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Effect of SIVA-1 Gene Overexpression on Cisplatin Resistance in Human Gastric Cancer Drug-Resistant Cell Lines Postprint

Authors: Shi Zhengyi, Huang Yuliang, Lei Yuanrui, Huang Haibin, Yang Jie, Xie Dongyi, Kong Fanbiao, Wang Xiaotong, Kong Fanbiao, Wang Xiaotong

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Abstract

Background Currently, there is a lack of in-depth research on the specific function of the apoptosis-inducing factor SIVA-1 in gastric cancer drug resistance, and whether it affects cellular drug sensitivity and proliferative activity through regulating the P53/E3 ubiquitin protein ligase (MDM2) pathway remains inconclusive. Elucidating the mechanism by which SIVA-1 regulates gastric cancer drug-resistant cells through the P53/MDM2 signaling pathway could provide novel theoretical basis and potential therapeutic targets for reversing chemotherapy resistance in gastric cancer.

Objective To investigate the effects of SIVA-1 overexpression on the expression levels of P53 and MDM2, observe its impact on drug sensitivity and cellular activity of the human gastric cancer drug-resistant cell line MKN-45/DDP, and explore the underlying mechanism.

Methods From September 2023 to March 2025, SIVA-1 overexpressing lentiviral vectors were constructed and transfected into human gastric cancer cells MKN-45/DDP. The cells were divided into SIVA-1 overexpression group (OE group), negative control group (NC group), and blank control group (CON group). The OE group was transfected with SIVA-1 overexpressing lentiviral particles; the NC group was transfected with control lentiviral particles; and the CON group consisted of untreated MKN-45/DDP cells. Cells from each group were collected, and CCK-8 assay was used to detect cell viability and changes in IC50 values for cisplatin (DDP), doxorubicin (ADM), and fluorouracil (5-FU). Bioinformatics analysis was performed using the STRING 12.0 and TIME 2.0 public databases. RT-qPCR and Western Blotting were employed to detect the mRNA and protein expression levels of SIVA-1, P53, and MDM2.

Results Sequencing results confirmed successful construction of the SIVA-1

overexpression recombinant vector with a lentiviral particle titer of 5$×$10^8 TU/mL. CCK-8 assay results demonstrated that compared with the NC and CON groups, the OE group exhibited decreased cell viability at 24 h, 48 h, 72 h, 96 h, and 120 h, along with reduced IC50 for DDP (P<0.05). Bioinformatics analysis revealed that compared with normal tissues, pan-cancer tissues showed increased expression of SIVA-1, P53, and MDM2 (P<0.05), while gastric cancer tissues exhibited increased expression of P53 and MDM2 (P<0.05). Protein-protein interaction analysis indicated interaction relationships between SIVA-1 and P53, as well as between P53 and MDM2. Correlation analysis results showed that in gastric cancer cells, SIVA-1 expression level was negatively correlated with MDM2 expression level (r=-0.114, P=0.020), P53 expression level was positively correlated with MDM2 expression level (r=0.293, P<0.001), while SIVA-1 expression was not correlated with P53 expression level (r=0.095, P=0.054). RT-qPCR and Western Blotting experimental results demonstrated that compared with the NC and CON groups, the OE group showed increased mRNA and protein expression levels of SIVA-1 and P53, and decreased mRNA and protein expression levels of MDM2 (P<0.05); there was no statistically significant difference in mRNA and protein expression levels of SIVA-1, P53, and MDM2 between the NC and CON groups (P>0.05).

Conclusion Elevating SIVA-1 expression in gastric cancer drug-resistant cells MKN-45/DDP can increase DDP sensitivity and inhibit their proliferative activity, with the mechanism being associated with SIVA-1-mediated upregulation of P53 gene expression and downregulation of MDM2 gene expression.

Full Text

The Effect of SIVA-1 Gene Overexpression on Cisplatin Resistance in Human Gastric Cancer Drug-Resistant Cell Lines

SHI Zhengyi[1,2], **HUANG Yuliang**[2], **LEI Yuanrui**[3], **HUANG Haibin**[2], **YANG Jie**[4], **XIE Dongyi**[5], **KONG Fanbiao**[2], ***WANG Xiaotong***[3]

[1] Guangxi Medical University, Nanning 530021, China
[2] Department of Colorectal and Anal Surgery, People' s Hospital of Guangxi Zhuang Autonomous Region/Institute of Minimally Invasive Technology and Applications, Guangxi Academy of Medical Sciences, Nanning 530021, China
[3] Departments of Gastrointestinal, Hernia and Enterofistula Surgery, People' s Hospital of Guangxi Zhuang Autonomous Region/Institute of Minimally Invasive Technology and Applications, Guangxi Academy of Medical Sciences, Nanning 530021, China
[4] Health Management Center, People' s Hospital of Guangxi Zhuang Autonomous Region/Institute of Health Management, Guangxi Academy of Medical Sciences, Nanning 530021, China
[5] Departments of Hepatobiliary, Pancreatic and Spleen Surgery, People' s Hospital of Guangxi Zhuang Autonomous Region/Institute of Minimally

Invasive Technology and Applications, Guangxi Academy of Medical Sciences, Nanning 530021, China

Corresponding authors: WANG Xiaotong, Associate Chief Physician; E-mail: 008.wxt@163.com; KONG Fanbiao, Associate Chief Physician; E-mail: kfb.32@163.com

Abstract

Background At present, there is a paucity of in-depth research on the specific role of the apoptosis-inducing factor SIVA-1 in gastric cancer drug resistance. The extent to which it modulates cellular drug sensitivity and proliferation activity by regulating the P53/E3 ubiquitin ligase (MDM2) pathway remains inconclusive. Elucidating the mechanism by which SIVA-1 regulates gastric cancer drug-resistant cells through the P53/MDM2 signaling pathway could provide new theoretical foundations and potential therapeutic targets for reversing chemotherapy resistance in gastric cancer.

Objective To investigate the effects of SIVA-1 overexpression on P53 and MDM2 expression levels, examine its impact on drug sensitivity and cellular viability in the human gastric cancer drug-resistant cell line MKN-45/DDP, and explore the underlying mechanisms.

Methods From September 2023 to March 2025, lentiviral vectors overexpressing SIVA-1 were constructed and transfected into human gastric cancer cells MKN-45/DDP. Cells were divided into three groups: the SIVA-1 overexpression group (OE group), negative control group (NC group), and blank control group (CON group). The OE group was transfected with SIVA-1 overexpressing lentiviral particles, the NC group with control lentiviral particles, and the CON group consisted of untreated MKN-45/DDP cells. Cells from each group were collected, and CCK-8 assay was used to assess cell viability and determine IC50 changes for cisplatin (DDP), doxorubicin (ADM), and fluorouracil (5-FU). Bioinformatics analysis was performed using the public STRING 12.0 and TIMER 2.0 databases. RT-qPCR and Western blotting were conducted to measure mRNA and protein expression levels of SIVA-1, P53, and MDM2.

Results Sequencing confirmed successful construction of the SIVA-1 overexpression recombinant vector with a lentiviral particle titer of 5×10^8 TU/mL. CCK-8 assay results demonstrated that compared with NC and CON groups, the OE group exhibited reduced cell viability at 24 h, 48 h, 72 h, 96 h, and 120 h, along with a decreased IC50 for DDP ($P < 0.05$). Bioinformatics analysis revealed elevated expression of SIVA-1, P53, and MDM2 in pan-cancer tissues compared with normal tissues ($P < 0.05$), with P53 and MDM2 expression significantly increased in gastric cancer tissues ($P < 0.05$). Protein interaction analysis showed interactions between SIVA-1 and P53, as well as between P53 and MDM2. Correlation analysis revealed that in gastric cancer cells, SIVA-1 ex-

pression negatively correlated with MDM2 expression ($r = -0.114$, $P = 0.020$), while P53 expression positively correlated with MDM2 expression ($r = 0.293$, $P < 0.001$); no correlation was found between SIVA-1 and P53 expression ($r = 0.095$, $P = 0.054$). RT-qPCR and Western blotting showed that compared with NC and CON groups, the OE group had increased mRNA and protein expression of SIVA-1 and P53, while MDM2 mRNA and protein expression were decreased ($P < 0.05$). No significant differences were observed between NC and CON groups for SIVA-1, P53, and MDM2 expression ($P > 0.05$).

Conclusion Enhancing SIVA-1 expression in gastric cancer drug-resistant cells MKN-45/DDP increases DDP sensitivity and inhibits proliferative activity. This mechanism is associated with SIVA-1-mediated upregulation of P53 gene expression and downregulation of MDM2 gene expression.

Keywords Gastric cancer; SIVA-1; P53; MDM2; Drug resistance; Bioinformatics; Lentiviral vector

Introduction

Gastric cancer is a common malignant tumor of the digestive system. According to 2020 global cancer statistics, both incidence and mortality rates ranked among the top five worldwide, with an estimated 968,784 new cases and 660,175 deaths globally. Analysis of 2024 cancer statistics projected 26,890 new gastric cancer patients and 10,880 related deaths in the United States. While the popularization of painless electronic gastroscopy and increased public health awareness have gradually improved 5-year relative survival rates, gastric cancer in China often presents with insidious onset and mild clinical symptoms during development, frequently leading to late-stage diagnosis when curative surgical opportunities have been lost. For such patients, chemotherapy remains a primary treatment modality. However, cancer cells often develop differential drug sensitivity or even drug resistance during chemotherapy, resulting in suboptimal treatment efficacy and severely impacting patient survival rates. Therefore, research aimed at reversing chemotherapy resistance and improving treatment outcomes holds significant clinical importance.

Apoptosis-inducing factor SIVA-1 is a pro-apoptotic protein that can be activated by the P53 tumor suppressor protein. Studies have shown that SIVA-1 expression correlates with apoptosis induction in various tumor cells, suggesting that SIVA-1 overexpression may negatively regulate certain cancer cells and enhance drug sensitivity in multidrug-resistant (MDR) cells. Research has demonstrated that silencing SIVA-1 expression enhances drug sensitivity in fluorouracil-resistant human colorectal cancer cells (HCT-8/5-FU), while also affecting apoptosis resistance mediated by the BCL2/BCL-XL signaling pathway, thereby enhancing resistance in cisplatin-resistant human gastric cancer cells (SGC-7901/DDP). However, whether SIVA-1 overexpression exerts similar effects across different gastric cancer drug-resistant cell populations remains

unclear. This study constructed an SIVA-1 overexpression vector and transfected it into the human gastric cancer drug-resistant cell line MKN-45/DDP to observe changes in drug sensitivity and proliferative activity, and to further elucidate the role and mechanism of SIVA-1 in gastric cancer chemoresistance.

Materials and Methods

1.2 Experimental Materials The human gastric cancer drug-resistant cell line MKN-45/DDP was induced and preserved in our laboratory. Reagents included GV358 lentiviral plasmid vector, pHelper 1.0 plasmid, pHelper 2.0 plasmid, 293T cells, AgeI/AgeI restriction enzymes (Shanghai Genechem Co., Ltd.), cisplatin (DDP, Shandong Qilu Pharmaceutical), doxorubicin (ADM, Shenzhen Wanle Pharmaceutical), fluorouracil (5-FU, Tianjin Jinyao Pharmaceutical), cell lysis buffer for immunoblotting and immunoprecipitation (Beyotime Biotechnology, Cat# P0013), phenylmethylsulfonyl fluoride (PMSF, Beyotime Biotechnology, Cat# ST506), SIVA-1 antibody (Cell Signaling Technology, #12532S), P53 antibody (Cell Signaling Technology, #9282S), MDM2 antibody (Cell Signaling Technology, #86934S), GAPDH antibody (Cell Signaling Technology, #5174), IRDye 800CW goat anti-rabbit secondary antibody (EarthOx Life Sciences), reverse transcription kit (Thermo Scientific, Cat# 4368813), amplification kit (Takara), TRIzol reagent kit (Thermo Scientific, Cat# 15596026), and polyvinylidene fluoride (PVDF) transfer membrane (Thermo Scientific, Cat# 88520). PCR primers were designed and synthesized by Takara, and sequencing analysis was performed by Shanghai Genechem.

1.3 Experimental Equipment Equipment included PCR thermal cycler (Applied Biosystems, Model 2720 Thermal Cycler), sequencer (Meiji Bio, Model ABI3730), high-speed centrifuge (Thermo Scientific, Model Legend Micro 17), real-time fluorescence quantitative PCR (RT-qPCR) instrument (Suzhou Yari Biotechnology, Model MA-6000), refrigerated centrifuge (Thermo Scientific, Model Micro 17R), electrophoresis apparatus (Thermo Scientific, Model PSC350MB), semi-dry transfer system (Thermo Scientific, Model PB0012), gel imaging system (Thermo Scientific, Model iBright CL750), metal bath (Qun' an Scientific Instrument, Model MBD100), and vortex mixer (Qun' an Scientific Instrument, Model VM-500pro).

1.4 Experimental Methods

1.4.1 Construction of SIVA-1 Overexpression Lentiviral Vector The linearized GV358 lentiviral plasmid vector was obtained by digestion with AgeI/AgeI restriction endonucleases. A chemically synthesized plasmid containing the SIVA-1 gene was similarly digested with AgeI/AgeI to obtain the SIVA-1 gene fragment. The GV358 vector and SIVA-1 gene fragment were recombined to achieve in vitro circularization. The recombinant product was directly transformed, and single colonies were selected as PCR amplification templates for identification. Primer sequences are shown in Table 1 .

Table 1 PCR Primer Sequences

Gene	Accession No.	Primer Sequence (5' -3')
SIVA-1	NM_{006427}	Forward: 5' -GGGTCAATATGTAATTTTCAGTG-3' Reverse: 5' -CCTTATAGTCCTTATCATCGTC-3'

1.4.2 Lentiviral Packaging and Titer Determination Positive clones were inoculated in appropriate medium and cultured overnight at 37°C. Plasmids were extracted using a plasmid extraction kit to obtain high-purity plasmids for downstream viral packaging. 293T cells in logarithmic growth phase were seeded in 10 cm culture dishes at a density of 333,333 cells/mL and cultured at 37°C with 5% CO_2. When cell density reached 70-80%, transfection was performed. The successfully constructed SIVA-1 overexpression recombinant plasmid vector, pHelper 1.0 plasmid, and pHelper 2.0 plasmid were mixed and added to 293T cell culture dishes for transfection, followed by continued culture for 48-72 h. Based on cell status, supernatant was collected and centrifuged at 4°C, 4,000 g for 10 min. The supernatant was filtered through a 0.45 m filter, then ultracentrifuged at 4°C, 4,000 g for 2 h. The supernatant was discarded, and viral pellets were resuspended in PBS solution at low temperature, aliquoted, and stored at -80°C. The viral solution was diluted in 10× concentration gradients and used to transfect logarithmic-phase 293T cells for 4 days. Green fluorescent protein (GFP) expression in each well was observed under a fluorescence microscope, and viral titer was calculated using the formula: Viral titer (TU/mL) = number of fluorescent cells / volume of original viral stock.

1.4.3 Lentiviral Transfection of Human Gastric Cancer Drug-Resistant Cells and Grouping Human gastric cancer drug-resistant cells MKN-45/DDP were cultured in RPMI1640 medium containing 10% fetal bovine serum. Logarithmic-phase cells were harvested and resuspended in 10% fetal bovine serum medium. 3.5×10^5 cells were seeded in 6-well plates. When cell confluence reached 35-55%, transfection was performed according to the manufacturer' s instructions. MKN-45/DDP cells were infected with SIVA-1 overexpression lentiviral particles or control lentiviral particles, and stable cell lines were selected. Cells with stable SIVA-1 overexpression were designated as the SIVA-1 overexpression group (OE group), cells infected with control lentiviral particles as the negative control group (NC group), and untreated cells as the blank control group (CON group).

1.4.4 CCK-8 Assay for Cell Viability Logarithmic-phase cells from OE, NC, and CON groups were harvested and adjusted to a concentration of 3×10^4 cells/mL. Cells were seeded in 96-well plates at 100 L/well with three replicate wells per group per plate and cultured in a 37°C, 5% CO_2 incubator. At 0,

24, 48, 72, and 96 h, 10 L of CCK-8 reagent was added to each well and incubated for 2 h in the incubator. Absorbance (A) at 450 nm was measured using a microplate reader. Cell viability was calculated using the formula: Cell viability = (An - Aempty) / (A0 - Aempty) × 100%, where An represents the mean absorbance of experimental groups, A0 represents the mean absorbance of wells without CCK-8 treatment, and Aempty represents the mean absorbance of blank control wells containing only complete medium.

1.4.5 CCK-8 Assay for Drug Resistance Cells from each group were routinely digested and adjusted to 1.5×10^4 cells/mL, then seeded in 96-well plates at 100 L/well with five replicate wells per group. After 24 h of culture at 37°C with 5% CO_2, five concentration gradients were established for DDP, ADM, and 5-FU: DDP at 0.25, 0.5, 1.0, 2.0, and 5.0 g/mL; ADM at 0.05, 0.1, 0.2, 0.5, and 1.0 g/mL; and 5-FU at 1.0, 2.0, 5.0, 10.0, and 20.0 g/mL. Drugs were added to corresponding wells. After 48 h of incubation, 10 L of CCK-8 reagent was added per well, followed by 2 h incubation. Absorbance at 450 nm was measured, and cell survival rate at each drug concentration was calculated as: Cell survival rate = (An - Aempty) / (A0 - Aempty) × 100%, where An represents the mean absorbance of experimental groups, A0 represents the mean absorbance of drug-untreated wells, and Aempty represents the mean absorbance of blank control wells. IC50 values (drug concentration causing 50% cell survival) were then calculated.

1.4.6 Protein Interaction Analysis, Pan-Cancer Differential Gene Analysis, and Correlation Analysis of Protein Expression The TIMER 2.0 online tool (http://timer.cistrome.org/) was used to analyze differential expression of SIVA-1, P53, and MDM2 between normal and pan-cancer tissues. The STRING 12.0 database (https://cn.string-db.org/) was used to search for SIVA-1, P53, and MDM2 with a minimum required interaction score of 0.40, using published literature as evidence for protein-protein interactions. Interaction scores were calculated and protein interaction network diagrams for SIVA-1, P53, and MDM2 were generated. TIMER 2.0 was also used for gene expression correlation analysis, with Spearman correlation analysis evaluating relationships among SIVA-1, P53, and MDM2.

1.4.7 RT-qPCR Detection of SIVA-1, P53, and MDM2 mRNA Expression Total RNA from OE, NC, and CON groups was extracted using TRIzol method, and RNA concentration and purity were measured using a nucleic acid detector. Reverse transcription was performed according to the TaKaRa reverse transcription kit instructions to obtain cDNA. Each group had four replicate wells. RT-qPCR was performed using a Roche RT-qPCR instrument. PCR conditions were: pre-denaturation at 95°C for 30 s (1 cycle); denaturation at 95°C for 3-10 s (40 cycles); annealing and extension at 60°C for 10-30 s (40 cycles). GAPDH was used as an internal reference for relative quantification, and the $2^{-\Delta\Delta C_t}$ method was used to calculate relative mRNA expression levels

of SIVA-1, P53, and MDM2.

1.4.8 Western Blotting Detection of SIVA-1, P53, and MDM2 Protein Levels Total protein from each group was extracted according to the protein extraction kit instructions, and protein concentration was determined using BCA assay. GAPDH antibody was used as an internal reference standard for SIVA-1, P53, and MDM2 protein levels. Twenty micrograms of protein were separated by 10% SDS-PAGE, transferred to PVDF membrane, blocked with 5% skim milk for 1 h, then incubated with SIVA-1, P53, and MDM2 antibodies (1:500) and GAPDH antibody (1:2000) overnight at 4°C on a shaker. The membrane was washed three times with TBST, incubated with goat anti-rabbit secondary antibody (IRDye 800) dilution (1:1000) at room temperature for 1 h, washed three times with TBST again, and scanned using an Odyssey 3.0 instrument (LI-COR).

1.5 Statistical Methods Data were analyzed using R 4.2.3 and SPSS 23.0 software. Normally distributed measurement data were expressed as mean ± standard deviation ($\bar{x} \pm s$). Comparisons among multiple groups were performed using one-way ANOVA. Pearson correlation analysis was used to explore correlations among SIVA-1, MDM2, and P53 protein expression levels. $P < 0.05$ was considered statistically significant.

Results

2.1 Construction of SIVA-1 Overexpression Recombinant Plasmid The human SIVA-1 target gene fragment was obtained, and sequencing results are shown in Figure 1 [Figure 1: see original paper]A. After digesting the plasmid containing the target gene fragment with AgeI/AgeI and ligating it into the linearized expression vector, sequencing results are shown in Figure 1B. Single colonies were used as PCR amplification templates, and positive clones were identified by DNA sequencing. Results demonstrated correct insertion of the SIVA-1 overexpression oligonucleotide sequence into the GV358 lentiviral plasmid vector, confirming successful construction of the SIVA-1 overexpression recombinant plasmid (Figure 1C).

2.2 Lentiviral Vector Packaging and Titer Determination The SIVA-1 overexpression recombinant plasmid vector, pHelper 1.0 plasmid, and pHelper 2.0 plasmid were co-transfected into 293T cells. After 48 h, cell supernatant was collected, purified, and concentrated to harvest lentiviral vectors. The titer of recombinant lentiviral particles was determined to be 5×10^8 TU/mL, meeting requirements for subsequent experiments. Fluorescence microscopy showed that both OE and NC groups achieved fluorescence rates above 85%, indicating successful transfection (Figure 2 [Figure 2: see original paper]). Cells were

transferred to culture flasks for further selection with puromycin and continued culture for subsequent experiments.

2.3 Comparison of Cell Viability Among Three Groups CCK-8 assay results showed no significant difference in cell viability among the three groups at 0 h ($P > 0.05$). However, significant differences were observed at 24 h, 48 h, 72 h, 96 h, and 120 h ($P < 0.05$). The OE group exhibited lower cell viability compared with NC and CON groups ($P < 0.05$), while no significant difference was found between NC and CON groups ($P > 0.05$) (Table 2).

Table 2 Comparison of Cell Viability Changes Among Groups After Lentiviral Transfection ($\bar{x} \pm s$)

Group	0 h	24 h	48 h	72 h	96 h	120 h
OE	0.49$±0.02|0.58±0.01^a|0.85±0.02^a|0.87±0.02^a|0.91±0.01^a|0.46±0.02^a||NC|0.51±0.01|0.67±0.03|0					
P-value	>0.05	<0.001	<0.001	<0.001	<0.001	<0.05

Note: indicates $P < 0.05$ compared with NC and CON groups. CON = blank control group, NC = negative control group, OE = SIVA-1 overexpression group.

2.4 Comparison of Drug Resistance to DDP, ADM, and 5-FU Among Three Groups CCK-8 assay was used to detect IC50 values after drug treatment. Results showed significant differences in IC50 for DDP among the three groups ($P < 0.05$), but no significant differences for ADM and 5-FU ($P > 0.05$). Under DDP treatment, the OE group showed lower IC50 values compared with NC and CON groups ($P < 0.05$) (Table 3).

Table 3 Comparison of IC50 Values Among Three Groups After Treatment with DDP, ADM, and 5-FU ($\bar{x} \pm s$)

Group	DDP IC50 (g/mL)	ADM IC50 (g/mL)	5-FU IC50 (g/mL)
OE	0.74$±0.12^a|0.14±0.03|3.73±1.08||NC|1.21±0.18|0.15±0.03|3.92±0.96||CON|1.60±0.25|0.17±0.03|		
P-value	<0.05	>0.05	>0.05

Note: indicates $P < 0.05$ compared with NC and CON groups.

2.5 Expression Levels and Correlation Analysis of SIVA-1, P53, and MDM2 in Gastric Cancer vs. Normal Cells Differential gene expression analysis revealed that SIVA-1, P53, and MDM2 showed significantly higher expression in pan-cancer tissues compared with normal tissues ($P < 0.05$). In gastric cancer tissues specifically, SIVA-1 expression did not significantly differ from normal tissues ($P > 0.05$), while P53 and MDM2 expression levels were

significantly elevated ($P < 0.05$) (Figure 3 [Figure 3: see original paper]). Protein interaction analysis demonstrated interactions between SIVA-1 and P53, as well as between P53 and MDM2 (Figure 4 [Figure 4: see original paper]). Gene expression correlation analysis showed that in gastric cancer cells, SIVA-1 expression negatively correlated with MDM2 expression ($r = -0.114$, $P = 0.020$), P53 expression positively correlated with MDM2 expression ($r = 0.293$, $P < 0.001$), while no correlation existed between SIVA-1 and P53 expression ($r = 0.095$, $P = 0.054$).

2.6 mRNA Expression of SIVA-1, P53, and MDM2 in Three Groups
RT-PCR results showed significant differences in SIVA-1, P53, and MDM2 mRNA expression levels among the three groups ($P < 0.05$). The OE group exhibited higher SIVA-1 and P53 mRNA expression and lower MDM2 mRNA expression compared with NC and CON groups ($P < 0.05$) (Table 4).

Table 4 Comparison of SIVA-1, P53, and MDM2 mRNA Expression Levels Among Three Groups

Group	SIVA-1	P53	MDM2
OE	5.29$±0.33^a	2.07±0.28^a	0.24±0.01^a
P-value	<0.001	<0.001	<0.001

Note: indicates $P < 0.05$ compared with NC and CON groups.

2.7 Protein Expression of SIVA-1, P53, and MDM2 in Three Groups
Western blotting results showed significant differences in relative protein expression levels of SIVA-1, P53, and MDM2 among the three groups ($P < 0.05$). The OE group had higher relative protein expression of SIVA-1 and P53 and lower MDM2 protein expression compared with NC and CON groups ($P < 0.05$) (Figure 5 [Figure 5: see original paper], Table 5).

Table 5 Comparison of SIVA-1, P53, and MDM2 Protein Expression Levels Among Three Groups

Group	SIVA-1	P53	MDM2
OE	1.70$±0.23^a	1.60±0.10^a	0.59±0.06^a
P-value	<0.001	<0.001	<0.001

Note: indicates $P < 0.05$ compared with NC and CON groups.

Discussion

As a pro-apoptotic gene, SIVA-1 plays different roles in various tumor development processes. Experimental evidence shows that SIVA-1 expression is significantly reduced in breast cancer cells compared with normal breast tissue, and similarly decreased in cervical cancer cells and gastric cancer tissues. This downregulation in tumor tissues may be attributed to reduced SIVA-1 expression in gastric chief cells, leading to prolonged cell survival, chronic accumulation of genetic damage, and increased likelihood of neoplastic transformation. In vitro experiments have shown that *Helicobacter pylori*-infected gastric cancer cells activate the PI3K/Akt signaling pathway, which phosphorylates XIAP, reduces SIVA-1 protein stability, promotes SIVA-1 ubiquitination, and ultimately inhibits apoptosis. Bioinformatics analysis revealed increased SIVA-1 expression in gastric cancer tissues compared with normal tissues, providing a theoretical basis for successful SIVA-1 overexpression in cisplatin-resistant gastric cancer cells.

Protein interaction and gene expression correlation analyses demonstrated interactions among SIVA-1, P53, and MDM2. Theoretical results indicated that SIVA-1 expression positively correlates with P53 and negatively correlates with MDM2 in gastric cancer tissues. These correlations were validated by qPCR and Western blotting experiments. Initially identified as a CD27-binding protein, SIVA-1 plays important roles in regulating apoptosis pathways and influencing drug resistance, indirectly affecting tumor cell fate and providing avenues for improving chemotherapeutic resistance. Recent studies show that the SIVA-1 promoter region can bind P53 protein in alveolar epithelial cells, leading to Caspase-3-dependent mitochondrial apoptosis. Notably, SIVA-1 exerts different effects across cell lines; silencing SIVA-1 enhances resistance in cisplatin-resistant gastric cancer cells, possibly due to dominant signaling pathways or other key apoptotic regulators. Thus, an SIVA-1/P53 pathway may indirectly alter tumor cell drug resistance by affecting apoptosis.

As one of the most important tumor suppressor genes, P53 maintains normal cellular function and tissue stability through apoptosis, cell cycle regulation, differentiation, and DNA repair. Early studies demonstrated that P53 mediates Reprimo (RPRM) to inhibit CDC2 activity and prevent CDC2/cyclin B1 translocation to the cytoplasm, arresting cells in G2/M phase and inducing gastric cancer cell apoptosis. Recent research shows that P53 upregulation, influenced by cell proliferation blocker BOP1, arrests gastric cancer cells in G0/G1 phase and induces apoptosis. Additionally, decreased P53 expression in gastric cancer cells causes loss of Caspase-9-dependent apoptosis function, leading to DDP resistance. Therefore, P53 indirectly reverses gastric cancer cell drug resistance by affecting cell cycle transition or apoptosis. As a protein that can structurally interact with P53, SIVA-1 may influence gastric cancer cell drug sensitivity through P53 mediation. Recent studies propose SIVA-1 as a predictive biomarker for cisplatin efficacy in gastric cancer. This study demonstrates that SIVA-1 overexpression in MKN-45/DDP cells increases P53 expression, reduces

cell viability, and enhances DDP sensitivity, further elucidating the mechanism of the SIVA-1/P53 signaling pathway in reversing chemotherapeutic resistance.

MDM2, the most important negative regulator of P53, can bind to P53 to form protein complexes that inhibit P53' s tumor suppressor function. Inhibition of MDM2 in human retinal pigment epithelial cells upregulates P53 and various pro-apoptotic proteins including SIVA-1, inducing apoptosis and inhibiting proliferation. The P53/MDM2 signaling pathway plays a critical role in cancer development. P53 positively regulates MDM2 expression as a transcription factor, while MDM2 negatively feedback-regulates P53 protein stability as an E3 ubiquitin ligase. Inhibition of MDM2 in human myeloma cell lines enhances anti-myeloma drug sensitivity and induces apoptosis. Previous studies showed that SIVA-1 overexpression in MKN-45/DDP cells reduced cell migration and invasion while reversing DDP resistance. The current study demonstrated significantly reduced IC50 values, successfully reversing DDP resistance in MKN-45/DDP cells. Results suggest that SIVA-1 overexpression, as an upstream regulator, upregulates P53 and downregulates MDM2 expression, thereby reversing DDP resistance in gastric cancer cells.

Cisplatin (DDP) is a first-line chemotherapy drug that effectively destroys DNA function and inhibits mitosis by inducing cross-linking. Studies show DDP induces endogenous thromboxane A2 (TXA2), which acts on TXA2 receptors (TP) and, under the influence of direct SIVA-1/TP interaction, promotes apoptosis in HeLa cells. DDP further increases intracellular SIVA-1 levels, confirming SIVA-1' s importance in DDP-induced apoptosis. Gastric cancer cisplatin resistance is associated with apoptosis, and P53 ubiquitination and degradation lead to resistance and cancer progression. Upregulated P53 expression induces apoptosis and enhances DDP sensitivity in gastric cancer resistant cells. As an E3 ubiquitin ligase, MDM2 inhibition significantly enhances chemotherapy-induced gastric cancer cell death. Previous studies showed SIVA-1 expression correlates positively with DDP sensitivity in gastric cancer resistant cells. Notably, SIVA-1' s molecular structure is necessary for enhancing cisplatin-induced apoptosis. The SIVA-1/MDM2 pathway also combats gastric cancer drug resistance by downregulating BCL2 and BCL-XL expression. BCL2 family proteins are key signaling nodes associated with evading drug resistance; BCL2 maintains mitochondrial outer membrane integrity through its BH domain, preventing release of pro-apoptotic proteins like cytochrome C and Caspase-9/Caspase-3 to avoid apoptosis.

In summary, this study demonstrates that increasing SIVA-1 expression in the human gastric cancer drug-resistant cell line MKN-45/DDP enhances DDP sensitivity and inhibits proliferative activity. The mechanism involves SIVA-1-mediated upregulation of P53 and downregulation of MDM2 expression, revealing the molecular mechanism by which SIVA-1 regulates gastric cancer chemosensitivity through the P53/MDM2 signaling pathway and providing a theoretical basis and potential biomarker for clinically targeting SIVA-1 to overcome chemoresistance. This study has limitations, as it only explored SIVA-1

overexpression in relation to P53 and MDM2 in a single cell line. The roles of other apoptosis-related genes or signaling pathways in tumor drug resistance and their potential involvement in SIVA-1-mediated regulation of gastric cancer resistance require further experimental validation.

Author Contributions: SHI Zhengyi was responsible for manuscript writing and experimental data analysis; HUANG Yuliang performed statistical analysis; LEI Yuanrui and HUANG Haibin conducted experimental operations; YANG Jie and XIE Dongyi collected data; KONG Fanbiao and WANG Xiaotong provided critical review and funding support.

Conflict of Interest: The authors declare no conflict of interest.

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