FOXQ1 Suppressing Apoptosis in Colorectal Cancer Cells by P53 Deacetylation

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ABSTRACT

Objective: To investigate the impact of Forkhead box Q1 (FOXQ1) expression on platinum-based chemoresistance in colorectal cancer (CRC) cells, and to examine the regulatory function of *FOXQ1* on *Sirtuin 1* (*SIRT1*) protein expression and P53 protein deacetylation levels during the DNA damage response (DDR).

Study Design: An experimental study.

Place and Duration of the Study: Second Department of Gastrointestinal Surgery, General Surgery Centre, Qingdao Municipal Hospital, Affiliated to Qingdao Medical College, Qingdao University, Qingdao, China, from October 2023 to December 2024.

Methodology: Gene expression levels of *FOXQ1* in CRC cells and SW620 cells treated with cisplatin (CDDP) were evaluated using quantitative real-time polymerase chain reaction (qRT-PCR). The t-test was used to compare the gene expression levels between the two groups. Three gene-edited SW620 cell models were established: *FOXQ1* overexpression (oe-FOXQ1), *FOXQ1* RNA interference (sh-FOXQ1), and a negative control (NC). CCK-8 assays measured CDDP's half-inhibitory concentration (IC50), while flow cytometry, calcein-AM/PI staining, and colony formation evaluated cell apoptosis and survival. Western blot analysed SIRT1 and acetylated p53, and the SIRT1 inhibitor (S)-Selisistat explored *FOXQ1*-related pathways.

Results: *FOXQ1* was highly expressed in CRC. CDDP treatment further increased its expression in SW620 cells. *FOXQ1* overexpression enhanced CDDP resistance, elevated SIRT1 levels, and promoted P53 deacetylation. (S)-Selisistat reversed P53 deacetylation and reduced CDDP resistance in oe-FOXQ1 cells.

Conclusion: *FOXQ1* promotes chemoresistance in CRC by upregulating SIRT1 expression and promoting P53 deacetylation, thereby inhibiting apoptosis triggered by DDR.

Key Words: *Colorectal cancer, Forkhead Box Q1, Sirtuin 1, P53 Acetylation, Apoptosis*.

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INTRODUCTION

Colorectal cancer (CRC) ranks among the three most prevalent cancers globally,[1] with rising incidence and mortality rates in recent years. The optimal treatment for CRC is complete surgical removal of the tumour; however, for patients with metastatic CRC, chemotherapy—particularly with platinum-based agents—remains the primary treatment option. This method has been shown to significantly enhance patient survival rates. Nonetheless, a significant obstacle in chemotherapy is the emergence of medicine resistance, which greatly impacts treatment effectiveness.[2]

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Most chemotherapy agents induce cell death by disrupting DNA replication and mitosis, harnessing the apoptotic mechanisms of the DNA damage response (DDR) pathway to target cancerous cells.

The gene with tumour suppressor function, *P53*, has a pivotal part to play in the DDR pathway. It stimulates cell apoptosis through mediating the halt of the cell cycle and initiating the downstream apoptotic pathways. The activity of *P53* is precisely controlled by many mechanisms. Among these mechanisms, acetylation is a key modification process for activating its function. *Sirtuin 1* (*SIRT1*), as a major regulator of *P53*, is capable of deacetylating the activated P53, thus reducing its activity.

FOXQ1 (*Forkhead Box Q1*) is a transcription factor that shows a high expression level in different kinds of malignant tumours. Researches have indicated that *FOXQ1* can elevate the expression degree of SIRT1.[3] Based on this, the hypothesis is proposed that *FOXQ1* may contribute to the deacetylation of P53 by increasing the expression of SIRT1, and consequently, inhibit the apoptosis induced by DDR.

This study focuses on exploring the mechanisms by which CRC cells evade apoptosis induced by chemotherapy medicines and analyses the role of the FOXQ1-SIRT1-P53 axis in the DDR-induced apoptotic pathway.

METHODOLOGY

The experiment took place from October 2023 to December 2024 at the Second Department of Gastrointestinal Surgery, General Surgery Centre, Qingdao Municipal Hospital, Affiliated to Qingdao Medical College, Qingdao University, Qingdao, China, ethical approval number: 2023-LW192. This work utilised cell lines CCD-18Co (normal colon cells), DLD-1, HT29, SW480, SW620, HCT116, and RKO, all of which were confirmed using STR analysis. The Chinese Academy of Sciences' Cell Bank supplied all of the colorectal cancer cell lines utilised in this study. The culture medium for each cell line comprised 89% base medium, 10% foetal bovine serum (FBS), and 1% penicillin-streptomycin solution. SW480 and SW620 cells were cultivated in DMEM with elevated glucose concentration. HT29 and HCT116 cells were cultured in McCoy 5A base medium; CCD-18Co and RKO cells were cultured in 89% MEM media (containing non-essential amino acids); and DLD-1 cells were cultured in RPMI 1640 base medium. The settings for the cell culture incubator were 37°C and 5% CO_2.

The clinical information and gene expression data utilised in this investigation were obtained from the database maintained by the TCGA (http://portal.gdc.cancer.gov/). Batch effects were eliminated from the normalised data, which was subsequently correlated with the relevant clinical samples. The inclusion criteria are colorectal cancer samples from this database and relevant healthy control samples. The criteria for sample inclusion are that the gene expression data integrity of the key gene regions reaches more than 90%, the samples should contain at least key information such as age, gender, and tumour stage, the RNA integrity number (RIN) is greater than 7, and the sequencing depth is reasonable. The exclusion criteria include duplicate samples, samples with extreme abnormal values in gene expression data, or samples with incorrect and contradictory clinical data, as well as samples with insufficient key information in clinical outcomes or gene expression profiles. The TCGA dataset consisted of 480 cancer samples and 41 healthy control samples. Statistical analyses were carried out employing R Software version 3.6.3. Differentially expressed genes (also called DEG were identified using the Limma package (version 3.40.2) in the R programming environment. To rectify false positives, the adjusted p-values within the TCGA context were applied. The selection criteria for DEGs were set as log2(FC) >2 and p lower than 0.05. A volcano plot was generated by utilising the ggplot2 package in the R software environment.

The *FOXQ1* overexpression and short hairpin RNA (shRNA) lentiviruses used in this experiment were designed and constructed by Shanghai GeneChem Co. The *FOXQ1* overexpression gene was loaded into the GV341 vector, and the functional module sequence of the vector was Ubi-MCS-3FLAG-SV40-puromycin. The primer sequences the targeted gene fragment:

F: 5'-CCAACTTTGTGCCAACCGGTCGCCACCATGAAGTTGGAG-GTGTTCGTC-3';

R: 5'-GTCAATGCCAACTCTGAGCTTGGCTAGGAGCGTCTCCAC-CGGGTAC-3'.

The *FOXQ1* interference RNA sequence was loaded into the GV112 vector, with the main functional module sequence being hU6-MCS-CMV-Puromycin. The designed target interference sequence was 5'-GTGCACGCAGCAAGCCATATA-3'. The GV lentiviral vector serves as the core of the viral genome, containing essential elements of HIV, including the 5'LTR and 3'LTR, viral packaging signals, and auxiliary elements. The company's manual for the recombinant lentiviral vector, together with the HiTransG P infection reagent, was utilised to infect the SW620 cell line with the aforementioned lentiviral vectors. There were three groups. In *FOXQ1* overexpression group (oe-FOXQ1), the cells were subjected to infection with the *FOXQ1* overexpression lentiviral vector. In *FOXQ1* interference group (sh-FOXQ1), the cells were infected with the *FOXQ1* shRNA lentiviral vector. In the control group (NC), the cells were infected with an empty virus vector as a reference.

To ascertain the IC50 value of cisplatin (CDDP; MCE, China), various concentrations of cisplatin solutions were initially produced to attain final concentrations of 0, 2, 4, 8, 16, 32, and 64 μmol/L in the growth medium. Each concentration was evaluated in five replicates. Incorporate the previously produced solution into the growing media of the cells in each group. After returning the cells to the incubator and allowing them to incubate for 24 hours, the cells were digested and resuspended in the entire media. Subsequently, the cells were enumerated, and 100 μL of the entire medium containing 10,000 cells was allocated onto a 96-well plate. Then, 10 μL of the CCK-8 reagent (MCE, China) was introduced into each well and the plate was gently swirled to achieve uniform distribution of the solution. Subsequently, the 96-well plate was returned to the cell incubator and incubated for one hour. Ultimately, the absorbance was assessed at 450 nm (OD450) using a microplate reader (Thermo Fisher Scientific, USA) to determine the cell viability and compute the IC50 value of cisplatin from this data. The vitality of the cells was assessed utilising the subsequent formula: Cell viability = [(OD value of experimental group - OD value of blank group) / (OD value of control group - OD value of blank group)] multiplied by 100%. The control group comprised culture media and CCK-8 solution devoid of medicines or cells.

The colony formation test was employed to assess the impact of *FOXQ1* on cellular proliferation. Following digestion and resuspension, cells were injected at a density of 3 × 10^4 cells/ml into a six-well plate and grown in a 37°C, 5% CO_2 atmosphere. One week later, the initial medium was eliminated, and the leftover medium was carefully rinsed away with PBS. Cells were fixed in methanol for 30 minutes and subsequently stained with a 0.2% crystal violet solution. Colonies consisting of around 30 cells were subsequently enumerated.

Total RNA was isolated from each cell group utilising an RNA extraction kit in accordance with the manufacturer's guidelines

(Vazyme, China). Following extraction, RNA samples were reconstituted in sterile, DEPC water. The RNA concentration was determined using a micro-volume spectrophotometre (Thermo Fisher Scientific, USA). The cDNA synthesis was performed using a reverse transcription kit (Takara, Japan), followed by qRT-PCR conducted with a qRT-PCR kit (Takara, Japan) on a real-time quantitative PCR device. The primers utilised for qRT-PCR were designed as follows:

FOXQ1: F: 5'-CGCGGACTTTGCACTTTGAA-3' R: 5'-CCTGAGAAG TTTAAATACT-3'

GAPDH: F: 5'-GCATGGCCTTCCGTGTCCCC-3' R: 5'-CAATGCCAG CCCCAGCGTCA-3'

GAPDH functioned as the standard gene, and the relative expression of *FOXQ1* in cells was assessed utilising the 2^-△△Ct method.

Target protein expression levels in each cellular group were assessed by Western blotting. RIPA buffer (Thermo Fisher Scientific, USA) supplemented with a protease inhibitor which was used to lyse cells on ice for 20 minutes. The supernatant was then extracted from the lysates by centrifuging them for 15 minutes at 4°C at 12,000 rpm. Protein concentration was measured using the BCA Protein Assay Kit (Elabscience, China). After the concentration of cell lysate in each group was standardised using RIPA buffer, protein samples were mixed with a quarter volume of 5× sample buffer (Elabscience, China) and heated in a heat block for five minutes at 95°C. Following separation using 10% SDS-PAGE, the protein samples were transferred to a polyvinylidene fluoride (PVDF) membrane.

A rapid protein-free blocking solution (Elabscience, China) was used to block the PVDF membrane for 15 minutes post-transfer. The sample was then treated at 4°C overnight with primary antibodies. The antibodies used, together with their respective concentrations, were FOXQ1 (1:1000) (Proteintech, China), SIRT1 (1:100) (Abcam, UK), P53 (1:1000) (Cell Signaling Technology, USA), AC-P53 (1:100) (Cell Signaling Technology, USA), and GAPDH (1:6000) (Elabscience, China). The membrane was thoroughly cleansed with TBST post-treatment and then incubated for one hour at ambient temperature with a goat anti-rabbit secondary antibody. Subsequently, TBST was used to wash the membrane. A chemiluminescence imaging apparatus (Vilber, France) was then used to visualise the protein after its fabrication using ECL chemiluminescent reagent (Elabscience, China). GAPDH served as the loading control for assessing target protein expression levels with ImageJ software.

Using the Calcein AM/PI Kit (Veyotime, China), cell toxicity was evaluated. At a density of 1.0 × 10^4 cells per well, cells from the control group (NC), the oe-FOXQ1 group, and the sh-FOXQ1 group were injected onto 96-well plates. The cells were exposed to a low concentration of cisplatin (CDDP) for 12 hours after they had enough time to adhere to the wells. The culture medium was aspirated after the treatment, and the buffer solution was used carefully to wash the wells. Each well was then filled with 100 μL of the Calcein AM/PI working solution. Red fluorescence is produced by PI (excitation wavelength/emission wavelength = 535/617 nm), whereas green fluorescence is produced by Calcein AM (excitation wavelength/emission wavelength = 494/517 nm). An inverted fluorescent microscope (Nikon, Japan) was used to see and count the cells after a 30-minute incubation period at 37°C in a dark atmosphere. The percentage of dead cells in each group was then determined.

Using flow cytometry, cell apoptosis was examined. After centrifugation and digestion, 5 × 10^5 cells were resuspended in 100 μL of 1× Annexin V binding buffer. The resuspended cell solution was combined with 2.5 μL of Annexin V-APC and 2.5 μL of 7-ADD reagent, gently stirred, and left to stand at room temperature for 15 minutes. After adding 400 μL of 1× Annexin V binding buffer, the mixture was assessed using a flow cytometre (CytoFLEX, USA). APC was detected using the R660 channel, whereas Percp-Cy5.5 was detected using the B690 channel.

Software called GraphPad Prism (GraphPad 9.5.1, Inc., USA) was used to perform statistical analyses. For each group, the data were shown as the mean ± standard deviation (SD) derived from three consecutive replicates. The t-test was used for the comparison between the two groups, and the one-way analysis of variance (ANOVA) method was adopted for the comparison among multiple groups. Immunohistochemistry was analysed using chi-square test. A p-value of 0.05 or lower was considered statistically significant.

RESULTS

In order to investigate the function of FOXQ1 in the pathophysiology of CRC, data were gathered from The Cancer Genome Atlas (TCGA) database, which included 480 cancer cases and 50 healthy control samples. The gene expression profiles of malignant and adjacent normal tissues from colorectal cancer patients were analysed to discover differentially-expressed genes (DEGs). Marked disparities in mRNA expression were observed, as shown by a heatmap (Figure 1A) and a volcano plot (Figure 1B). The identification of DEGs was predicated on a specified fold-change, a p-value of less than 0.05 and a q-value of less than 0.05.

RT - qPCR was used to determine the expression of *FOXQ1* in six distinct colorectal cancer cell lines (Figure 1C). The results indicated that the DLD-1, HT29, SW480, and SW620 cell lines had significantly elevated FOXQ1 expression compared to normal colon cells (CCD-18Co), but the HCT116 and RKO cell lines showed reduced expression ($p < 0.001$). The SW620 cell line, originating from the metastatic lymph nodes of colorectal cancer, was selected for further investigation.

SW620 cells were subjected to low concentrations of CDDP, and RNA was extracted for qRT - PCR analysis to determine if the chemotherapeutic treatment induces alterations in *FOXQ1* expression. Results showed that *FOXQ1* expression in SW620 cells significantly increased after CDDP treatment ($p = 0.0002$, Figure 1D).

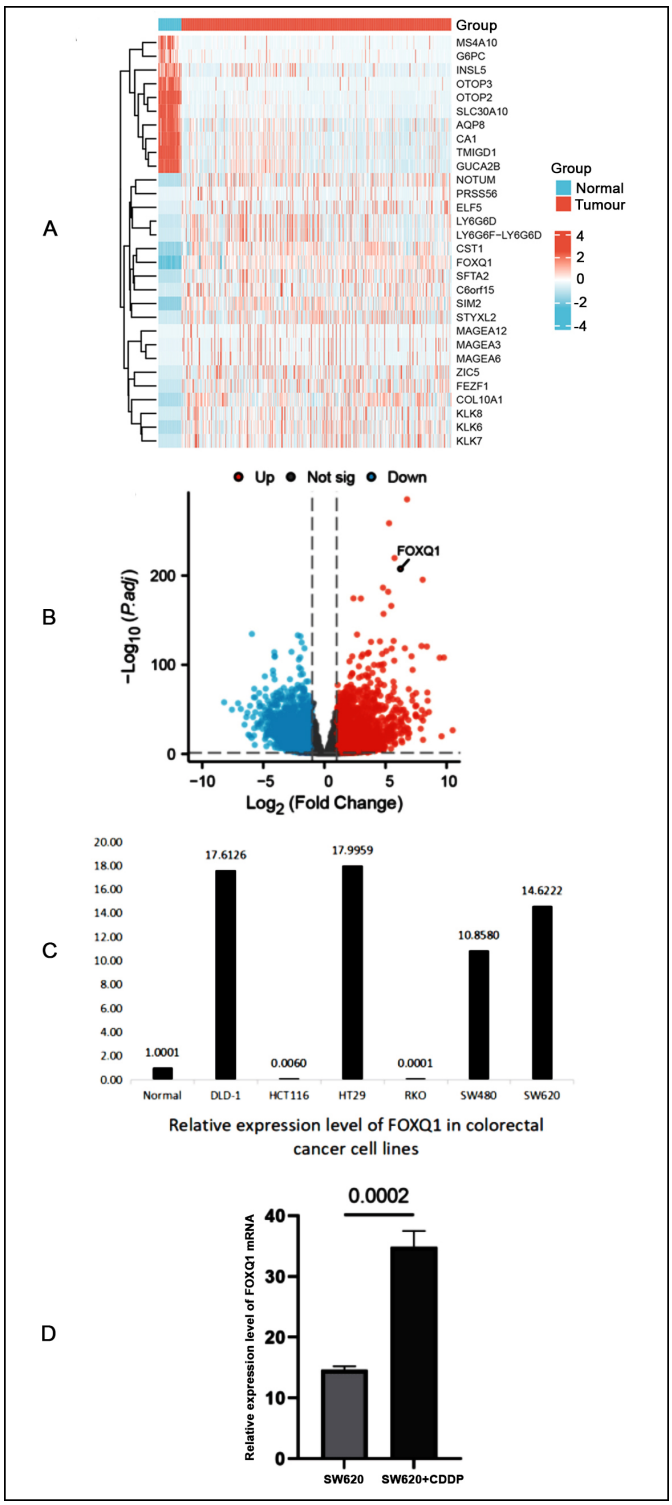

Figure 1: CDDP stimulation may elevate *FOXQ1* expression in SW620 cells, whereas *FOXQ1* is significantly expressed in CRC cells. (A) Heatmap illustrating the variations in *FOXQ1* expression levels between CRC tissues and nearby normal tissues. (B) A volcano plot showing that CRC tissues had higher levels of *FOXQ1* than the surrounding tissues. Normal: A sample of normal colon tissue; tumour: A sample of CRC tissue. Genes that are down-regulated are shown by blue, and those that are elevated by red. (C) The proportional expression levels of *FOXQ1* mRNA in six CRC cell lines. (One-way analysis of variance (ANOVA) was used (p <0.001). (D) Modifications in FOXQ1 mRNA expression in SW620 cells following a 24-hour treatment with 15 µmol/L CDDP. The paired samples t-test was used for analysis (p = 0.0002).

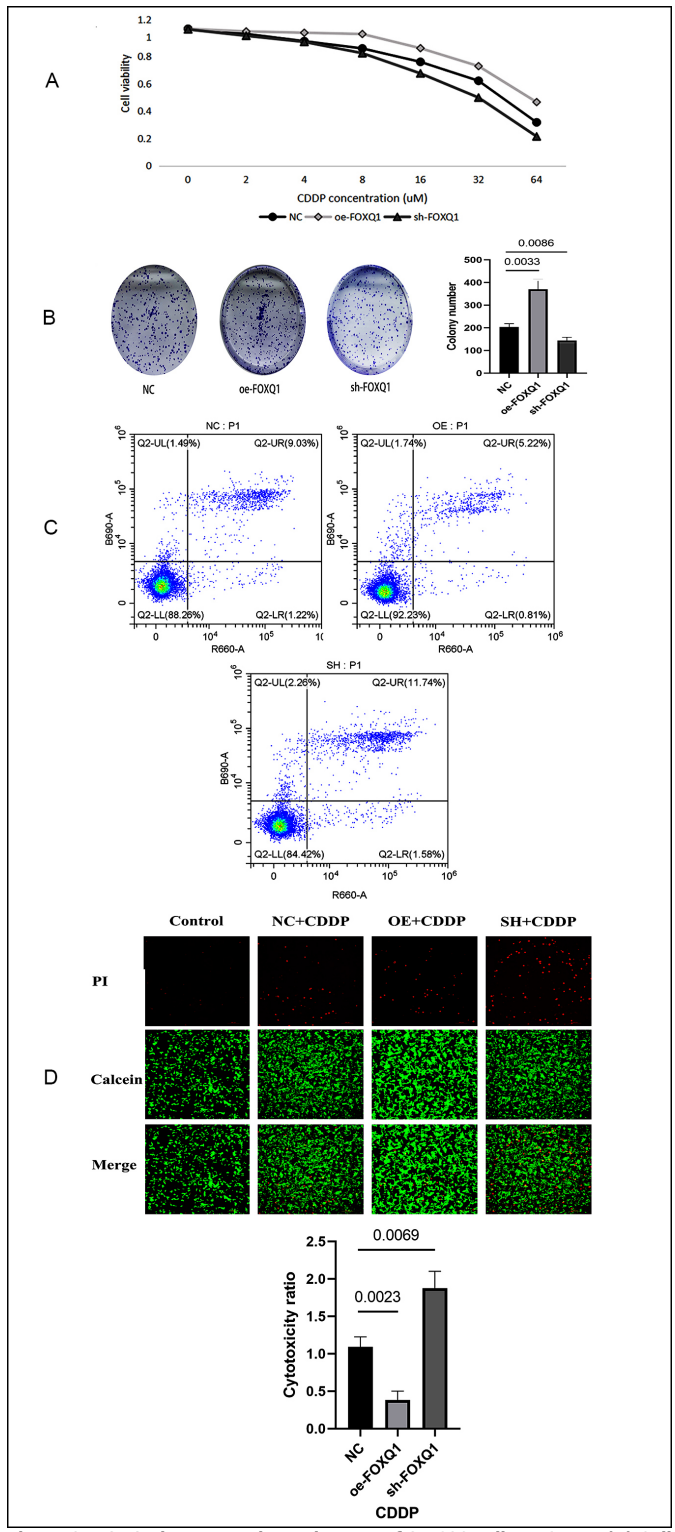

Figure 2: *FOXQ1* increases the resistance of SW620 cells to CDDP. (A) Cell viability in the NC, oe-FOXQ1, and sh-FOXQ1 groups was assessed using the CCK-8 assay after a 24-hour treatment with a range of CDDP doses. (B) The NC, oe-FOXQ1, and sh-FOXQ1 groups' colony counts were determined using the colony formation assay. (The paired samples t-test was used for analysis). (C) To investigate apoptosis in cells after a 24-hour treatment with 15 µmol/L CDDP, flow-cytometry analysis was performed. Live cells are denoted by Q2-LL, whereas late apoptotic cells are denoted by Q2-UR. (D) Fluorescent labelling of cells was conducted to ascertain the percentage of deceased cells following treatment (15 µmol/L, 24-hours). Red indicates necrotic cells, while green signifies viable cells (×200), in comparison to the NC group (p = 0.0002).

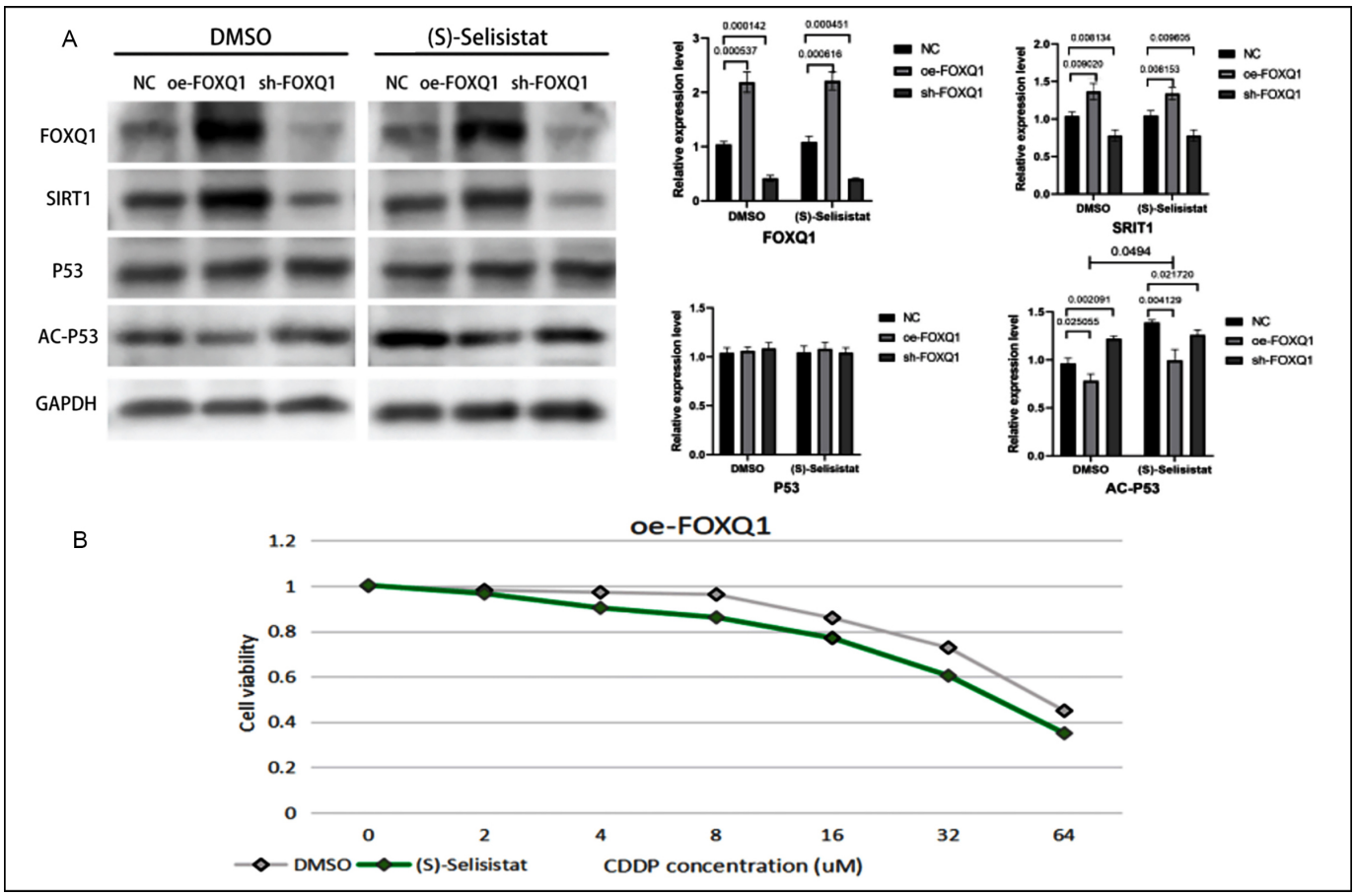

Figure 3: *FOXQ1* promotes SIRT1 expression, and *FOXQ1* overexpression facilitates P53 deacetylation; *SIRT1* pathway inhibition suppresses this process. (A) The protein expression of *FOXQ1*, SIRT1, P53, acetylated P53 (AC-P53), and GAPDH in the NC, oe-*FOXQ1*, and sh-FOXQ1 groups treated with DMSO or the SIRT1 pathway inhibitor (S)-Selisistat (38 nmol/L, DMSO). GAPDH was utilised for normalisation. The paired samples t-test was used for analysis. (B) Cell viability in the oe-FOXQ1 group treated with a gradient of CDDP concentrations for 24 hours, either with or without (S)-Selisistat.

The authors used the CCK-8 assay to determine the IC50 values of CDDP across different experimental groups in order to investigate the influence of *FOXQ1* on the resistance of CRC cells to platinum-based chemotherapy regimens. The findings indicated that the impact of CDDP on the viability of SW620 cells intensified with increasing concentrations of CDDP. After subjecting the cells to a 24 hours treatment with a range of CDDP concentrations, the cell viability decreased. The 24 hours IC50 value of CDDP for SW620 cells was determined to be 43.7 µmol/L. In comparison to the NC group, the IC50 value in the oe-FOXQ1 group increased to 58.3 µmol/L. Conversely, in the sh-FOXQ1 group, the IC50 value decreased to 36.4 µmol/L (Figure 2A).

To further explore the role that *FOXQ1* plays in CRC cells, 15 µM (IC20) was chosen as the CDDP concentration for the subsequent experiments. The overexpression of *FOXQ1* significantly enhanced the number of colonies formed, while the knockdown of *FOXQ1* had the opposite impact ($p < 0.01$, Figure 2B).

The impact of *FOXQ1* on CRC cell apoptosis was shown by the flow cytometry data. After being exposed to a low dose of CDDP for 24 hours, the oe-FOXQ1 group had considerably less late apoptotic cells than the NC group ($p = 0.008$), whereas the sh-FOXQ1 group had more late apoptotic cells ($p = 0.016$). The quantity of early apoptotic cells in each group did not differ significantly ($p > 0.05$, Figure 2C).

Fluorescence staining results demonstrated that *FOXQ1* diminished CDDP's cytotoxic effects on CRC cells. The proportion of dead cells in the oe-FOXQ1 group was significantly lower than in the NC group ($p = 0.002$) under identical treatment conditions (15 µmol/L, 24 hours), but the proportion of dead cells in the sh-FOXQ1 group was higher ($p = 0.007$, Figure 2D).

The acetylation state of P53 in the NC, oe-FOXQ1, and sh-FOXQ1 groups was evaluated using Western blot analysis to investigate the mechanism by which *FOXQ1* supports CRC cell survival after platinum-induced DNA damage. Furthermore, the expression levels of SIRT1, an essential protein that promotes P53 deacetylation, were evaluated (Figure 3A). The results demonstrate that the P53 acetylation level in the oe-FOXQ1 group, marked by increased FOXQ1 expression, was considerably lower than that of the control group ($p = 0.025$). In contrast, the sh-FOXQ1 group's acetylation level of P53, shown by a reduction in FOXQ1 expression, was

significantly higher than that of the control group ($p = 0.002$). The expression level of the SIRT1 protein was significantly increased in the oe-FOXQ1 group relative to the control group ($p = 0.009$), whereas it was reduced in the sh-FOXQ1 group ($p = 0.008$).

The expression levels of *FOXQ1,* SIRT1, and acetylated P53 were measured after treatment with the SIRT1 pathway inhibitor (S)-Selisistat (38 nmol/L, DMSO) to clarify the relationship between the SIRT1 pathway and P53 protein acetylation. The results demonstrated that the expression levels of SIRT1 and FOXQ1 were constant after (S)-Selisistat treatment. (S)-Selisistat increased the sensitivity of oe-FOXQ1 group cells to CDDP (Figure 3B) and restored the acetylation of P53 in the oe-FOXQ1 group ($p = 0.0494$).

The results suggested that the increased resistance of CRC cells to chemotherapy due to *FOXQ1* overexpression may be mitigated by (S)-Selisistat, and that the *FOXQ1*-mediated inhibition of CRC cell apoptosis requires the involvement of the SIRT1 pathway.

DISCUSSION

Because of its role in controlling gene transcription, *FOXQ1*, a transcription factor belonging to the FOX family,[4] has garnered a lot of attention lately.[5-7] According to earlier research, *FOXQ1* is crucial for the development and spread of many cancer types, including colorectal cancer. Its expression is markedly upregulated and strongly associated with the prognosis, invasiveness, and level of malignancy of tumours.[8–10] Throughout this work, it was noted that *FOXQ1* was significantly expressed in the SW620 colorectal cancer cell line, particularly subsequent to chemotherapy treatment, where the expression of *FOXQ1* increased significantly. This observation indicated that the expression of *FOXQ1* may be associated with the cellular reaction to stress induced by chemotherapy.

Cell lines with *FOXQ1* overexpression have a much higher CDDP IC50 than the control group, suggesting that *FOXQ1* overexpression increases cellular resistance to CDDP. Conversely, in the cell lines where *FOXQ1* was knocked down, the IC_{50} of CDDP was notably lower, which implies that silencing *FOXQ1* heightens the cells' sensitivity to chemotherapy. Flowcytometry and morphological staining further confirmed that overexpression of *FOXQ1* significantly suppressed apoptosis induced by CDDP, while silencing *FOXQ1* accelerated the apoptotic process. Cytotoxicity assays also demonstrated that inhibiting FOXQ1 enhances the cytotoxic effects of CDDP on CRC cells. These results suggest that FOXQ1 regulate chemotherapy-induced apoptosis, thereby affecting CRC cell sensitivity to chemotherapy agents.

The aim was also to dissect the underlying mechanisms through which *FOXQ1* suppresses chemotherapy-induced apoptosis. CDDP causes DNA damage by establishing intra- and inter-strand cross-links, thereby initiating the DNA damage response (DDR). When DNA repair is unsuccessful, the cell activates the apoptotic programme to maintain genomic stability. This process is mainly driven by P53-mediated intrinsic apoptotic signalling.[11-13] P53 suppresses tumourigenesis through multiple mechanisms, such as inducing cell-cycle arrest, promoting DNA repair processes, and activating the apoptotic pathway.[14-17] Acetylation of P53 is a critical activation event.[18] After DNA damage occurs, human P53 undergoes acetylation at Lys382 (AC-P53).[19] Research has shown that SIRT1, an NAD^+-dependent deacetylase, can inhibit the activity of P53 by removing its acetylation modifications, which influences the cells' ability to initiate the apoptotic process after DNA damage.[20] Additionally, the high expression of *SIRT1* in various cancers is often associated with enhanced resistance to chemotherapy,[21] suggesting that *SIRT1* could play a crucial regulatory function in assisting cancer cells in avoiding death.

Interestingly, previous studies have proposed that *FOXQ1* may bind to the promoter region of *SIRT1*, leading to upregulation of its transcription. Based on these findings, the hypothesis suggests that *FOXQ1* suppresses chemotherapy-induced apoptosis *via* the SIRT1-P53 pathway.

To verify this proposed hypothesis, the influence of *FOXQ1* expression on *SIRT1* and the downstream P53 signalling cascade was first investigated. The experimental results indicated that in the SW620 cell line with first overexpressed *FOXQ1*, *SIRT1* expression was dramatically upregulated, but the AC-P53 levels were significantly downregulated.

Conversely, with the *FOXQ1* knockdown cell line, *SIRT1* expression decreased, whereas AC-P53 levels were reinstated. These data suggest that FOXQ1 may enhance SIRT1 expression and decrease the acetylation of P53.

The SIRT1-specific inhibitor (S)-Selisistat was used on the cells to further confirm the upstream and downstream relationships of this pathway.[22] The results indicated that (S)-Selisistat, although not affecting *SIRT1* or *FOXQ1* expression, reinstated the decreased AC-P53 expression levels caused by *FOXQ1* overexpression. This further substantiates the idea that *FOXQ1* enhances *SIRT1* production, which indirectly reduces P53 acetylation levels, hence inhibiting CRC cell death post-chemotherapy.

Although this study has provided evidence for the role of the *FOXQ1-SIRT1*-P53 signalling pathway in suppressing chemotherapy-induced apoptosis, there are still some limitations. This study mainly focused on the SW620 cell line, which may not be able to fully represent all colorectal cancer cell lines or other types of cancer. Different cell lines may have unique genetic backgrounds and characteristics, which may affect the research results. Additionally, the experiments were mainly conducted *in vitro*, which means that the

research findings may not be directly applicable to *in vivo* situations. In the body, cancer cells exist in a complex microenvironment, involving interactions with other cell types (such as immune cells and stromal cells), blood vessels, and components of the extracellular matrix. These *in vivo* factors may regulate the impact of *FOXQ1* on the chemotherapy response and the regulation of apoptosis. Moreover, there may be other mechanisms that have not been investigated. *FOXQ1* may interact with other proteins or signalling pathways to regulate chemoresistance and apoptosis of colorectal cancer cells.

CONCLUSION

Based on these findings, a model is proposed: When CRC cells are exposed to chemotherapy medicines and undergo DNA damage response (DDR), the expression level of *FOXQ1* increases significantly. *FOXQ1*, then binds to the promoter of SIRT1, activating its transcription. Consequently, SIRT1 interacts with AC-P53, removing the acetylation modifications on P53 and inhibiting the P53 signalling pathway. This inhibition prevents the initiation of apoptosis, which is normally triggered by DDR. This process provides a molecular foundation for CRC cell survival under chemotherapy-induced stress and promotes the development of chemotherapy resistance.

ETHICAL APPROVAL:
This study obtained approval from the Ethics Committee of the Qingdao Municipal Hospital, Affiliated to Qingdao Medical College, Qingdao University, Qingdao, China.

COMPETING INTEREST:
The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:
GY, HC, XM: Completed the experimental section and wrote the manuscript.
FW, GM: Carried out the statistical analysis.
HQ: Conceived the study.
All authors approved the final version of the manuscript to be published.

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