistics. The data are presented as the mean ± standard deviation. Student's *t*-test or one-way analysis of variance followed by Tukey's post hoc test was employed for data comparison. A value of $p < 0.05$ was defined as statistically significant.

Bioinformatics analysis

The miRNAs binding to OGFRP1 as well as target genes of miR-149-5p were predicted utilizing a bioinformatics tool starBase (http://starbase.sysu.edu.cn/) (Li et al. 2014).

Results

OGFRP1 is upregulated in GC tissues and cells

To explore the role of OGFRP1 in GC, its expression in GC tissues was first detected. FISH and RT-qPCR revealed that OGFRP1 expression was higher in GC tissues than in adjacent noncancerous tissues (Fig. 1a). Associations between OGFRP1 expression and clinical characteristics of patients with GC are provided in Table 1. High OGFRP1 expression correlates with higher Tumor, Lymph Node, Metastasis (TNM) stage, greater tumor size and positive lymph node metastasis. Additionally, high expression of OGFRP1 was observed in GC cells (HGC-27, AGS,

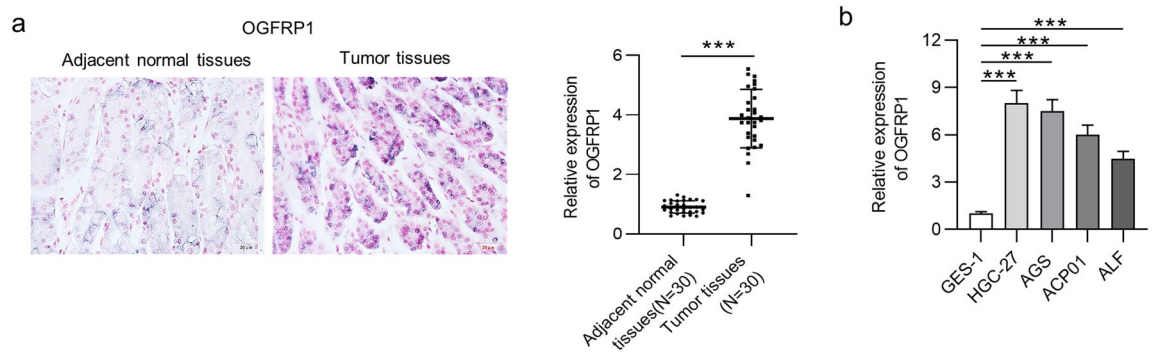


Fig. 1 OGFRP1 is upregulated in GC tissues and cells. **a** OGFRP1 expression in GC and noncancerous tissues was examined by RT-qPCR. **b** OGFRP1 expression in GC cells (HGC-27, AGS, ACP01 and ALF) and normal human gastric mucosal cells (GES-1) was determined by RT-qPCR. ***$p < 0.001$

Table 1 Associations between OGFRP1 expression and clinical characteristics of gastric cancer

Clinicopathological characteristics	Number of patients (N = 30)	High expression (n = 18)	Low expression (n = 12)	*p* value
Age				0.266
≤ 50	11	5	6	
> 50	19	13	6	
Gender				0.176
Male	17	12	5	
Female	13	6	7	
TNM stage				0.045*
I–II	10	3	7	
III–IV	20	15	5	
Tumor size				
≤ 4 cm	12	4	8	0.024*
> 4 cm	18	14	4	
Lymph node metastasis				
Negative	13	4	9	
Positive	17	14	3	0.004*

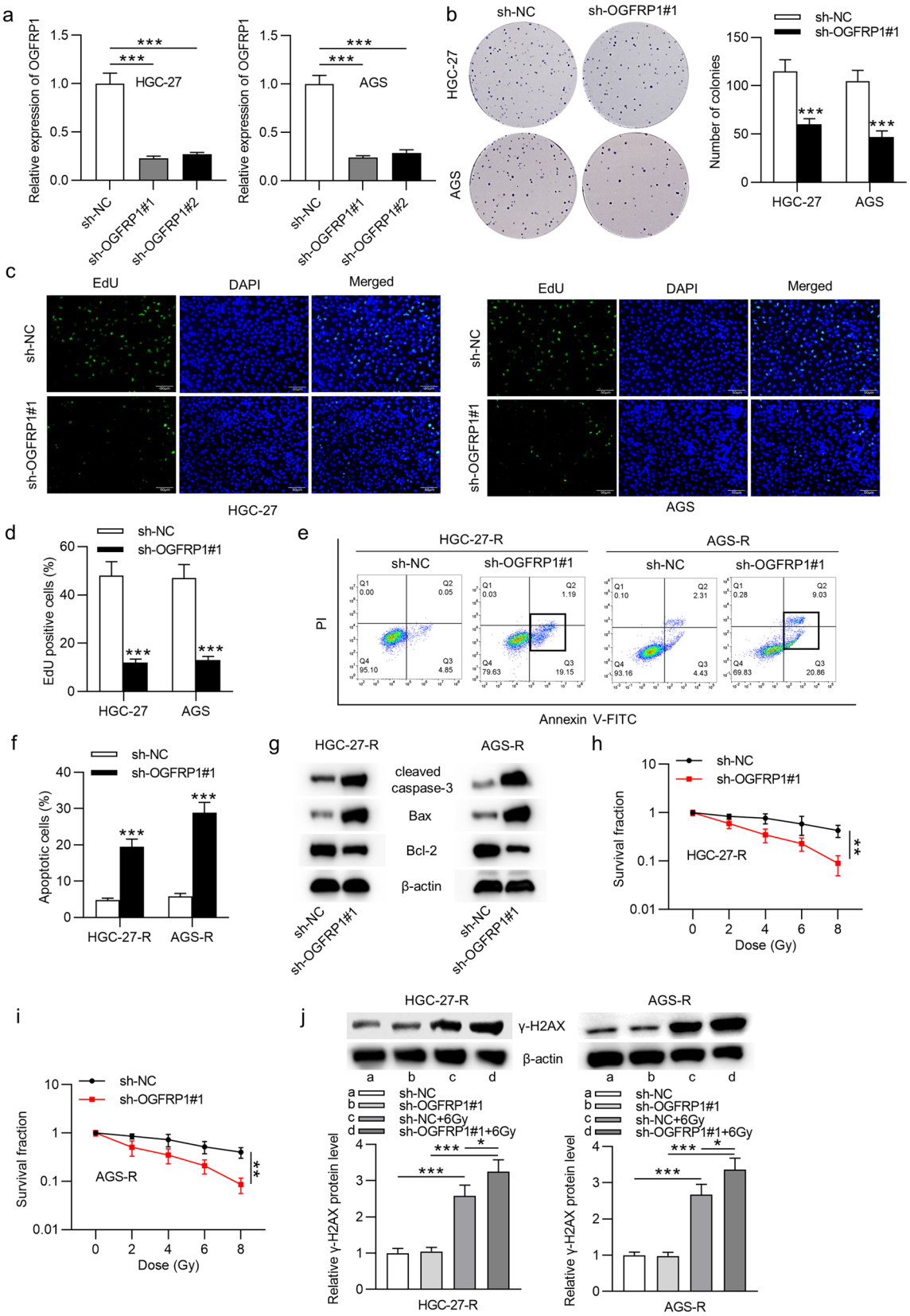
TNM tumor, lymph node, metastasis

*$p < 0.05$

ACP01 and ALF) compared with its expression in normal human gastric mucosal cells (GES-1) (Fig. 1b).

OGFRP1 knockdown inhibits cell proliferation and promotes cell apoptosis and radiosensitivity in GC

Afterwards, we investigated the biological functions of OGFRP1 in GC. First, HGC-27 and AGS cells were chosen for the loss-of-function assays due to their relatively high OGFRP1 expression among the four GC cell lines. RT-qPCR suggested that OGFRP1 expression was decreased by sh-OGFRP1#1/2 compared with OGFRP1 expression in cells treated with the control shRNA (Fig. 2a). Colony formation and EdU assays implied that silencing OGFRP1 expression hampered the proliferation of GC cells (Fig. 2b-2d). Next, flow cytometry analysis suggested that OGFRP1 depletion enhanced the apoptosis of radioresistant GC cells (Fig. 2e, f). Additionally, the expression levels of proteins related to cell apoptosis were detected utilizing western blot analysis. Protein levels of factors facilitating cell apoptosis (cleaved caspase-3 and Bax) were enhanced by OGFRP1 knockdown, while the expression level of the protein inhibiting cell apoptosis (Bcl-2) was reduced by silencing OGFRP1 expression in HGC-27-R and AGS-R cells (Fig. 2g). Moreover, OGFRP1-silenced HGC-27-R and AGS-R cells received different doses of radiation, and clonogenic cell survival was evaluated. The results demonstrated that the survival fraction was lower in OGFRP1-silenced cells than in the control group, implying an increase in radiosensitivity after


a
Relative expression of OGFRP1
HGC-27
AGS
sh-NC
sh-OGFRP1#1
sh-OGFRP1#2
b
sh-NC
sh-OGFRP1#1
HGC-27
AGS
Number of colonies
c
EdU
DAPI
Merged
sh-NC
sh-OGFRP1#1
HGC-27
AGS
d
EdU positive cells (%)
e
HGC-27-R
AGS-R
PI
Annexin V-FITC
f
Apoptotic cells (%)
g
cleaved caspase-3
Bax
Bcl-2
β-actin
h
Survival fraction
Dose (Gy)
i
j
γ-H2AX
β-actin
sh-NC+6Gy
sh-OGFRP1#1+6Gy
Relative γ-H2AX protein level

◂**Fig. 2** OGFRP1 knockdown inhibits cell proliferation and promotes radiosensitivity in GC. **a** Sh-OGFRP1#1 or sh-OGFRP1#2 was transfected into HGC-27 and AGS cells, and RT-qPCR analysis was performed to evaluate the transfection efficiency. **b** The colony formation assay was used to assess the proliferation of HGC-27 and AGS cells. **c, d** An EdU assay was employed to examine the proliferation of HGC-27 and AGS cells. **e**, **f** The apoptosis of HGC-27 and AGS cells was detected by flow cytometry analysis. **g** The levels of proteins related to cell apoptosis were quantified by western blot analysis. h, **i** Clonogenic survival of HGC-27 and AGS cells transfected with sh-OGFRP1#1 or sh-NC after 2 weeks of exposure to indicated doses of radiation. **j** The protein level of γ-H2AX in HGC-27 and AGS cells after receiving different treatments. $*p<0.05$, $**p<0.01$, $***p<0.001$

knockdown of OGFRP1 expression (Fig. 2h, i). To further explore the hypothesis that OGFRP1 influenced the radiosensitivity of GC cells, we assessed the effect of OGFRP1 suppression on irradiation-induced DNA damage. Radiation can induce DNA damage by producing unrepaired DNA double-strand breaks, leading to the formation of γ-H2AX (Santivasi and Xia 2014). In other words, the formation of γ-H2AX reflects the inhibition of DNA damage repair. Therefore, the effect of OGFRP1 depletion on γ-H2AX levels was examined in this study. The results showed that irradiation treatment (6 Gy) led to an increase in γ-H2AX protein levels, and γ-H2AX level was significantly upregulated by OGFRP1 depletion together with irradiation treatment compared with that in sh-NC + 6 Gy group (Fig. 2j). The results indicated that OGFRP1 depletion enhanced the radiosensitivity of GC cells by augmenting DNA damage. In summary, OGFRP1 knockdown promotes GC cell proliferation and inhibits GC cell apoptosis and radiosensitivity.

OGFRP1 overexpression promotes cell proliferation while inhibiting cell apoptosis and radiosensitivity in GC

To explore the effect of OGFRP1 overexpression on GC cellular processes, the efficiency of OGFRP1 overexpression was first examined by RT-qPCR, showing that OGFRP1 was markedly increased after the transfection of the pcDNA3.1/OGFRP1 plasmid (Fig. 3a). OGFRP1 overexpression significantly increased the number of colonies and EdU-positive cells in colony formation and EdU assays, respectively (Fig. 3b–d). Flow cytometry analysis indicated that GC cell apoptosis after irradiation was suppressed by OGFRP1 overexpression (Fig. 3e). Cleaved caspase-3 and Bax expression levels were downregulated while Bcl-2 expression was upregulated by OGFRP1 overexpression in HGC-27-R and AGS-R cells, as shown in the western blot analysis (Fig. 3f). In addition, the survival fraction in the pcDNA3.1/OGFRP1 group was increased in a dose-dependent manner compared with that in the control group, suggesting a decrease in radiosensitivity induced by OGFRP1 overexpression (Fig. 3g). Western blot analysis showed that OGFRP1 overexpression enhanced the reduction of γ-H2AX level in radioresistant GC cells induced by irradiation treatment at 6 Gy dose. The findings suggested that OGFRP1 overexpression suppressed the radiosensitivity of GC cells (Fig. 3h).

OGFRP1 interacts with miR-149-5p

Subsequently, we explored the molecular regulatory mechanism of OGFRP1 in GC. Since lncRNAs have been proven to serve as ceRNAs by binding to miRNAs to regulate the expression of mRNAs in many cancers (Lei et al. 2019), we hypothesized that OGFRP1 regulates GC development by serving as a ceRNA. To confirm our hypothesis, we first conducted a subcellular fraction assay and found that OGFRP1 mostly existed in the cytoplasm of GC cells (Fig. 4a). Subsequently, we found 6 miRNAs that share binding sites with OGFRP1 using the starBase database (condition: Pan-Cancer: 2 cancer types). The interaction between OGFRP1 and 6 candidate miRNAs in HGC-27 and AGS cells was probed by RNA pulldown assays followed by RT-qPCR analyses. The results illustrated that miR-149-5p was markedly enriched in the OGFRP1 probe group in both HGC-27 and AGS cells (Fig. 4b); thus, miR-149-5p was chosen for the following study. Then, we conducted a luciferase reporter assay to further validate the binding capacity between OGFRP1 and miR-149-5p. The predicted binding site is presented in Fig. 4c. The results suggested that miR-149-5p overexpression weakened the luciferase activity of the OGFRP1-Wt vector, while the luciferase activity of the OGFRP1-Mut vector showed no obvious change, confirming that OGFRP1 could bind with miR-149-5p (Fig. 4c). Next, FISH and RT-qPCR revealed that miR-149-5p expression was decreased in GC tissues and cells (Fig. 4d). We also explored the effects of OGFRP1 expression on miR-149-5p expression in GC cells. MiR-149-5p expression as measured by RT-qPCR was markedly upregulated by the knockdown of OGFRP1 expression and was decreased by OGFRP1 overexpression (Fig. 4e). In conclusion, miR-149-5p is the downstream miRNA of OGFRP1 in GC cells.

MAP3K3 is the downstream target of miR-149-5p

To identify the downstream target of miR-149-5p, we searched the starBase website and found 6 mRNAs that shared binding sites with miR-149-5p under the condition of CLIP Data: strict stringency (> = 5), Pan-Cancer: 10 cancer types, Predicted Program: PITA + RNA22. As shown by Venn diagram, gene prediction program PITA reveals that there are 124 mRNAs binding to miR-149-5p, and 8 downstream mRNAs of miR-149-5p are predicted by another prediction tool RNA 22. The 6 mRNAs predicted by both PITA and RNA 22 databases were identified for our subsequent

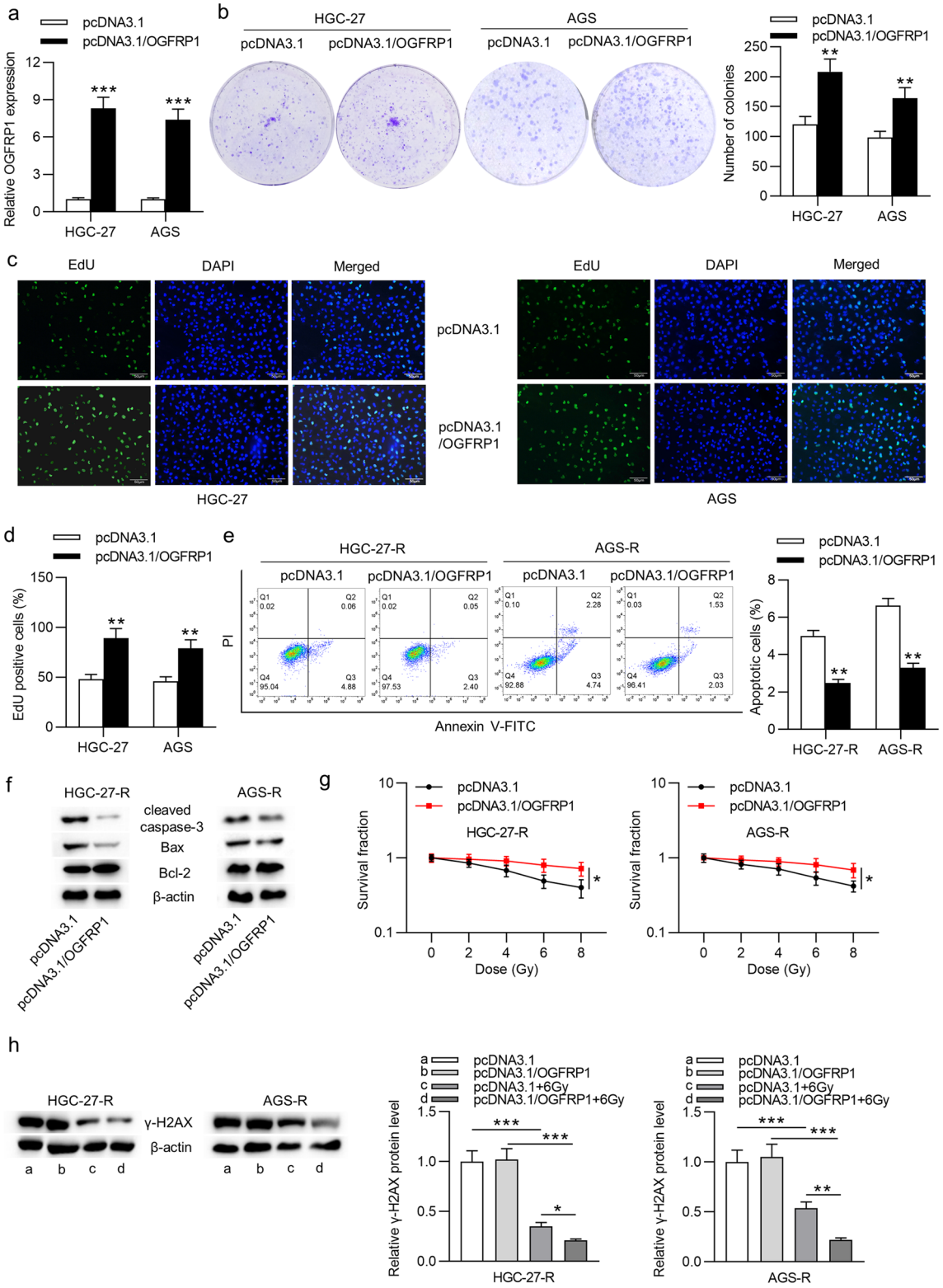


Fig. 3 OGFRP1 overexpression promotes cell proliferation while inhibiting cell apoptosis and radiosensitivity in GC. **a** RT-qPCR was conducted to explore the efficiency of OGFRP1 overexpression in HGC-27 and AGS cells. **b–d** Colony formation and EdU assays were conducted to explore the effects of OGFRP1 overexpression on cell apoptosis. **e** The apoptosis of GC cells transfected with pcDNA3.1 or pcDNA3.1/OGFRP1 was assessed by flow cytometry analysis. **f** western blot analysis was utilized to examine the levels of apoptosis-associated proteins in HGC-27 and AGS cells. **g** Clonogenic survival of HGC-27 and AGS cells transfected with pcDNA3.1/OGFRP1 or pcDNA3.1 after exposure to 0, 2, 4, 6, or 8 doses of radiation. **h** The protein level of γ-H2AX in HGC-27 and AGS cells after different treatments was quantified by western blotting. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

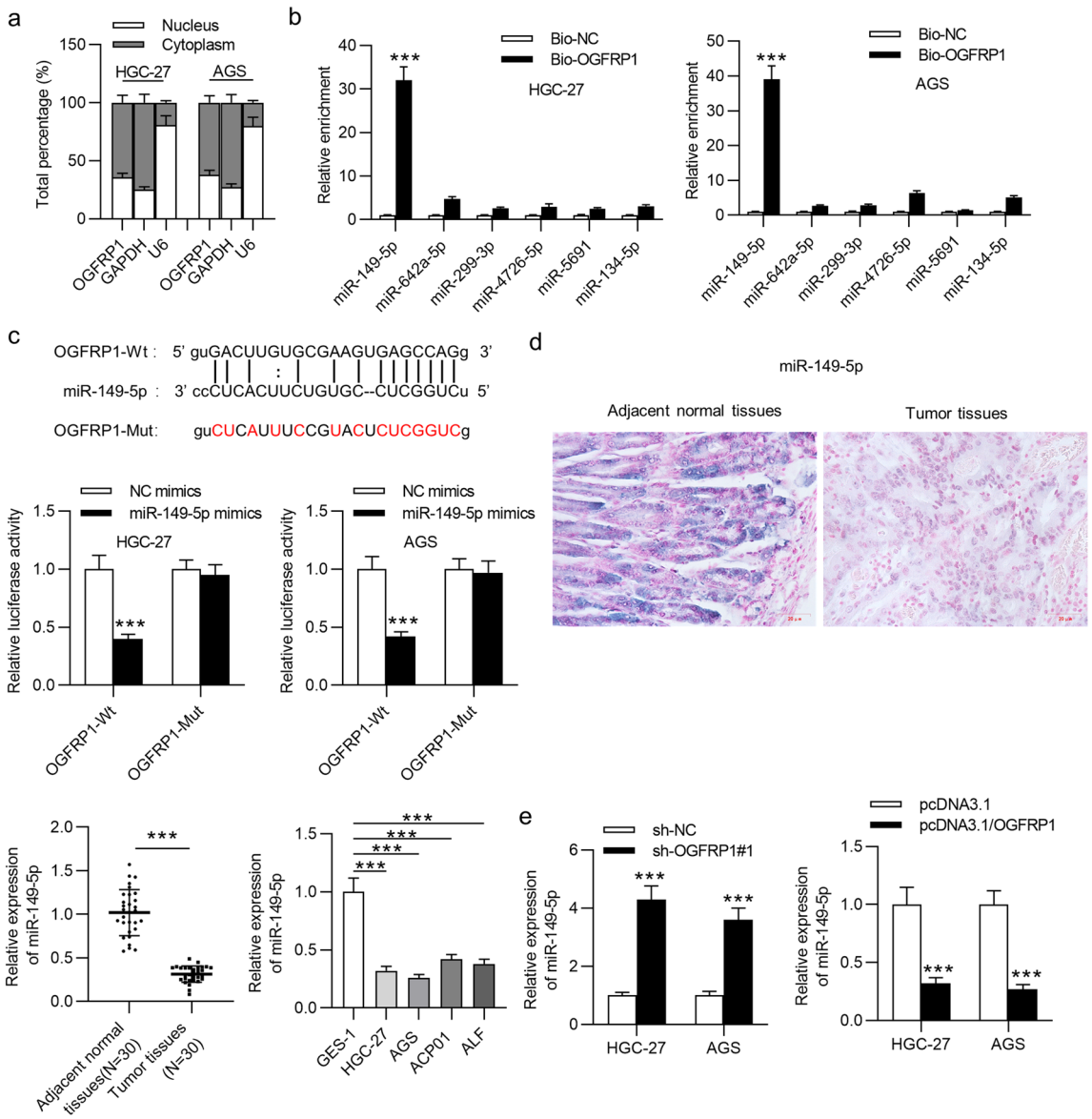
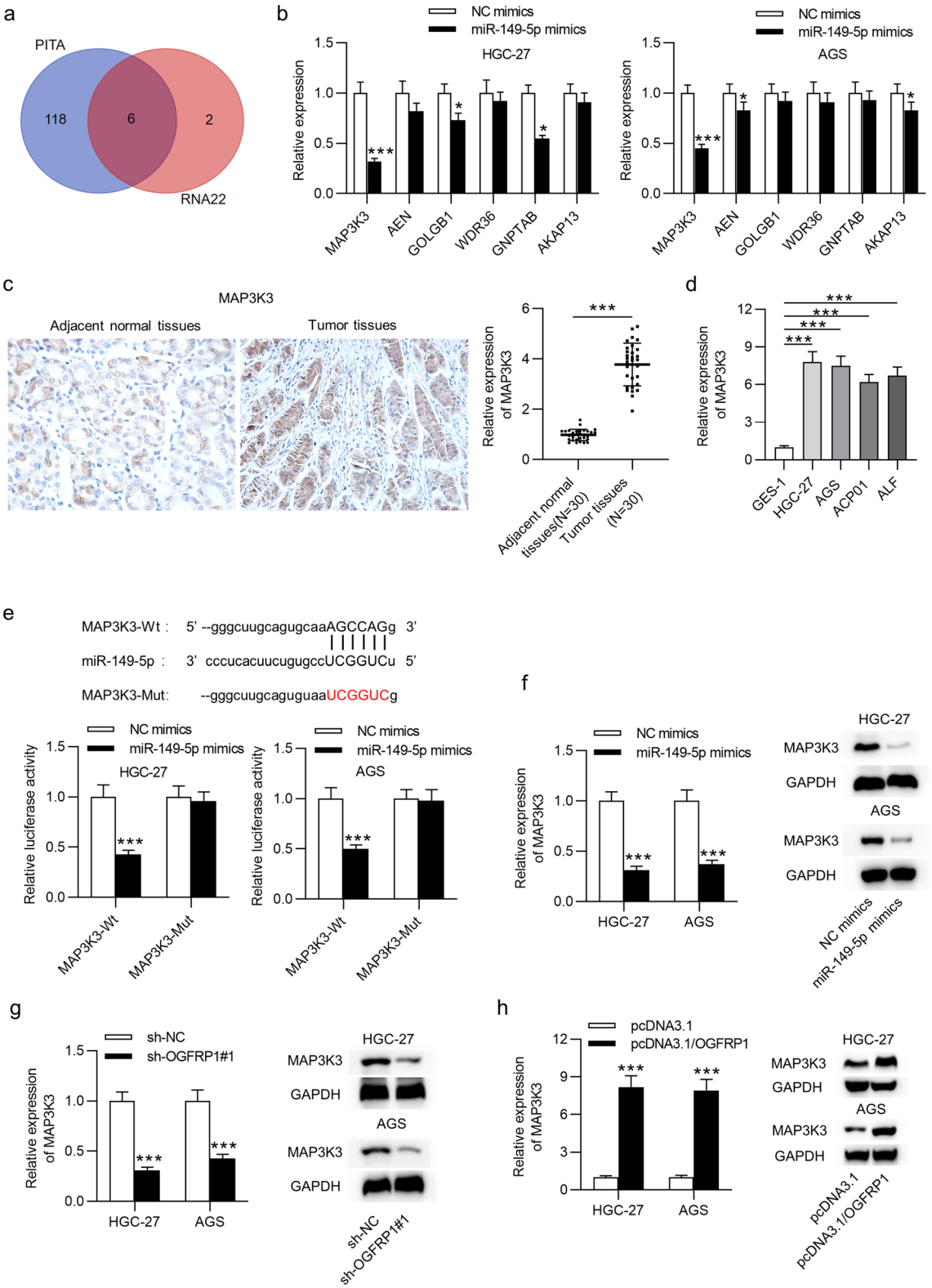


Fig. 4 OGFRP1 combines with miR-149-5p in GC cells. **a** Subcellular fractionation assays were applied to determine the location of OGFRP1 in GC cells. **b** Potential miRNAs that share binding sites with OGFRP1 were predicted from starBase, and an RNA pull-down assay was applied to probe the enrichment of miRNAs. **c** A luciferase reporter assay was utilized to evaluate the binding ability between OGFRP1 and miR-149-5p. **d** The expression of miR-149-5p in GC tissues and cells was detected by RT-qPCR. **e** RT-qPCR was employed to explore the influences of OGFRP1 knockdown or overexpression on miR-149-5p expression in GC cells. ***$p < 0.001$

experiments (Fig. 5a). We discovered by RT-qPCR analysis that MAP3K3 expression was reduced by the upregulation of miR-149-5p expression in both HGC-27 and AGS cells (Fig. 5b). In addition, MAP3K3 expression was increased in GC tissues and GC cells, as illustrated by FISH and RT-qPCR (Fig. 5c, d). A luciferase reporter assay demonstrated that the luciferase activity of the MAP3K3-Wt plasmid was significantly reduced by miR-149-5p overexpression, while miR-149-5p overexpression exerted no distinct effect on the MAP3K3-Mut plasmid, suggesting that MAP3K3 could bind with miR-149-5p (Fig. 5e). Subsequently, RT-qPCR and western blot analyses reflected that miR-149-5p overexpression reduced the mRNA expression and protein level of MAP3K3 in HGC-27 and AGS cells (Fig. 5f), indicating that MAP3K3 was targeted by miR-149-5p. Moreover, the mRNA expression and protein level of MAP3K3 were downregulated by the knockdown of OGFRP1 expression and increased by OGFRP1 overexpression in HGC-27 and AGS cells (Fig. 5g, h), which revealed that MAP3K3 expression was positively correlated with OGFRP1 expression in GC cells. In summary, MAP3K3 is a downstream target gene of miR-149-5p in GC cells.


a
PITA
118
6
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RNA22
b
NC mimics
miR-149-5p mimics
HGC-27
AGS
Relative expression
MAP3K3
AEN
GOLGB1
WDR36
GNPTAB
AKAP13
c
MAP3K3
Adjacent normal tissues
Tumor tissues
Relative expression of MAP3K3
Adjacent normal tissues(N=30)
Tumor tissues (N=30)
d
Relative expression of MAP3K3
GES-1
HGC-27
AGS
ACP01
ALF
e
MAP3K3-Wt : 5' --gggcuugcagugcaaAGCCAGg 3'
miR-149-5p : 3' cccucacuucugugccUCGGUCu 5'
MAP3K3-Mut: --gggcuugcaguguaaUCGGUCg
Relative luciferase activity
MAP3K3-Wt
MAP3K3-Mut
f
Relative expression of MAP3K3
HGC-27
AGS
MAP3K3
GAPDH
NC mimics
miR-149-5p mimics
g
sh-NC
sh-OGFRP1#1
h
pcDNA3.1
pcDNA3.1/OGFRP1

◂**Fig. 5** MAP3K3 is the downstream target of miR-149-5p in GC cells. **a** The starBase website was utilized to identify the downstream target gene of miR-149-5p. A Venn diagram displayed 6 mRNAs that may be target genes of miR-149-5p. **b** RT-qPCR was applied to probe the expression levels of candidate mRNAs. **c** MAP3K3 expression in GC tissues was markedly higher than its expression in normal tissues, as shown by RT-qPCR. **d** MAP3K3 expression in GC cells and normal human gastric mucosal cells was detected utilizing RT-qPCR. **e** A luciferase reporter assay was conducted to evaluate the binding ability between MAP3K3 and miR-149-5p. **f** The effect of miR-149-5p overexpression on the mRNA expression and protein level of MAP3K3 was probed by RT-qPCR and western blot analyses. **g, h** The effect of OGFRP1 knockdown or overexpression on the mRNA expression and protein level of MAP3K3 was detected by RT-qPCR and western blot analyses. $*p < 0.05$, $***p < 0.001$

MAP3K3 overexpression counteracts the effects of OGFRP1 silencing on GC cell proliferation, apoptosis and radiosensitivity

Rescue assays were performed to further investigate the molecular regulatory mechanism of OGFRP1 in GC. HGC-27 cells were transfected with the pcDNA3.1/MAP3K3 plasmid (Fig. 6a). Colony formation and EdU assays suggested that MAP3K3 overexpression rescued the suppressive effect of silencing OGFRP1 expression on the proliferation of GC cells (Fig. 6b, c). Additionally, the upregulation of MAP3K3 expression offset the promoting effect of OGFRP1 depletion on the apoptosis of radioresistant GC cells (Fig. 6d, e). Moreover, the decreased survival fraction induced by silencing OGFRP1 expression was abolished by MAP3K3 overexpression in HGC-27-R cells (Fig. 6f). Furthermore, we observed that the improved γ-H2AX protein level arising from irradiation treatment was offset by increased MAP3K3 expression in HGC-27-R cells (Fig. 6g). In summary, the above findings indicated that OGFRP1 promotes cell proliferation and suppresses radiosensitivity of GC cells by upregulating MAP3K3.

Discussion

GC is the most common malignancy of the human digestive system and a substantial threat to human health (Siegel et al. 2017). Previous studies have reported that lncRNAs are actively implicated in regulating the development of various cancers (Chi et al. 2019; Du et al. 2020; Kim et al. 2018; Li et al. 2018a; Liu et al. 2018; Sun et al. 2019; Tang et al. 2019b). LncRNA OGFRP1 has been proven to serve as an oncogenic gene in several cancer types. For example, OGFRP1 acts as a ceRNA to facilitate the growth of prostate cancer cells by mediating SARM1 levels (Yan et al. 2020). OGFRP1 is closely associated with the prognosis of patients with lung adenocarcinoma (Li et al. 2018b). Herein, we discovered that OGFRP1 expression was upregulated in GC tissues and cells. Moreover, knockdown of OGFRP1 expression distinctly hampered GC cell proliferation and facilitated GC cell apoptosis. It is well known that radiation can induce DNA damage by producing unrepaired DNA double-strand breaks, contributing to the formation of Gamma-H2A histone family member X (γ-H2AX) (Santivasi and Xia 2014). γ-H2AX is regarded as a biomarker for irradiation-induced DNA double-strand breaks (Kawashima et al. 2020; Nagelkerke and Span 2016). Western blot analysis illustrated that OGFRP1 depletion together with 6 Gy radiation increased γ-H2AX expression in GC cells, implying that the knockdown of OGFRP1 expression enhanced irradiation-induced DNA damage. Since irradiation mainly affects cellular activities by DNA damage, we concluded that the knockdown of OGFRP1 expression enhanced the radiosensitivity of GC cells.

Since the ceRNA involvement has been discovered frequently in GC (Qi et al. 2020; Zheng et al. 2020), and OGFRP1 was reported to have an oncogenic role in prostate and lung cancer (Tang et al. 2018; Yan et al. 2020), we hypothesized that OGFRP1 may influence GC cell proliferation, apoptosis and radiosensitivity by serving as a ceRNA. Our subcellular fractionation assay suggested that OGFRP1 was primarily distributed in the cytoplasm of GC cells. It has been reported that lncRNAs in the cytoplasm often act as ceRNAs in cancers (Chen et al. 2019; Christensen et al. 2016; Teng et al. 2019; Wang et al. 2020a; Wei et al. 2020; Xue et al. 2018); therefore, it is possible that OGFRP1 acts as a ceRNA in GC.

LncRNAs function as ceRNAs to combine with miRNAs and thus antagonize the suppressive effect of miRNAs on mRNAs (Li et al. 2016; Lou et al. 2020). Herein, we identified a combination between OGFRP1 and miR-149-5p in GC. It has been previously reported that miR-149-5p suppresses tumor progression in different cancers. For example, miR-149-5p is negatively regulated by lncRNA OGFRP1, which aggravates tumorigenesis and metastasis in hepatocellular carcinoma (Dong et al. 2018). MiR-149-5p overexpression abolished the oncogenic effect of circNRIP1 (Zhang et al. 2019a). MiR-149-5p is targeted by lncRNA PCAT-1 and thus facilitates the proliferation and inhibiting the apoptosis of colorectal cancer cells (Wang et al. 2019). Herein, we validated the binding of OGFRP1 with miR-149-5p, and miR-149-5p expression was negatively associated with OGFRP1 expression in GC cells. Additionally, miR-149-5p expression was proven to be downregulated in GC tissues and cells.

Subsequently, downstream targets of the OGFRP1/miR-149-5p axis were explored. Previously, interleukin 6 (IL-6) was reported to be the target of OGFRP1/miR-149-5p in the ceRNA pattern in prostate cancer, and the OGFRP1/miR-149-5p/IL-6 contributes to therapeutic approaches aimed at prostate cancer chemosensitization and treatment

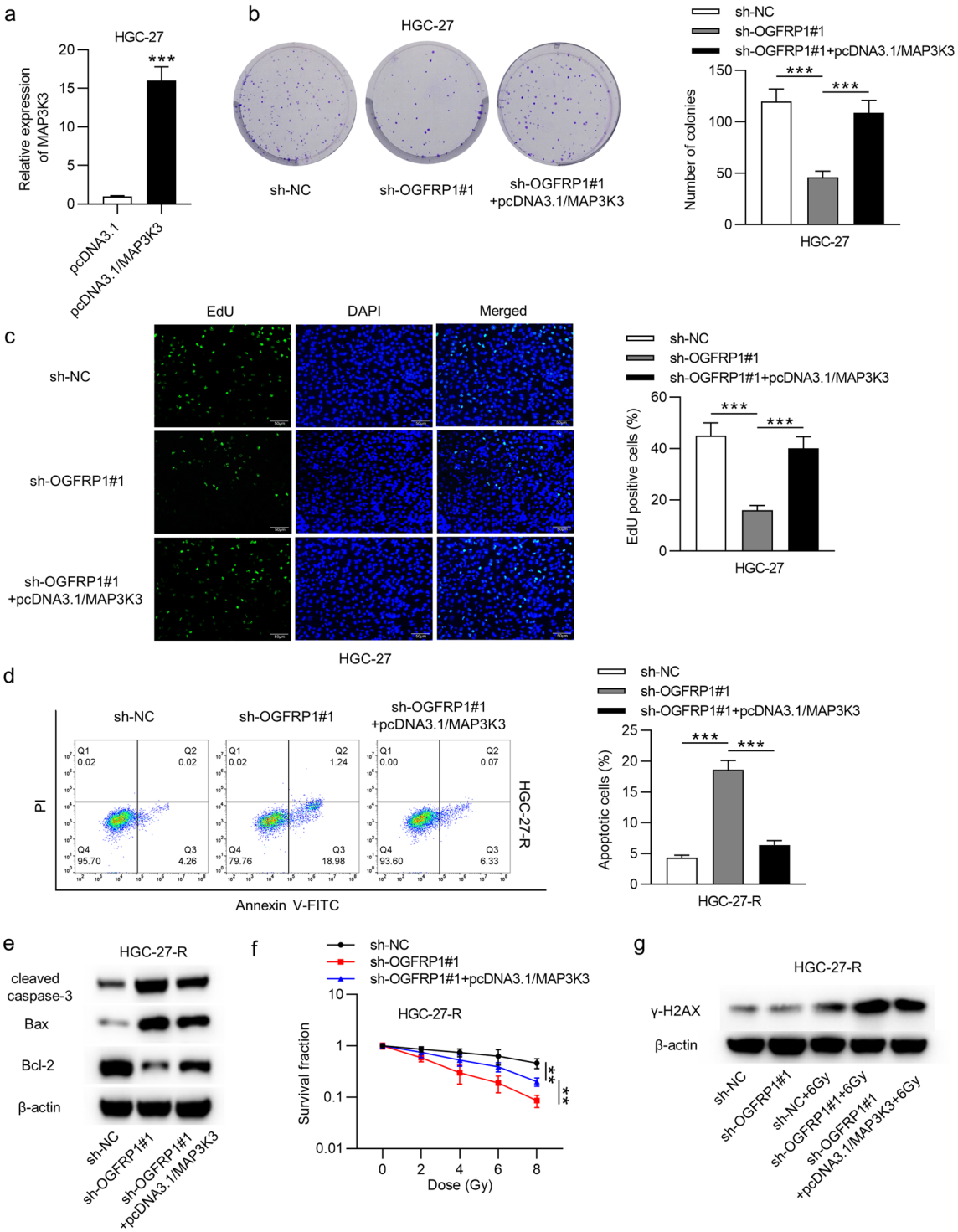

Fig. 6 MAP3K3 overexpression counteracts the effects of OGFRP1 silencing on GC cell proliferation, apoptosis and radiosensitivity. **a** The plasmid pcDNA3.1 or pcDNA3.1/MAP3K3 was transfected into HGC-27 cells, and RT-qPCR analysis was performed to test the efficiency of MAP3K3 overexpression. **b** A colony formation assay was utilized to determine the proliferation of HGC-27 cells transfected with sh-NC, sh-OGFRP1#1, or sh-OGFRP1#1 + pcDNA3.1/MAP3K3. **c** The EdU assay was applied to examine the proliferation of HGC-27 cells transfected with sh-NC, sh-OGFRP1#1, or sh-OGFRP1#1 + pcDNA3.1/MAP3K3. **d** The apoptosis of HGC-27 cells transfected with sh-NC, sh-OGFRP1#1, or sh-OGFRP1#1 + pcDNA3.1/MAP3K3 was probed by flow cytometry analysis. **e** The levels of proteins related to cell apoptosis were assessed by western blot. **f** Clonogenic survival of HGC-27 cells transfected with the indicated plasmids after exposure to different doses of radiation was measured. **g** The protein level of γ-H2AX in HGC-27 cells with different transfections was measured by western blot analyses. $^{**}p < 0.01$, $^{***}p < 0.001$

(Wang et al. 2021). Amounting evidence verified that target effect may be various from tissues to tissues in different cancers (Deepak et al. 2020; Lee et al. 2018; Zhou et al. 2020). Herein, we demonstrated that miR-149-5p targeted mitogen-activated protein kinase kinase kinase 3 (MAP3K3) in GC cells. We suspected that the target effects might be tissue specific in cancers, which still requires more investigation in future studies. MAP3K3 belongs to the serine/threonine protein kinase family (Zhang et al. 2019b). MAP3K3 is frequently reported to promote the development of certain cancers. For example, MAP3K3 overexpression promotes cell proliferation and facilitates tumor growth in ovarian carcinoma (Zhang et al. 2019b). MAP3K3 expression is upregulated by CDKN2B-AS1 expression via miR-4458 in osteosarcoma (Gui and Cao 2020). MAP3K3 is targeted by and negatively regulated by miR-188, and knockdown of MAP3K3 expression suppresses tumorigenesis in non-small cell lung cancer (Zhao et al. 2018). Herein, MAP3K3 expression was elevated in GC tissues and cells, which was positively associated with OGFRP1 expression and negatively correlated with miR-149-5p expression in GC cells. Mechanistically, MAP3K3 is a downstream target of miR-149-5p, and OGFRP1 upregulated MAP3K3 expression by combining with miR-149-5p. Furthermore, through rescue experiments, we validated that enhanced MAP3K3 could reverse the suppressive effect on GC cell proliferation and the enhancement of GC cell apoptosis and radiosensitivity induced by OGFRP1 depletion.

Many signaling pathways were reported to be implicated in GC development, including PI3K/AKT/mTOR signaling, Wnt/β-catenin signaling, stem cell pathway, EGFR signaling, VEGF signaling, HGF/MET signaling, RHOA signaling, JAK/STAT signaling and hedgehog signaling pathways (Fattahi et al. 2020; Riquelme et al. 2015; Yu et al. 2021). Investigation of signaling pathways is beneficial to improve treatment responses to targeted therapy (Riquelme et al. 2015). In this study, we did not explore the potential downstream signaling pathway of the OGFRP1/miR-149-5p/MAP3K3 axis in GC. We hypothesized that MAP3K3 might affect GC development through a certain signaling pathway according to previous studies. MAP3K3 can regulate cell proliferation, migration, immune response, and cell cycle by regulating MAPK pathway and activated MAPKs, such as ERK1/ERK2, c-Jun N-terminal kinase, p38 and ERK5 (Tang et al. 2019a). MAP3K3 was reported to promote tumor growth by activating the NF-κB signaling way in ovarian cancer (Zhang et al. 2019b). MAP3K3 activates the AKT/NF-κB signaling in lung cancer, contributing to cell growth and epithelial-mesenchymal transition (Tang et al. 2019a). Hence, we hypothesized that OGFRP1 might promote cell proliferation and inhibit cell apoptosis and radiosensitivity by upregulating MAP3K3 through a certain signaling pathway which will be further explored in our future studies.

In conclusion, OGFRP1 promoted GC cell proliferation and inhibited GC cell apoptosis and radiosensitivity by targeting the miR-149-5p/MAP3K3 axis. Our study may provide novel insight for the molecular investigation of GC.

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Declarations

Conflict of interest None.

References

Baskar R, Lee KA, Yeo R, Yeoh KW (2012) Cancer and radiation therapy: current advances and future directions. Int J Med Sci 9:193–199

Brown PD, Ahluwalia MS, Khan OH, Asher AL, Wefel JS, Gondi V (2018) Whole-brain radiotherapy for brain metastases: evolution or revolution? J Clin Oncol 36:483–491

Cats A et al (2018) Chemotherapy versus chemoradiotherapy after surgery and preoperative chemotherapy for resectable gastric cancer (CRITICS): an international, open-label, randomised phase 3 trial. Lancet Oncol 19:616–628

Chen W, Zheng R, Zeng H, Zhang S (2015) The updated incidences and mortalities of major cancers in China, 2011. Chin J Cancer 34:502–507

Chen W, You J, Zheng Q, Zhu YY (2018) Downregulation of lncRNA OGFRP1 inhibits hepatocellular carcinoma progression by AKT/mTOR and Wnt/β-catenin signaling pathways. Cancer Manag Res 10:1817–1826

Chen S et al (2019) Silencing of long noncoding RNA LINC00958 prevents tumor initiation of pancreatic cancer by acting as a sponge of microRNA-330-5p to down-regulate PAX8. Cancer Lett 446:49–61

Chi J et al (2019) Long non-coding RNA LUCAT1 promotes proliferation and invasion in gastric cancer by regulating miR-134–5p/YWHAZ axis. Biomed Pharmacother 118:109201

Christensen LL et al (2016) SNHG16 is regulated by the Wnt pathway in colorectal cancer and affects genes involved in lipid metabolism. Mol Oncol 10:1266–1282

Correa P (2013) Gastric cancer: overview. Gastroenterol Clin N Am 42:211–217

Deepak KG, Vempati R, Nagaraju GP, Dasari VR, Nagini S, Rao DN, Malla RR (2020) Tumor microenvironment: challenges and opportunities in targeting metastasis of triple negative breast cancer. Pharmacol Res 153:104683

Dong J, Teng F, Guo W, Yang J, Ding G, Fu Z (2018) lncRNA SNHG8 Promotes the Tumorigenesis and Metastasis by Sponging miR-149-5p and Predicts Tumor Recurrence in Hepatocellular Carcinoma. Cell Physiol Biochem 51:2262–2274

Du J, Liang Y, Li J, Zhao JM, Lin XY (2020) LINC00858 knockdown inhibits gastric cancer cell growth and induces apoptosis through reducing WNK2 promoter methylation. Cell Oncol (dordr) 43:709–723

Eusebi LH, Telese A, Marasco G, Bazzoli F, Zagari RM (2020) Gastric cancer prevention strategies: A global perspective. J Gastroenterol Hepatol 35:1495–1502

Fattahi S, Amjadi-Moheb F, Tabaripour R, Ashrafi GH, Akhavan-Niaki H (2020) PI3K/AKT/mTOR signaling in gastric cancer: Epigenetics and beyond. Life Sci 262:513

Gui D, Cao H (2020) Long non-coding RNA CDKN2B-AS1 promotes osteosarcoma by increasing the expression of MAP3K3 via sponging miR-4458. In Vitro Cell Dev Biol Anim 56:24–33

Kamangar F, Dores GM, Anderson WF (2006) Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. J Clin Oncol 24:2137–2150

Kawashima S et al (2020) γ-H2AX as a potential indicator of radiosensitivity in colorectal cancer cells. Oncol Lett 20:2331–2337

Kim J et al (2018) Long noncoding RNA MALAT1 suppresses breast cancer metastasis. Nat Genet 50:1705–1715

Lee YT, Tan YJ, Oon CE (2018) Molecular targeted therapy: treating cancer with specificity. Eur J Pharmacol 834:188–196

Lei F, Zhang H, Xie X (2019) Comprehensive analysis of an lncRNA-miRNA-mRNA competing endogenous RNA network in pulpitis. PeerJ 7:e7135

Li JH, Liu S, Zhou H, Qu LH, Yang JH (2014) starBase v2.0: decoding miRNA-ceRNA, miRNA-ncRNA and protein-RNA interaction networks from large-scale CLIP-Seq data. Nucleic Acids Res 42:D92-97

Li T, Mo X, Fu L, Xiao B, Guo J (2016) Molecular mechanisms of long noncoding RNAs on gastric cancer. Oncotarget 7:8601–8612

Li Y et al (2018a) Long non-coding RNA SNHG5 promotes human hepatocellular carcinoma progression by regulating miR-26a-5p/GSK3β signal pathway. Cell Death Dis 9:888

Li YY, Yang C, Zhou P, Zhang S, Yao Y, Li D (2018b) Genome-scale analysis to identify prognostic markers and predict the survival of lung adenocarcinoma. J Cell Biochem 119:8909–8921

Liu M, Jia J, Wang X, Liu Y, Wang C, Fan R (2018) Long non-coding RNA HOTAIR promotes cervical cancer progression through regulating BCL2 via targeting miR-143-3p. Cancer Biol Ther 19:391–399

Liu C, Zhang H, Liu H (2019) Long noncoding RNA UCA1 accelerates nasopharyngeal carcinoma cell progression by modulating miR-124-3p/ITGB1 Axis. Onco Targets Ther 12:8455–8466

Lordick F, Nilsson M, Leong T (2021) Adjuvant radiotherapy for gastric cancer-end of the road? Ann Oncol 32:287–289

Lou W, Ding B, Fu P (2020) Pseudogene-Derived lncRNAs and Their miRNA Sponging Mechanism in Human Cancer. Front Cell Dev Biol 8:85

Nagelkerke A, Span PN (2016) Staining against phospho-H2AX (γ-H2AX) as a marker for DNA damage and genomic instability in cancer tissues and cells. Adv Exp Med Biol 899:1–10

Qi M, Yu B, Yu H, Li F (2020) Integrated analysis of a ceRNA network reveals potential prognostic lncRNAs in gastric cancer. Cancer Med 9:1798–1817

Qian L et al (2020) MicroRNA-200c nanoparticles sensitized gastric cancer cells to radiotherapy by regulating PD-L1 expression and EMT. Cancer Manag Res 12:12215–12223

Ren Z, Liu X, Si Y, Yang D (2020) Long non-coding RNA DDX11-AS1 facilitates gastric cancer progression by regulating miR-873-5p/SPC18 axis. Artif Cells Nanomed Biotechnol 48:572–583

Riquelme I et al (2015) Molecular classification of gastric cancer: Towards a pathway-driven targeted therapy. Oncotarget 6:24750–24779

Roviello F, Caruso S, Neri A, Marrelli D (2013) Treatment and prevention of peritoneal carcinomatosis from gastric cancer by cytoreductive surgery and hyperthermic intraperitoneal chemotherapy: overview and rationale. Eur J Surg Oncol 39:1309–1316

Santivasi WL, Xia F (2014) Ionizing radiation-induced DNA damage, response, and repair. Antioxid Redox Signal 21:251–259

Sasaki A et al (2020) Enhanced tumor response to radiotherapy after PD-1 blockade in metastatic gastric cancer. Gastric Cancer 23:893–903

Sexton RE, Al Hallak MN, Diab M, Azmi AS (2020) Gastric cancer: a comprehensive review of current and future treatment strategies. Cancer Metastasis Rev 39:1179–1203

Siegel RL, Miller KD, Jemal A (2017) Cancer Statistics, 2017. CA Cancer J Clin 67:7–30

Song Z, Wu Y, Yang J, Yang D, Fang X (2017) Progress in the treatment of advanced gastric cancer. Tumour Biol 39:1010428317714626

Sun Q, Li J, Li F, Li H, Bei S, Zhang X, Feng L (2019) LncRNA LOXL1-AS1 facilitates the tumorigenesis and stemness of gastric carcinoma via regulation of miR-708–5p/USF1 pathway. Cell Prolif 52:e12687

Sun CB et al (2020) LINC00511 promotes gastric cancer cell growth by acting as a ceRNA. World J Gastrointest Oncol 12:394–404

Tang LX, Chen GH, Li H, He P, Zhang Y, Xu XW (2018) Long noncoding RNA OGFRP1 regulates LYPD3 expression by sponging miR-124-3p and promotes non-small cell lung cancer progression. Biochem Biophys Res Commun 505:578–585

Tang H, Lv W, Sun W, Bi Q, Hao Y (2019a) miR-505 inhibits cell growth and EMT by targeting MAP3K3 through the AKT-NFκB pathway in NSCLC cells. Int J Mol Med 43:1203–1216

Tang L, Wen JB, Wen P, Li X, Gong M, Li Q (2019b) Long noncoding RNA LINC01314 represses cell migration, invasion, and angiogenesis in gastric cancer via the Wnt/β-catenin signaling pathway by down-regulating KLK4. Cancer Cell Int 19:94

Teng J, Ai X, Jia Z, Wang K, Guan Y, Guo Y (2019) Long noncoding RNA ARAP1-AS1 promotes the progression of bladder cancer by regulating miR-4735-3p/NOTCH2 axis. Cancer Biol Ther 20:552–561

Valentini V et al (2009) Survival after radiotherapy in gastric cancer: systematic review and meta-analysis. Radiother Oncol 92:176–183

Wang AH, Fan WJ, Fu L, Wang XT (2019) LncRNA PCAT-1 regulated cell proliferation, invasion, migration and apoptosis in colorectal cancer through targeting miR-149-5p. Eur Rev Med Pharmacol Sci 23:8310–8320

Wang B et al (2020a) Long intergenic non-protein coding RNA 324 prevents breast cancer progression by modulating miR-10b-5p. Aging (Albany NY) 12:6680–6699

Wang P, Yang Z, Ye T, Shao F, Li J, Sun N, He J (2020b) lncTUG1/miR-144-3p affect the radiosensitivity of esophageal squamous cell carcinoma by competitively regulating c-MET. J Exp Clin Cancer Res 39:7

Wang C, Ding T, Yang D, Zhang P, Hu X, Qin W, Zheng J (2021) The lncRNA OGFRP1/miR-149-5p/IL-6 axis regulates prostate cancer chemoresistance. Pathol Res Pract 224:153535

Wei C, Zhao L, Liang H, Zhen Y, Han L (2020) Recent advances in unraveling the molecular mechanisms and functions of HOXA11-AS in human cancers and other diseases (review). Oncol Rep 43:1737–1754

Wu D, Li Y, Zhang H, Hu X (2017) Knockdown of Lncrna PVT1 enhances radiosensitivity in non-small cell lung cancer by sponging Mir-195. Cell Physiol Biochem 42:2453–2466

Xu W, Yang Z, Lu N (2016) Molecular targeted therapy for the treatment of gastric cancer. J Exp Clin Cancer Res 35:1

Xue JY, Huang C, Wang W, Li HB, Sun M, Xie M (2018) HOXA11-AS: a novel regulator in human cancer proliferation and metastasis. Onco Targets Ther 11:4387–4393

Yan K et al (2020) lncRNA OGFRP1 functions as a ceRNA to promote the progression of prostate cancer by regulating SARM1 level via miR-124-3p. Aging (albany NY) 12:8880–8892

Yang T et al (2019) TGF-β receptor inhibitor LY2109761 enhances the radiosensitivity of gastric cancer by inactivating the TGF-β/SMAD4 signaling pathway. Aging (albany NY) 11:8892–8910

Yu Z et al (2021) A novel UBE2T inhibitor suppresses Wnt/β-catenin signaling hyperactivation and gastric cancer progression by blocking RACK1 ubiquitination. Oncogene 40:1027–1042

Zhang X et al (2019a) Circular RNA circNRIP1 acts as a microRNA-149-5p sponge to promote gastric cancer progression via the AKT1/mTOR pathway. Mol Cancer 18:20

Zhang Y et al (2019b) Overexpression of MAP3K3 promotes tumour growth through activation of the NF-κB signalling pathway in ovarian carcinoma. Sci Rep 9:8401

Zhang J, Piao HY, Guo S, Wang Y, Zhang T, Zheng ZC, Zhao Y (2020a) LINC00163 inhibits the invasion and metastasis of gastric cancer cells as a ceRNA by sponging miR-183 to regulate the expression of AKAP12. Int J Clin Oncol 25:570–583

Zhang K et al (2020b) A ceRNA network and a potential regulatory axis in gastric cancer with different degrees of immune cell infiltration. Cancer Sci 111:4041–4050

Zhao L et al (2018) MiroRNA-188 acts as tumor suppressor in non-small-cell lung cancer by targeting MAP3K3. Mol Pharm 15:1682–1689

Zheng R et al (2016) Upregulation of long noncoding RNA small nucleolar RNA host gene 18 promotes radioresistance of glioma by repressing semaphorin 5A. Int J Radiat Oncol Biol Phys 96:877–887

Zheng X et al (2020) Construction and analysis of the tumor-specific mRNA-miRNA-lncRNA network in gastric cancer. Front Pharmacol 11:1112

Zhou X, Dong R, Zhang JY, Zheng X, Sun LP (2020) PROTAC: a promising technology for cancer treatment. Eur J Med Chem 203:112539

Zhu YT et al (2020) Chemoradiotherapy is inferior to chemotherapy alone in adjuvant setting for signet ring cell containing gastric cancer. Front Oncol 10:570268

Zou K, Yu H, Chen X, Ma Q, Hou L (2019) Silencing long noncoding RNA OGFRP1 inhibits the proliferation and migration of cervical carcinoma cells. Cell Biochem Funct 37:591–597