### Original Article RP11-874J12.4, a novel IncRNA, confers chemoresistance in human gastric cancer cells by sponging miR-3972 and upregulating SSR2 expression

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Abstract: Increasing evidence has revealed the contributions of long noncoding RNAs (IncRNAs) in the modulation of drug resistance in gastric cancer. In the present study, we explored the role of a novel IncRNA, RP11-874J12.4, in regulating chemoresistance in gastric cancer and determined the underlying molecular mechanisms. We observed that compared with normal controls, human gastric cancer tissues and cell lines, including MKN-45 and AGS cells, expressed higher RP11-874J12.4 levels. RP11-874J12.4 knockdown sensitized MKN-45 and AGS cells to docetaxel and cisplatin in terms of cell viability and apoptosis rate. In addition, RP11-874J12.4 was found to be a competing endogenous RNA that sponged microRNA (miR)-3972, which showed significantly reduced expression in human gastric cancer tissues and cell lines. Furthermore, signal sequence receptor subunit 2 (SSR2) was identified as a downstream target of miR-3972, and the miR-3972/SSR2 axis was found to regulate chemoresistance in MKN-45 and AGS cells. SSR2 downregulation further sensitized gastric cancer cells with RP11-874J12.4 knockdown to chemotherapeutic drugs via enhanced apoptosis, which was evidenced by significantly upregulated expressions of cleaved caspase-3, cleaved caspase-9, and Bax and downregulated expression of Bcl-2. Furthermore, RP11-874J12.4 knockdown markedly inhibited the growth of xenograft MKN-45 cells in nude mice, which was associated with an increased expression of miR-3972 and decreased expression of SSR2 in tumors. Therefore, the RP11-874J12.4/miR-3972/SSR2 axis plays important roles in the regulation of chemoresistance in MKN-45 and AGS cells and may serve as a target for the diagnosis and treatment of human gastric cancer.

Keywords: Long noncoding RNA, gastric cancer, chemoresistance, RP11-874J12.4, miR-3972, SSR2

#### Introduction

Gastric cancer is one of the most common types of malignant cancers as well as one of the leading causes of cancer-related deaths worldwide [1, 2]. In several Asian countries, particularly in East Asian countries such as China, gastric cancer is one of the most prevalent cancers in terms of incidence and mortality. Statistical analyses have shown that only lung cancer is associated with a higher incidence and mortality than gastric cancer in China [3]. In the USA, over 27,000 cases of gastric cancer were diagnosed in 2019, of which 11,140 were expected to result in death [4]. Although advanced surgical treatments can significantly improve the prognosis of patients in the early stages of gastric cancer, this malignancy is frequently diagnosed at an advanced stage, thereby necessitating radiotherapy and chemotherapy [5, 6].

Despite aggressive frontline treatments with surgery and adjuvant chemotherapy, most patients with gastric cancer ultimately progress to an incurable, metastatic, chemotherapy-resistant disease state, which results in a low 5-year survival rate [7]. Chemotherapy for patients with gastric cancer broadly encompasses two types of drugs: cisplatin, an anticancer agent that can destroy the target DNA of tumor cells [8], and docetaxel, a drug that promotes cell apoptosis after  $\beta$ -tubulin interaction and Bcl-2 phosphorylation [9]. The treatment of gastric

cancer is frequently hampered by resistance to docetaxel or cisplatin, causing a remarkable decline in the success rate of chemotherapy and endangering the survival of patients. However, mechanisms underlying this drug resistance have not yet been completely understood [10], although previous studies have proposed multiple mechanisms, including epithelial-mesenchymal transition and presence of cancer stem cells [7, 11, 12].

Recently, an increasing number of studies have revealed that noncoding RNAs (ncRNAs) significantly contribute to the regulation of chemotherapy resistance in patients with cancer [13-17]. Two major types of ncRNAs, long noncoding RNAs (IncRNAs) and microRNAs (miRNAs or miRs), reportedly play essential roles in the mechanism of drug resistance during the treatment of several cancers, including gastric cancer [13, 16-18]. Further, IncRNAs are defined as transcripts measuring >200 nucleotides in length and have been identified to function as key regulators of diverse cellular processes such as development, differentiation, and cell fate as well as disease pathogenesis [19, 20]. Moreover, IncRNAs can serve as signal mediators, molecular decoys and scaffolds, or enhancers of transcription. Intriguingly, a considerable number of IncRNAs reportedly function as competing endogenous RNAs (ceRNAs) that regulate gene expression by sponging miRNAs [21]. miRNAs are a group of short noncoding RNAs that are highly conserved among a wide range of species and are typically involved in post-transcriptional gene regulation. They play important roles in maintaining normal human physiological conditions, and abnormal miRNA expression levels have been found to be associated with several human diseases, ranging from psychiatric disorders to malignant cancers [22-24].

Numerous studies have investigated the contributions of IncRNAs and miRNAs in the modulation of drug resistance in gastric cancer. For instance, the IncRNA HOTAIR has been reported to inhibit miR-34a expression for promoting resistance to cisplatin in gastric cancer via the Wnt/ $\beta$ -catenin and PI3K/Akt signaling pathways [25]. Another study has proposed that HOTAIR functions as a ceRNA of miR-126 and increases vascular endothelial growth factor A expression to activate the PI3K/Akt/MRP1 sig-

naling pathway in gastric cancer [26]. These studies suggest a novel direction of screening potential markers and targets for overcoming chemotherapy resistance in gastric cancer. Nevertheless, because of the large and complex network of IncRNAs, further extensive and comprehensive investigations are required to elucidate the detailed mechanisms underlying anticancer chemoresistance in human gastric cancer.

In a previous study, high-throughput screening of IncRNA expression profiles in gastric adenocarcinoma and gastric squamous cell carcinoma revealed abnormally high expression levels of numerous IncRNAs, among which a novel IncRNA-RP11-874J12.4-was found to be upregulated five times in stomach adenocarcinoma compared with that in normal gastric tissues [27]. In the present study, we evaluated RP11-874J12.4 expression levels in clinically resected samples of patients with gastric cancer and several human gastric cancer cell lines and identified that higher RP11-874J12.4 expression was associated with malignant behaviors. Through small interfering RNA (siRNA)- or short hairpin RNA (shRNA)-mediated knockdown of RP11-874J12.4 expression, we identified the critical roles of RP11-874J12.4 in promoting the proliferation and survival of human gastric cancer cells following chemotherapy under in vitro culture conditions and in a xenograft mouse model. Furthermore, we explored the interaction between RP11-874J12.4 and the miRNA miR-3972 as well as that between miR-3972 and its target signal sequence receptor subunit 2 (SSR2) in human gastric cancer cells. Our findings demonstrated the critical roles played by the RP11-874J12.4/miR-3972/SSR2 axis in conferring chemoresistance to human gastric cells against docetaxel and cisplatin.

#### Materials and methods

#### Cell culture

The human normal gastric epithelial cell line GES-1 [28] was purchased from Beijing Institute of Cancer Research (Beijing, China). The human gastric cancer cell lines MKN-45, AGS, and HGC-27 were purchased from China General Microbiological Culture Collection Center (Beijing, China), American Type Culture Collection (ATCC<sup>®</sup> CRL-1739<sup>™</sup>, ATCC, Manassas, VA, USA), and Type Culture Collection of the Chinese

Academy of Sciences (Shanghai, China), respectively. These cell lines were authenticated using short tandem repeat DNA profiling analysis and verified to be mycoplasma contamination-free by the venders. Cells were maintained in Dulbecco's modified Eagle's medium (Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (Gibco, Gaithersburg, MD, USA), 100 U/mL penicillin, and 100  $\mu$ g/mL streptomycin (Gibco) at 37°C in a humidified chamber containing 5% CO<sub>2</sub>.

Cells were treated with docetaxel or cisplatin at the indicated concentrations. Docetaxel (Sigma-Aldrich, Cleveland, OH, USA) was dissolved in dimethyl sulfoxide (DMSO) to prepare a stock solution of 1 mg/mL. Cisplatin (Sigma-Aldrich) was dissolved in 0.9% NaCl to prepare a stock solution of 1 mg/mL. Both stock solutions were stored in tightly sealed sterile tubes at -20°C and diluted to the indicated concentration in phosphate-buffered saline (PBS) before each experiment.

#### Clinical samples

Between May 2017 and July 2018, 20 pairs of human gastric cancer tissue samples and adjacent noncancerous tissue samples were collected from patients who underwent surgery at Shaanxi Provincial People's Hospital (Xi'an, Shaanxi, China). All samples were confirmed by pathological analysis. Written informed consent was obtained from all participants. This study was approved by the Ethics Committee of Shaanxi Provincial People's Hospital and was performed in accordance with the ethical standards of the Declaration of Helsinki and its later amendments.

#### siRNA, miRNA inhibitor, and mimic

Three siRNA oligonucleotides against RP11-874J12.4 or SSR2 and one negative control oligonucleotide (scramble) were used to transfect MKN-45 cells to evaluate the knockdown efficiency. The sequences of these siRNAs were as follows: si-RP11-874J12.4-1, antisense strand sequence: 5'-GCATGCTTGTAAGAGTTCCAC-GTTT-3'; si-RP11-874J12.4-2, antisense strand sequence: 5'-CGGAGTTTGGGATTATCAAT-CTAAA-3'; si-RP11-874J12.4-3, antisense strand sequence: 5'-CAGAGGTGAGAATTCATCAG-TGTTA-3'; and scramble siRNA, antisense strand sequence: 5'-CCTAAGGTTAAGTCGCCCTC- GCTC-3'. These siRNAs were synthesized by GenePharma Co., Ltd (Shanghai, China). SSR2 siRNA was purchased from Sigma-Aldrich (Catalog number: EHU154141). miR-3972 mimic and inhibitor were purchased from Thermo Fisher Scientific (Catalog number: AM17000).

## Cell transfection and generation of stable cell lines

Transfections of the abovementioned siRNAs and inhibitor were performed using Lipofectamine 3000 (Thermo Fisher Scientific) according to the manufacturer's instructions. To construct MKN-45 cells with stable RP11-874J12.4 knockdown, cells were infected with the lentivirus expressing RP11-874J12.4-specific shRNA. Briefly, the selected shRNA sequence (si-RP11-874J12.4-2; antisense strand sequence: 5'-CGGAGTTTGGGATTATCAATCTAAA-3') and a nonspecific control scramble shRNA (antisense strand sequence: 5'-CCTAAGGTTAAGTCGCCC-TCGCTC-3') were separately cloned into the lentiviral vector pLK0.1 with the U6-vshRNA-CMV frame. Lentiviral particles were produced in HEK 293T cells (ATCC<sup>®</sup> CRL-3216<sup>™</sup>) by transiently co-transfecting the control scramble shRNA lentiviral vector (scramble-shRNA) or the RP11-874J12.4-knockdown lentiviral vector (RP11-874J12.4-shRNA) together with the helper plasmids pHelper 1.0 (Gag and Pol) and pHelper 2.0 (VSVG) using Lipofectamine 3000. MKN-45 cells in the logarithmic growth phase were infected by the lentivirus with a multiplicity of infection of 70, and the stable MKN-45 cell line with RP11-874J12.4 knockdown was obtained using the limited dilution method after screening with puromycin (2 µg/mL) for 14 days.

#### Reverse transcription quantitative PCR

Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA), and miRNA was extracted using the miRNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Using total RNA (1 µg for each sample), cDNA was synthesized using the PrimeScript® RT Master Mix Perfect Real-Time Reagent Kit (Takara Bio Inc., Shiga Prefecture, Japan). For reverse transcription of miRNA, cDNA was synthesized with a universal tag using the miScript II RT Kit (Qiagen). Reverse transcription quantitative PCR (RT-qPCR) assays for miRNA and mRNA were performed

using a standard protocol described in the SYBR Green PCR Kit (Toyobo, Osaka, Japan) on an AB7500 RT-PCR instrument (Applied Biosystems, Foster City, CA, USA). Relative quantification was determined via normalization to GAPDH or U6. The primers used for RT-qPCR analysis were as follows: RP11-874J12.4-1, forward 5'-ATTCGCACATGGCGTCAAAC-3', reverse 5'-GAGAAGCCTGGAAAAGGCGA-3': SSR2, forward 5'-GCTGTCACTCAAGCAGAGGAAGG-3', reverse 5'-GTTGTACTGCAAGGTCAGGTCTCG-3'; miR-3972, 5'-CTGCCAGCCCCGTTCCA-3'; GAP-DH, forward 5'-ACTTTGTCAAGCTCATTTCCTG-3'; reverse 5'-CTCTCTTCCTCTTGGCTCTTG-3'; and U6. forward 5'-ATTGGAACGATACAGAGAAGATT-3', reverse 5'-GGAACGCTTCACGAATTTG-3'. These PCR primers were synthesized by Sangon Biotech Co., Ltd (Shanghai, China). The PCR protocol comprised two steps: one, initial denaturation at 95°C for 30 s, and two, denaturation at 95°C for 5 s, followed by annealing and extension at 60°C for 31 s and measurement of the fluorescence signal. The reactions comprised a total of 40 cycles and ended with a melting curve, which consisted of reaction for 15 s at 95°C, 1 min at 60°C, 15 s at 95°C, and 15 s at 60°C. The experiments were repeated three times, and each sample was run in triplicates. Specificity of the PCR products was confirmed by melting curve analysis. Gene expression levels were calculated using the 2-DACT method.

#### RNA immunoprecipitation

RNA immunoprecipitation (RIP) assay was conducted using the Dynabeads<sup>™</sup> Protein G Immunoprecipitation Kit (Thermo Fisher Scientific). Briefly, anti-Argonaute-2 (AGO2) antibody (Catalog number: ab186733, Abcam, Cambridge, MA, USA) was mixed with Dynabeads<sup>™</sup> magnetic beads, and the mixture was incubated for 20 min at room temperature. After washing with the Wash Buffer provided, the beads were added into MKN-45 cell lysates and incubated for an additional 10 min at room temperature. Subsequently, following a non-denaturing elution at room temperature, TRIzol reagent was used for RNA purification, and qPCR assay was conducted.

#### Cell viability assay

At 24 h after transfection, MKN-45 and AGS cells were seeded into 96-well plates at a den-

sity of 5  $\times$  10<sup>3</sup>/well and were treated with various doses of docetaxel or cisplatin for 48 h. As an indicator of cell viability, the activity of mitochondrial succinate dehydrogenase was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. At the end of the culture period, 20 µL of 5 mg/ mL MTT solution (5 mg/mL; KeyGen Biotech, Nanjing, China) was added, and the cells were cultured for an additional 4 h. The culture medium was removed, and 150 µL of DMSO (Sangon Biotech, Inc., Shanghai, China) was added to dissolve formazan. Cell viability was quantified by measuring the absorbance at 490 nm using a microplate reader. The optical density values of samples in the experimental groups were normalized to those of each group without drug treatments.

#### Apoptosis assay

At 24 h after transfection, MKN-45 and AGS cells were seeded into 6-well plates and treated with docetaxel or cisplatin at the indicated concentration for another 48 h. Cells were harvested after detachment by incubating with 0.25% trypsin-EDTA solution (Wolsen Biotechnology Inc., Xian'an, Shanxi, China). Next, flow cytometric analysis was conducted to measure cell apoptosis using the Annexin V-Fluorescein Isothiocyanate (FITC)/Propidium Iodide (PI) Apoptosis Kit (BD Biosciences, Franklin Lakes, NJ, USA) according to the manufacturer's protocol. Briefly, cells were washed once each with PBS and binding buffer and were then stained with Annexin V-FITC/PI for 20 min at room temperature in the dark. This was followed by washing once with the binding buffer. and then the labeled cells were immediately detected using a flow cytometer (FACSCalibur, BD Biosciences). Data were analyzed using the FlowJo software (FlowJo, LLC). In addition, terminal deoxynucleotidyl transferase dUTP nickend labeling (TUNEL) assay was used to detect apoptotic cells that underwent extensive DNA degradation during the late stages of apoptosis. The Click-iT® TUNEL Alexa Fluor® 488 Imaging Assay kit (Thermo Fisher Scientific) was used to determine the percentage of apoptotic cells according to the manufacturer's instructions. Fluorescent images were obtained using a Nikon Eclipse TI fluorescence microscope, and data were processed using ImageJ software (version 1.49; National Institutes of Health, Bethesda, MD, USA).

#### Luciferase reporter assay

Because miRNAs potentially interact with RP11-874J12.4, the binding sites between RP11-874J12.4 and miR-3972 were predicted using LncBase version 2.0 [29], and the interaction between miR-3972 and the 3' untranslated region (UTR) of SSR2 mRNA was predicted using the online tools TargetScan (http: //www.targetscan.org/vert\_72/) and miRDB (http://www.mirdb.org/). The predicted miR-3972 binding site region of RP11-874J12.4 and the corresponding mutated region as well as the predicted miR-3972 binding site region at the 3' UTR of SSR2 mRNA and the corresponding mutated region were cloned into the luciferase-expressing vector pGL3 (Promega, Madison, WI). HEK293 T cells (ATCC<sup>®</sup> CRL-3216<sup>™</sup>) were co-transfected with the indicated vector together with the miR-3972 inhibitor, miR-3972 mimic, or their scramble controls. At 48 h after transfection, the culture supernatant was harvested and subjected to luciferase activity analysis using a Dual-Luciferase Reporter Assay System (Promega, Fitchburg, WI, USA) according to the manufacturer's instructions.

#### Western blot assay

Cells were washed with cold PBS and lysed in radioimmunoprecipitation assay lysis buffer (Sigma-Aldrich) containing a protease and phosphatase inhibitor cocktail on ice for 30 min. Cells were centrifuged at 13,000 × g for 20 min at 4°C, following which the proteins in the supernatants were quantified and equal amounts of total proteins were loaded. The protein samples were resolved using 10% SDS-PAGE and transferred to polyvinylidene difluoride membranes. The membranes were incubated overnight with the indicated primary antibodies. Anti-SSR2 antibody (1:1000 dilution: Catalog number: NBP1-69471) was purchased from Novus Biologicals (Centennial, CO, USA), and the antibody for the loading control GAPDH (1:10,000 dilution; Catalog number: ab9484) was purchased from Abcam. Antibodies against procaspase-3 (Catalog number: ab32499, Abcam), cleaved caspase-3 (Catalog number: ab32042, Abcam), procaspase-9 (Catalog number: ab138412, Abcam), cleaved caspase-9 (Catalog number: ab2324, Abcam), Bcl-2 (Catalog number: ab32124, Abcam), and Bax (Catalog number: ab32503, Abcam) were used for evaluating apoptosis. The secondary antibodies consisted of horseradish peroxidase-conjugated anti-rabbit IgG (1:10,000; Catalog number: HS101, TransGen Biotech, Inc., Beijing, China) or anti-mouse IgG (1:10,000; Catalog number: HS201, TransGen Biotech, Inc.). Proteins of interest were visualized using an enhanced chemiluminescence kit (EMD Millipore, Burlington, MA, USA). Band intensities were quantified by densitometry using ImageJ software. Western blot experiments were repeated at least three times, and one representative blotting result was shown for each experiment.

#### Xenograft tumor model

Male BALB/c nude mice aged 6 weeks that were purchased from Charles River Laboratories (Beijing, China) were maintained at room temperature (22°C ± 1°C) with a 12/12 h light/ dark cycle and access to food and water ad libitum. Stable MKN-45 cells with RP11-874J12.4 knockdown (RP11-874J12.4-shRNA) or control MKN-45 cells (scramble-shRNA) were subcutaneously injected into the right flank of each mouse  $(5 \times 10^6 \text{ cells/mouse})$  to establish the xenograft model. Thereafter, intraperitoneal injections of docetaxel at a dose of 10 mg/kg body weight once a week or cisplatin at a dose of 5 mg/kg body weight once a week were administered to the mice. The tumor volume (V) was monitored once every 4 days by measuring the tumor diameters and was calculated using the following formula:  $V = ab^2/2$ , where a is the long diameter, and b is the short diameter. At 17 days after tumor inoculation, mice were euthanized and tumor tissues were subjected to further analyses. All animal experiments were conducted in accordance with the Declaration of Helsinki, and all procedures involving the experimental animals were approved by the University Committee on the Use and Care of Animals.

#### Statistical analysis

Data were presented as the mean  $\pm$  standard error of mean. Student's *t*-test was used to analyze the differences between two groups. Differences among more than two groups were analyzed using analysis of variance with Bonferroni correction. All statistical analyses were performed using GraphPad Prism software (GraphPad Software Inc., San Diego, CA, USA), and differences were considered to be statistically significant at *P* < 0.05.



**Figure 1.** Human gastric cancer tissues and cell lines showed higher RP11-874J12.4 expression levels than normal controls. A. The relative RP11-874J12.4 expression level in tumor tissues (GC) and adjacent normal gastric tissues (Adjacent) collected from patients with gastric cancer (n = 20) was determined using RT-qPCR. B. The relative RP11-874J12.4 expression level in the normal human gastric epithelial cell line GES-1 and three human gastric cancer cell lines (MKN-45, AGS, and HGC-27) were determined using RT-qPCR. n = 3 for each group; \*\**P* < 0.01, compared with the indicated controls.

#### Results

#### Human gastric cancer tissues and cells exhibit higher RP11-874J12.4 expression levels than normal controls

To explore the potential roles of RP11-874J12.4 in the pathogenesis and chemoresistance of human gastric cancer, we first evaluated the relative transcription level of RP11-874J12.4 in the clinical gastric cancer samples and in human gastric cancer cell lines. Cancer tissues and adjacent normal gastric tissues were collected from 20 patients who had undergone surgery. RT-qPCR results demonstrated that RP11-874J12.4 expression in gastric cancer tissues was more than 3-fold higher than that in adjacent normal gastric tissues (Figure 1A). Moreover, RP11-874J12.4 expression was remarkably higher in the three common human gastric adenocarcinoma cell lines MKN-45, AGS, and HGC-27 than in the human normal gastric epithelial cell line GES-1. RP11-874J12.4 expression level was an approximately 7-fold higher in HGC-27 cells than in GES-1 cells, whereas it was >15-fold higher in MKN-45 and AGS cells than in GES-1 cells (Figure 1B). As both MKN-45 and AGS cells exhibited

evidently higher RP11-874J-12.4 expression than HGC-27 cells, we selected these two cell lines to establish *in vitro* models for evaluating the potential contribution of RP11-874J12.4 in regulating chemoresistance in human gastric adenocarcinoma in subsequent experiments.

#### RP11-874J12.4 knockdown sensitizes human gastric cancer cells to docetaxel and cisplatin treatments

To assess whether RP11-87-4J12.4 contributes to chemoresistance in human gastric cancer, we determined the effects of RP11-874J12.4 knockdown on the viability and apoptosis of MKN-45 and AGS cells following treatment with chemotherapeutic drugs. First, we evaluated the knockdown efficiency of three siR-

NAs against RP11-874J12.4 in MKN-45 cells. As shown in **Figure 2A**, the siRNA si-RP11-874J12.4-2 exhibited the most efficient knockdown of RP11-874J12.4, with a silencing efficacy of up to 80%. Therefore, this siRNA was used for silencing RP11-874J12.4 in subsequent experiments. MKN-45 and AGS cells transfected with scramble siRNAs or RP11-874J12.4-specific siRNAs were treated with various doses of docetaxel or cisplatin, following which the cell viability was measured to determine the contribution of RP11-874J12.4 expression to chemoresistance in these cells. At >20 nM docetaxel (Figure 2B) and >5 µM cisplatin (Figure 2C), the viability of both MKN-45 and AGS cells with RP11-874J12.4 knockdown was significantly reduced compared with that of corresponding control cells (P < 0.05), suggesting that RP11-874J12.4 silencing markedly inhibited the proliferation of human gastric cancer cells treated with relatively higher doses of docetaxel or cisplatin. Notably, viability was almost completely repressed in MKN-45 cells with RP11-874J12.4 knockdown treated with >40 nM docetaxel or >15  $\mu$ M cisplatin. Similarly, minimal viability was detected in AGS cells with RP11-874J12.4 knockdown treated with 20 µM cisplatin (Figure 2B and 2C). Moreover, siRNA-



**Figure 2.** RP11-874J12.4 knockdown sensitized the human gastric cancer cells MKN-45 and AGS to docetaxel and cisplatin. (A) The relative expression level of RP11-874J12.4 in MKN-45 cells transfected with the indicated siRNA was determined using RT-qPCR. At 48 h after transient transfection of control siRNAs (scramble) or RP11-874J12.4-specific siRNAs (si-RP11-874J12.4-1, si-RP11-874J12.4-2, and si-RP11-874J12.4-3), MKN-45 cells were harvested to determine the knockdown efficiency. n = 3 for each group. (B, C) MKN-45 and AGS cells were transfected with

control siRNAs (scramble) or RP11-874J12.4-specific siRNAs (RP11-874J12.4 siRNA). At 24 h after transfection, cells were treated with docetaxel (B) or cisplatin (C) at the indicated concentration for 48 h, and the cell viability was measured using the MTT assay. n = 3 for each group. (D, E) MKN-45 and AGS cells were transfected with control siRNAs (scramble) or RP11-874J12.4-specific siRNAs (RP11-874J12.4 siRNA). At 24 h after transfection, cells were treated with 40 nM docetaxel (D) or 10  $\mu$ M cisplatin (E) for 48 h and subjected to flow cytometric analysis after staining with Annexin V and PI. The apoptosis rate (percentages of Annexin V<sup>+</sup> cells) was calculated. n = 3 for each group. (F, G) Experiments were performed as described in (D and E), except that cell apoptosis was evaluated using the TUNEL assay, and representative images are shown. Magnification, 20 ×. \**P* < 0.05, \*\**P* < 0.01, compared with the indicated control group or between the indicated groups.

mediated functional inactivation of RP11-874J12.4 significantly increased the apoptosis rate of MKN-45 and AGS cells treated with 40 nM docetaxel (Figures 2D and S1A) or 10 µM cisplatin (Figures 2E and S1B). Remarkably, all cells with RP11-874J12.4 knockdown exhibited an apoptosis rate of ~70% compared with ~40% in control cells. Furthermore, the TUNEL assay confirmed that RP11-874J12.4 knockdown evidently increased the apoptosis rate of both MKN-45 (Figure 2F) and AGS (Figure 2G) cells following treatment with either docetaxel or cisplatin. Collectively, these results indicate that RP11-874J12.4 conferred chemoresistance to MKN-45 and AGS cells against docetaxel and cisplatin.

### RP11-874J12.4 acts as a ceRNA that sponges miR-3972

Further, we predicted the possible binding partner of RP11-874J12.4 using the tool LncBase version 2.0 and identified that miR-3972 is one of the most promising candidate miRNAs that could be sponged by RP11-874J12.4, considering that miR-3972 exhibited the most increased expression following RP11-874J12.4 knockdown in MKN-45 cells (Figure 3A). To assess this possibility, we constructed luciferase reporter vectors expressing either wild-type RP-11-874J12.4 or mutant RP11-874J12.4 (Figure 3B) and co-transfected these plasmids with either scramble or miR-3972 inhibitor to 293T cells. As shown in Figure 3C, the luciferase assay results demonstrated that the luciferase activities were significantly increased after cotransfection of wild-type RP11-874J12.4 and miR-3972 inhibitor (P < 0.05) but they were only slightly changed after co-transfection of wildtype RP11-874J12.4 and scramble or mutant RP11-874J12.4 and miR-3972 inhibitor, implying that RP11-874J12.4 is a ceRNA of miR-3972. Further, this interaction was validated by downregulating RP11-874J12.4 expression in MKN-45 and AGS cells based on the observation that cells with siRNA transfectioninduced RP11-874J12.4 knockdown exhibited profoundly increased levels of miR-3972 (Figure 3D). In addition, RIP assay showed that the complex broken down by the anti-AGO2 antibody exhibited over 100-fold higher levels of both RP11-874J12.4 and miR-3972 compared with the control normal tissues with anti-IgG antibody (Figure 3E). Furthermore, compared with the control normal tissues or gastric epithelial cells, the gastric cancer tissues of patients (Figure 3F) and human gastric cancer cell lines (Figure 3G) with higher RP11-874J12.4 expression levels exhibited significantly reduced miR-3972 expression (P < 0.05), suggesting an inverse correlation between RP11-874J12.4 and miR-3972 expression levels. Collectively, our results indicate that RP11-874J12.4 and miR-3972 have a targeted relationship in human gastric cancer cells.

#### SSR2 is a downstream target of miR-3972, and the miR-3972/SSR2 axis regulates the chemoresistance of human gastric cancer cells to docetaxel and cisplatin

We next attempted to elucidate the molecular mechanisms underlying RP11-874J12.4 expression in conferring chemoresistance to human gastric cancer cells. We searched for the protein-coding mRNA targets of miR-3972 using the online tools TargetScan (http://www.targetscan.org/) and miRDB (http://mirdb.org/) and identified 12 candidate mRNAs that were predicted by both tools (Figure 4A). A previous study reported that SSR2, one of the most promising target mRNAs, is required for the survival of human melanoma cells during endoplasmic reticulum stress [30]. Considering the stresses that gastric cancer cells are exposed to after treatment with chemotherapeutic drugs, we predicted that SSR2 is involved in the chemoresistance of gastric carcinomas. Moreover, among the 12 candidate mRNAs, SSR2 showed the most upregulated expression



Figure 3. The IncRNA RP11-874J12.4 functioned as a competing endogenous RNA by sponging miR-3972 in human gastric cancer cells. A. A total of 13 candidate miRNAs of RP11-874J12.4 were predicted using the online tool LncBase version 2.0. Relative expression levels of these miRNAs in MKN-45 cells at 24 h after transfection of control siRNAs (scramble) or RP11-874J12.4-specific siRNAs (RP11-874J12.4 siRNA) were determined using RT-qPCR. n = 3 for each group. B. Schematic diagram shows the matching base pairs between miR-3972 and wild-type (WT) or mutant (MUT) RP11-874J12.4. C. The luciferase activity of the reporter vectors was detected in 293T cells after co-transfection of the plasmid expressing WT or MUT RP11-874J12.4 together with scramble or miR-3972 inhibitor. n = 3 for each group. D. The relative miR-3972 expression level in MKN-45 and AGS cells was determined at 48 h after the transient transfection of control scramble siRNA or RP11-874J12.4-specific siRNAs using RT-qPCR. n = 3 for each group. E. RNA immunoprecipitation was performed using anti-AGO2 or anti-IgG antibody and MKN-45 cell lysates. The relative RP11-874J12.4 and miR-3972 expression levels in the broken-down complexes were quantitated using qPCR. n = 3 for each group. F. The relative miR-3972 expression level in tumor tissues (GC) and adjacent normal gastric tissues (Adjacent) collected from patients with gastric cancer (n = 20) was determined using RT-qPCR. G. The relative expression level of miR-3972 in the normal human gastric epithelial cell line GES-1 and three human gastric cancer cell lines (MKN-45, AGS, and HGC-27) was determined using RT-qPCR. n = 3 for each group; \*P < 0.05, \*\*P < 0.01, compared with the indicated control groups.

following miR-3972 inhibition in MKN-45 cells (**Figure 4B**). Accordingly, we further investigated the interaction between miR-3972 and SSR2 in MKN-45 and AGS cells; miR-3972 was

found to share seven nucleotide-binding sequences with the 3' UTR of SSR2 mRNA (**Figure 4C**). We cloned the wild-type and mutant 3' UTR of SSR2 mRNA into a luciferase reporter plas-



**Figure 4.** SSR2 is a downstream target of miR-3972, and the expression of miR-3972 and SSR2 regulated the sensitization of human gastric cancer cells to docetaxel and cisplatin. (A) A Venn diagram shows the predicted numbers of candidate mRNA targets of miR-3972 using the online tools TargetScan and miRDB. There were 12 common candidate targets. (B) The relative mRNA levels of the 12 candidate targets in MKN-45 cells at 48 h after treatment with miR-3972 inhibitor or scramble were determined using RT-qPCR. n = 3 for each group. (C) A schematic diagram shows the matching base pairs between miR-3972 and wild-type (WT) or mutant (MUT) 3' UTR of SSR2 (signal sequence receptor subunit 2) mRNA. (D) The luciferase activity of the reporter vectors was detected in 293T cells after co-transfection of the plasmid expressing wild-type or mutant 3' UTR of SSR2 mRNA together with the scramble or the miR-3972 mimic. n = 3 for each group. (E, F) Functional inhibition of miR-3972 enhanced SSR2 protein expression in MKN-45 and AGS cells. Representative western blot images are shown (E), and the relative band intensity of SSR2 evaluated by densitometry analysis was calculated (F). n = 3 for each group. (G, H) The relative SSR2 expression level in MKN-45 (G) and AGS (H) cells transfected with the indicated siRNAs was determined using RT-qPCR. At 48 h after the transient transfection of the control siRNAs (scramble siRNA) or SSR2-specific siRNAs (SSR2 siRNA-1, SSR2 siRNA-2, and SSR2 siRNA-3), cells were harvested to determine the knockdown efficiency. n = 3 for each group; \**P* < 0.05, \*\**P* < 0.01, compared with the scramble siRNA group. (I, J) SSR2 knockdown increased the apoptosis rate in MKN-45 and AGS cells treated with docetaxel (I) or cisplatin (J). Cells were transfected with control siRNA (scramble) or SSR2-specific siRNA (SSR2 siRNA). At 24 h after transfection, cells were treated with 40 nM docetaxel (I) or 10 µM cisplatin (J) for 48 h and then subjected to flow cytometric analysis after staining with Annexin V and PI. The apoptosis rate (percentages of Annexin V<sup>+</sup> cells) was calculated. n = 3 for each group. \**P* < 0.05, \*\**P* < 0.05, \*\**P* < 0.05, \*\**P* < 0.01, compared with the indicated control groups.

mid pGL3 and co-transfected the plasmids together with miR-3972 inhibitor or scramble to assess the regulation of SSR2 expression by miR-3972. The luciferase reporter assay results showed that only the co-transfection of miR-3972 inhibitor and the plasmid expressing the wild-type 3' UTR of SSR2 resulted in a significant increase of luciferase activity compared with that in other controls (P < 0.05), thereby confirming the binding of miR-3972 to the 3' UTR of SSR2 (Figure 4D). Consistent with this result. MKN-45 and AGS cells treated with miR-3972 inhibitor showed significantly increased SSR2 protein expression (Figure 4E and 4F). Further, we evaluated the silencing efficiency of three siRNAs against SSR2 and selected the most efficient SSR2 siRNA-3 to achieve SSR2 interference in gastric cancer cells (Figure 4G and **4H**). siRNA-mediated silencing of SSR2 markedly increased the apoptosis rates of MKN-45 and AGS cells treated with 40 nM docetaxel (Figures 4I and S2A) and 10 µM cisplatin (Figures 4J and S2B). Moreover, inactivation of miR-3972 via miR-3972 inhibitor transfection markedly sensitized MKN-45 and AGS cells to docetaxel (Figure 4I) and cisplatin (Figure 4J). Therefore, our results demonstrate that miR-3972 modulates SSR2 expression and that the expression levels of both molecules are strongly associated with the chemoresistance ability of human gastric cancer cells.

## SSR2 downregulation further sensitizes gastric cancer cells with RP11-874J12.4 knockdown to chemotherapy

To validate the regulatory role of the RP11-874J12.4/miR-3972/SSR2 axis in the progression of human gastric cancer, we investigated whether miR-3972 functional inactivation and SSR2 silencing affected the viability and apoptosis rate of MKN-45 and AGS cells with RP11-874J12.4 knockdown treated with docetaxel or cisplatin. Compared with control cells, RP11-874J12.4 knockdown in both MKN-45 and AGS

cells led to a marked reduction of cell viability after treatment with 40 nM docetaxel (Figure 5A, 5B) or 10 µM cisplatin (Figure 5C, 5D). However, further suppression of miR-3972 using the inhibitor almost completely reversed the antiproliferative ability of RP11-874J12.4specific siRNA, implying that the targets of miR-3972 functioned downstream of RP11-874J12.4. Indeed, additional SSR2 knockdown further impaired the viability of MKN-45 and AGS cells with RP11-874J12.4 knockdown treated with docetaxel (Figure 5A, 5B) or cisplatin (Figure 5C, 5D). Similarly, RP11-874J12.4 silencing resulted in enhanced apoptosis of both MKN-45 and AGS cells after treatment with docetaxel (Figures 5E and S3A) or cisplatin (Figures 5F and S3B). The additional suppression of miR-3972 almost completely reversed the apoptosis-promoting effects of RP11-874J12.4-specific siRNA, whereas the additional SSR2 silencing further increased the apoptotic rates of cells treated with docetaxel (Figure 5E) or cisplatin (Figure 5F). Similar trends were observed regarding the effects of apoptosis caused by the additional miR-3972 suppression or additional SSR2 silencing in MKN-45 (Figure 6A and 6C) and AGS (Figure 6B and 6D) cells with RP11-874J12.4 knockdown treated with docetaxel (Figure 6A and 6B) or cisplatin (Figure 6C and 6D), as demonstrated by the altered expressions of apoptosis-related proteins, including cleaved caspase-3, cleaved caspase-9, Bax, and Bcl-2. Collectively, these findings indicate that SSR2 downregulation could further sensitize gastric cancer cells with RP11-874J12.4 knockdown to chemotherapy.

# RP11-874J12.4 knockdown inhibits the growth of xenograft MKN-45 gastric cancer cells in nude mice

To further investigate the role of RP11-874J12.4 in conferring chemoresistance to human gastric cancer cells *in vivo*, we inoculated scramble-shRNA lentivirus-infected control MKN-45



**Figure 5.** SSR2 downregulation sensitized gastric cancer cells with RP11-874J12.4 knockdown to docetaxel and cisplatin. (A-D) The effects of miR-3972 inhibition and SSR2 knockdown on the viability of MKN-45 cells (A, C) and AGS cells (B, D) with RP11-874J12.4 knockdown treated with docetaxel (A, B) and cisplatin (C, D). Cells were transfected with negative control (scramble) or RP11-874J12.4-specific siRNA (RP11-874J12.4 siRNA) or with RP11-874J12.4-specific siRNA and miR-3972 inhibitor (RP11-874J12.4 siRNA + miR-3972 inhibitor) or with RP11-874J12.4-specific siRNA and SSR2-specific siRNA (RP11-874J12.4 siRNA + miR-3972 inhibitor) or with RP11-874J12.4-specific siRNA and SSR2-specific siRNA (RP11-874J12.4 siRNA + SSR2 siRNA). At 24 h after transfection, cells were treated with 40 nM docetaxel (A, B) or 10  $\mu$ M cisplatin (C, D) for 48 h, and cell viability was measured using the MTT assay. (E, F) Experiments were performed as described in (A-D), except that cells were harvested to evaluate the effects of miR-3972 inhibition and SSR2 knockdown on the apoptosis of cells with RP11-874J12.4 knockdown after treatment with docetaxel (E) or cisplatin (F) by flow cytometry. n = 3 for each group; \**P* < 0.05, \*\**P* < 0.01, compared with the scramble group or between the indicated groups.

RP11-874J12.4 induces chemoresistance in GC





**Figure 6.** Effects of miR-3972 inhibition and SSR2 knockdown on the expression levels of apoptosis-related proteins in gastric cancer cells with RP11-874J12.4 knockdown treated with docetaxel or cisplatin. (A-D) The experiments were performed as described in **Figure 5**. At 24 h after transfection of the indicated siRNAs or inhibitors, MKN-45 cells (A, C) or AGS cells (B, D) were treated with 40 nM docetaxel (A, B) or 10  $\mu$ M cisplatin (C, D) for 48 h. Cells were harvested to determine the protein levels of cleaved caspase-3, cleaved caspase-9, procaspase-9, Bcl-2, and Bax by western blot assays. Representative images of bands are shown, and the relative protein levels of cleaved caspase-3, cleaved caspase-3, Bcl-2, and Bax are summarized from three experiments with similar results. n = 3 for each group; \**P* < 0.05, compared between the indicated groups.



**Figure 7.** RP11-874J12.4 knockdown significantly retarded the growth of xenograft MKN-45 cells in nude mice that received chemotherapy. (A-I) Stable MKN-45 cells with RP11-874J12.4 knockdown (RP11-874J12.4-shRNA) or control MKN-45 cells (scramble) were injected subcutaneously into the right flank of each mouse ( $5 \times 10^6$  cells/ mouse) to establish the xenograft model. Mice were treated with intraperitoneal injection of docetaxel at a dose of 10 mg/kg body weight once a week or cisplatin at a dose of 5 mg/kg body weight once a week. Tumor volume was monitored once every 4 days, and tumor tissues were collected at 17 days after tumor inoculation. The image of tumors (A) and the tumor growth curves in the RP11-874J12.4-shRNA and scramble groups treated with docetaxel (B) and cisplatin (C) are shown. (D) Tumor weight in the indicated groups. (E, F) The SSR2 protein level in tumors of the indicated groups was determined by western blotting. The representative band images (E) and summarized data on the relative intensity of SSR2 bands (F) are shown. n = 5 for each group. (G-I) The RP11-874J12.4 (G), miR-3972 (H), and SSR2 (I) expression levels in tumor tissues were determined using RT-qPCR. n = 5 for each group; \**P* < 0.05, \*\**P* < 0.01, compared with the control group.

cells and RP11-874J12.4-shRNA lentivirus-infected stable MKN-45 cells in the flank of nude mice and treated these xenograft mice with docetaxel or cisplatin. As shown in **Figure 7A**, RP11-874J12.4 knockdown caused significantly reduced growth of xenograft MKN-45 cells at 17 days after drug treatment. The tumor volumes in the RP11-874J12.4-shRNA group were smaller than those in the scramble-shRNA group, particularly starting from 9 days after tumor inoculation (**Figure 7B** and **7C**). Similarly, the tumor volumes in the RP11-874J12.4-shRNA group were markedly reduced by >85% compared with those in the control scramble-shR- NA group at 17 days after tumor inoculation (Figure 7D). Further, we found that the tumor tissues in the RP11-874J12.4-shRNA group showed significantly decreased SSR2 protein expression regardless of the treatments with docetaxel or cisplatin (Figure 7E and 7F). Moreover, RT-PCR analyses showed that compared with the scramble-shRNA group, the tumor tissues in the RP11-874J12.4-shRNA group had significantly decreased RP11-874J12.4 expression (Figure 7G), increased miR-3972 expression (Figure 7I). Therefore, the altered expression of molecules in the RP11-874J12.4/

miR-3972/SSR2 axis contributed to the sensitization of xenograft MKN-45 cells to docetaxel and cisplatin *in vivo*.

#### Discussion

Gastric cancer remains a serious threat to global health, indicating the urgent need to identify novel molecular biomarkers for early diagnosis, therapeutic response, prognosis, and overcoming chemoresistance [5, 6]. The occurrence and development of gastric cancer is a complex process that is determined by multiple factors and multiple steps and involves both coding and noncoding genes. Increasing evidence has indicated that abnormal IncRNA expression is associated with disease initiation and development, tumor metastasis, and invasion of gastric cancer [31-34]. In the present investigation, we identified a novel IncRNA, RP11-874J12.4, whose expression was significantly upregulated in gastric cancer cell lines and clinical tissues compared with that in corresponding nontumor cells and tissues. Our results demonstrated that RP11-874J12.4 knockdown markedly enhanced the sensitivity of human gastric cancer cells to chemotherapeutic drugs, including docetaxel and cisplatin, both in vitro and in vivo. Further, miR-3972, a putative target gene of RP11-874J12.4, exhibited an inverse correlation with RP11-874J12.4 in terms of expression levels in gastric cancer tissues and cell lines. Luciferase reporter assay results also demonstrated the direct binding between miR-3972 and the 3' UTR mRNA of SSR2, whose downregulation was confirmed to be associated with increased apoptosis of gastric cancer cells after treatment with docetaxel or cisplatin. Our study identified the role of the RP11-874J12.4/ miR-3972/SSR2 axis in regulating the chemoresistance of human gastric cancer cells and suggested that the molecules in this axis are potential novel biomarkers for the diagnosis, prognosis, and treatment of gastric cancer.

The recent development of high-throughput screening technologies, such as microarray and RNA sequencing, has facilitated the identification of the critical roles of IncRNAs and miR-NAs in the pathogenesis of gastric cancer [31, 35, 36]. The present study demonstrated that RP11-874J12.4 was highly expressed in human gastric cancer cells. RP11-874J12.4 expression appears to be inversely correlated with the viability of gastric cancer cells, suggesting that

RP11-874J12.4 is a possible biomarker for predicting the overall and disease-free survival outcomes of patients with gastric cancer. Moreover, RP11-874J12.4 might act as an oncogenic IncRNA based on our observation that its knockdown could significantly repress the proliferation and reduce the viability of human gastric cancer cells. Thus, RP11-874J12.4 expression in tumor tissues may be associated with gastric cancer progression and act as an important biomarker for predicting this progression. Importantly, RP11-874J12.4 might confer chemoresistance to human gastric cancer cells against docetaxel and cisplatin by modulating apoptosis, which further strengthens the rationale of targeting RP11-874J12.4 for gastric cancer therapy.

A previous study documented that some IncRNAs with dysregulated function in gastric cancer also contribute to chemoresistance of cancer cells [15]. For example, MDR-related and upregulated IncRNA (MRUL) was found to exhibit higher expression in the chemoresistant SGC7901/VCR and SGC7901/ADR cells than in the parental SGC7901 cells [37]. Moreover, MRUL downregulation enhanced the chemosensitivity of multidrug-resistant (MDR) gastric cancer cell sub-lines to P-gp-related chemotherapeutic drugs, which was associated with a reduced Bcl-2/Bax ratio [37]. Moreover, in another study, silencing of AK022798, a Notch 1 over-expression positively regulated IncRNA, remarkably reduced the viability and increased the apoptosis of the cisplatin-resistant cell lines SGC7901/DDP and BGC823/DDP [38]. Collectively, these results suggested that similarly to the IncRNAs MRUL and AK022798, RP11-874J12.4 is involved in regulating the expression of MDR-related genes, such as MDR1, MRP1, LRP, and BCRP, as well as cell proliferation-dependent pathways, such as the mammalian target of rapamycin pathway. Further research investigating the effects of abnormal RP11-874J12.4 expression in human gastric cancer cells is required to clearly elucidate the molecular mechanisms underlying the role of RP11-874J12.4 in modulating the resistance to multiple drugs. Interestingly, in a large-scale screening for IncRNAs involved in epigenetic regulation in response to platinum treatment, RP11-874J12.4 was found to be downregulated in cisplatin-resistant lung cancer and ovarian cancer cell lines [39]. Therefore, it is necessary to elucidate the mechanisms underlying the differential regulation of RP11-874J12.4 expression and the corresponding roles of RP11-874J12.4 in distinct cancer types.

It is known that IncRNAs play vital roles in regulating gene expression via various mechanisms and at multiple levels. They can interact with proteins, DNAs, or RNAs to exert versatile functions [34, 40]. Several studies have shown that some IncRNAs can act as miRNA sponges by competitively interacting with miRNAs to reduce the availability of miRNA to their target mRNAs [41, 42]. Using bioinformatics databases, we identified miR-3972 as one of the target miR-NAs of RP11-874J12.4 in human gastric cancer cells, which showed a relatively lower expression level in gastric carcinomas. Notably, the luciferase reporter assays conducted in our study corroborated that SSR2 is a target of miR-3972, and its expression negatively correlated with the apoptosis rate of MKN-45 and AGS cells following treatment with docetaxel or cisplatin. Apparently, miR-3972 functions as a tumor suppressor and SSR2 functions as a tumor promoter in the pathogenesis of human gastric cancer. Indeed, SSR2 has been revealed to exert a pro-survival function in human melanoma cells, and high SSR2 expression levels were associated with an unfavorable disease outcome in patients with primary melanoma [30]. Importantly, in the same study, SSR2 upregulation was observed in human melanoma cells during the occurrence of drug resistance to BRAF inhibitors; moreover, the survival of BRAF inhibitor-resistant melanoma cells was dependent on SSR2 expression [30]. Therefore, it is plausible that SSR2 plays an essential role in promoting the survival of human gastric cancer cells challenged with docetaxel or cisplatin by antagonizing the chemotherapy-induced cellular stresses such as endoplasmic reticulum stress. Supporting this assumption, in the present study, the MKN-45 xenograft mice with reduced tumor burden demonstrated markedly decreased SSR2 mRNA and protein expression in tumors. It is worth noting that in MKN-45 or AGS cells with RP11-874J12.4 knockdown. additional SSR2 knockdown further increased the apoptosis rate of cells following treatments with docetaxel or cisplatin. This might be due to the inadequate knockdown of RP11-874J12.4 by the transient transfection of the siRNA or to the possible modulation of SSR2 expression in gastric cancer by other upstream molecules other than RP11-874J12.4/miR-3972. Nevertheless, the novel and precise therapeutic strategies targeting SSR2 are extremely promising for treating patients with chemoresistant gastric cancer in a more effective manner.

The current investigation has several strengths and limitations. The major strength is its novelty. To the best of our knowledge, this is the first study to elucidate the critical role of the RP11-874J12.4/miR-3972/SSR2 axis in regulating chemoresistance in human gastric cancer. This is also the first investigation reporting the association between the function of RP11-874J12.4/ miR-3972 and the pathogenesis of gastric cancer. Further, this study provides a feasible framework for the exploration of chemoresistance-related candidate IncRNA genes to better facilitate the diagnosis and implementation of more effective therapeutic strategies for patients with various types of cancer. On the other hand, the small sample size of clinical tissues and the single data source used for evaluating RP11-874J12.4 and miR-3972 expression levels in tumor and nontumor tissues is a limitation of this study. Therefore, additional research validating the therapeutic effects of in vivo RP11-874J12.4 or SSR2 knockdown as well as the delivery of miR-3972 mimics in animals is warranted to further validate the potential of the RP11-874J12.4/miR-3972/SSR2 axis-targeted therapy for patients with gastric cancer.

In conclusion, for the first time, our study demonstrated the involvement of the RP11-874-J12.4/miR-3972/SSR2 axis in modulating the proliferation and apoptosis of human gastric cancer cells both *in vitro* and *in vivo*. The IncRNA RP11-874J12.4 exhibited oncogenic properties by directly targeting miR-3971, which functions as a putative tumor suppressor by targeting the tumor-promoting factor SSR2 in gastric cancer cells treated with the chemotherapeutic drugs docetaxel and cisplatin. Our findings reveal a role of the RP11-874J12.4/miR-3972/SSR2 axis in regulating the chemoresistance in cancer as well as provide a novel insight into the diagnosis and therapy of gastric cancer.

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#### Disclosure of conflict of interest

None.

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**Figure S1.** Representative flow cytometry profiles of apoptosis assay by Annexin V/PI staining (related to **Figure 2D** and **2E**). MKN-45 and AGS cells were transfected with control siRNAs (scramble) or RP11-874J12.4-specific siRNAs (RP11-874J12.4 siRNA). At 24 h after transfection, cells were treated with 40 nM docetaxel (A) or 10 µM cisplatin (B) for 48 h and subjected to flow cytometric analysis after staining with Annexin V and PI.



**Figure S2.** Representative flow cytometry profiles of apoptosis assay by Annexin V/PI staining (related to **Figure 4I** and **4J**). SSR2 knockdown increased the apoptosis rate in MKN-45 and AGS cells treated with docetaxel (A) or cisplatin (B). Cells were transfected with control siRNA (scramble) or SSR2-specific siRNA (SSR2 siRNA). At 24 h after transfection, cells were treated with 40 nM docetaxel (A) or 10 µM cisplatin (B) for 48 h and subjected to flow cytometric analysis after staining with Annexin V and PI.



**Figure S3.** Representative flow cytometry profiles of apoptosis assay by Annexin V/PI staining (related to **Figure 5E** and **5F**). MKN-45 and AGS cells were transfected with negative control (scramble) or RP11-874J12.4-specific siRNA (RP11-874J12.4 siRNA) or RP11-874J12.4-specific siRNA and miR-3972 inhibitor (RP11-874J12.4 siRNA + miR-3972 inhibitor) or with RP11-874J12.4-specific siRNA and SSR2-specific siRNA (RP11-874J12.4-siRNA + SSR2 siRNA). At 24 h after transfection, cells were treated with 40 nM docetaxel (A) or 10  $\mu$ M cisplatin (B) for 48 h, and cell apoptosis was determined by flow cytometry after staining with Annexin V and PI.