

MEF2C Promotes Intrinsic Apoptosis and Restores Cisplatin Sensitivity in A2780 Ovarian Cancer Cells

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Abstract

Ovarian cancer is still the most deadly cancer among gynecologic tumors, mainly due to the frequent development of resistance to cisplatin that greatly reduces treatment effectiveness. Gaining deeper insight into the molecular basis of this resistance is vital for creating improved therapies. Our RNA sequencing analysis identified MEF2C as a differentially expressed gene associated with the acquisition of cisplatin resistance. We confirmed this observation using RT-qPCR, which revealed significant differences in MEF2C transcript levels between the cisplatin-sensitive A2780 line and its resistant counterpart, A2780cp. Forcing MEF2C expression in the resistant A2780cp cells lowered the cisplatin IC₅₀ value, boosted caspase activation, raised amounts of the pro-apoptotic nuclear receptor NR4A1 (also known as Nur77), and strongly promoted programmed cell death. These outcomes were verified through MTT viability assays, western blotting, and Annexin V/PI flow cytometry. Taken together, the data show that MEF2C levels are reduced in cisplatin-resistant ovarian cancer cells, and that restoring its expression can resensitize the cells to the drug by engaging the intrinsic apoptosis machinery. Clinically, uncovering the drivers of chemoresistance and therapeutically targeting molecules such as MEF2C holds promise for reversing resistance phenotypes, boosting the performance of standard chemotherapy, and ultimately improving patient responses in ovarian cancer management.

Keywords: Ovarian cancer, Chemoresistance, Cisplatin, Apoptosis, MEF2C

Introduction

Ovarian cancer stands out as the gynecological malignancy with the highest mortality rate. Roughly 60% of affected women do not survive, and the overall five-year survival remains low at 20–40%, varying with stage at diagnosis and how well the tumor responds to treatment [1-3]. Recognized risk contributors include older age, infertility, excess body weight, and genetic

factors such as variants in BRCA1 or BRCA2 [4, 5]. High-grade serous carcinoma represents the dominant histological subtype [6]. The conventional treatment strategy combines aggressive surgical removal of tumor tissue with platinum-containing regimens, most often cisplatin paired with paclitaxel [7]. Although many patients show good initial responses, the disease frequently recurs [8, 9], largely due to acquired chemoresistance [10].

The processes behind chemoresistance are intricate and still only partially mapped. Important mechanisms include impaired drug uptake, active drug efflux, intracellular detoxification, enhanced DNA repair systems, enrichment of ovarian cancer stem cells, rewiring of signaling networks, and inhibition of apoptosis, allowing malignant cells to survive chemotherapy [11-14]. Transcription factors are

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Received: 14 July 2025; Accepted: 22 November 2025

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How to cite this article: Nilsson P, Johansson E, Andersson L. MEF2C Promotes Intrinsic Apoptosis and Restores Cisplatin Sensitivity in A2780 Ovarian Cancer Cells. Arch Int J Cancer Allied Sci. 2025;5(2):223-33. <https://doi.org/10.51847/LWswEsbLgO>

increasingly recognized as central players in these phenomena. For example, Wu *et al.* [15] found that lowering ZNF11 expression increases cisplatin resistance in ovarian cancer cells. The FoxO transcription factor family has similarly been implicated in controlling sensitivity to cisplatin [16].

The MEF2 family of transcription factors (MEF2A, MEF2B, MEF2C, and MEF2D) can act as either tumor promoters or suppressors depending on the tissue and cellular setting [17]. In liver cancer, MEF2C can fuel malignant behavior through VEGF signaling while simultaneously restraining tumor growth by blocking Wnt/ β -catenin signaling. Higher MEF2C reduces β -catenin entry into the nucleus, and elevated β -catenin in turn limits MEF2C nuclear accumulation [18]. MEF2C and MEF2D also drive apoptosis in activated T cells, at least partly by increasing expression of NR4A1/Nur77 orphan nuclear receptors that are essential for thymocyte elimination. Further evidence indicates tumor-suppressor functions for MEF2C and MEF2D in rhabdomyosarcoma [19].

Even so, the role of MEF2 proteins in cancer chemoresistance remains poorly studied. Research in mice has shown that platinum drugs can suppress MEF2C levels, suggesting a link to cellular responses during platinum treatment [20]. Overexpression of MEF2D has been linked to cisplatin resistance in ovarian cancer [21], whereas both MEF2C and HIP3 appear reduced in oxaliplatin-resistant gastric cancer cells [22]. Transcriptomic profiling has also indicated lower MEF2C expression in paclitaxel- or cisplatin-resistant ovarian cancer cells [23]. However, a detailed understanding of how MEF2C expression or function affects cisplatin response remains limited.

In this study, we demonstrate that MEF2C downregulation is associated with cisplatin resistance in the A2780cp ovarian cancer model. Re-expressing MEF2C in these cells restores drug sensitivity and markedly elevates apoptosis. This re-sensitization likely involves functional crosstalk with the pro-apoptotic nuclear receptor Nur77, as supported by earlier literature [24, 25].

Materials and Methods

RNA-seq analysis

We analyzed three publicly available GEO datasets (GSE98230, GSE173201, and GSE176218) to compare gene expression between the parental A2780 and

resistant A2780cp ovarian cancer cells. Quality checks on raw reads were performed with FastQC (Version 0.73 + galaxy0). Adapters and poor-quality bases were trimmed using Trim Galore (Version 0.6.7 + galaxy0) and Trimmomatic (Version 0.38.0). Clean reads were aligned to the hg38 human genome assembly with HISAT2 (Version 2.1.0 + galaxy7). Gene counts were obtained using HTSeq-count (Version 0.9.1 + galaxy1) and then normalized. Differential expression testing and fold-change calculations were carried out with DESeq2 (2.11.40.7 + galaxy1) on the Galaxy platform (<https://usegalaxy.org/>). DEGs identified in each dataset were intersected using R, which consistently highlighted MEF2C as downregulated in resistant cells. Because of its transcription factor function, MEF2C was selected for follow-up experiments to explore its role in cisplatin sensitivity.

Transcription factor binding site (TFBS) analysis

MEF2C's DNA recognition motif was obtained from the JASPAR database to evaluate its potential regulatory targets. Binding site predictions were generated from this motif, and Nur77 emerged as a candidate target gene. This prompted further investigation into whether MEF2C regulates Nur77 in the A2780 cell background.

Cell culture and cisplatin treatment

A2780 and A2780cp cell lines were sourced from the Pasteur Institute in Tehran, Iran. Both lines were grown in RPMI-1640 (Gibco, USA) supplemented with 10% FBS (Gibco), penicillin (100 U/mL), and streptomycin (100 U/mL) (Sigma, UK) under standard conditions (37 °C, 5% CO₂). Cisplatin treatments were administered at concentrations tailored to each specific experimental setup.

RNA isolation and cDNA synthesis

Total cellular RNA was harvested with TRIzol™ reagent (Zaverzistazma, Iran) according to the supplier's instructions. RNA quality was assessed by agarose gel electrophoresis, and concentration/purity were measured using a NanoDrop spectrophotometer (Thermo Scientific, USA). Complementary DNA was synthesized from 1 µg total RNA using reverse transcriptase enzyme (Invitrogen, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

Quantitative PCR was performed on a StepOne™ Real-Time PCR instrument (Applied Biosystems, USA) with Real-Time PCR Master Mix (Amplicon, Denmark). Relative expression values were determined by the $2^{-\Delta\Delta C_t}$ method and normalized to the housekeeping gene GAPDH (**Table 1**). All reactions were run in three independent biological replicates.

Table 1. List of primers.

Gene name	Forward primer	Reverse primer
MEF2C.cloning	ACTGAGCTGTGCAA GTGCT	TTTTCCACACACGGCA CATAT
MEF2C.re al time	CTGGTGTAACACAT CGACCTC	GATTGCCATACCCGTT CCT
GAPDH	GCCACATCGCTCAG ACAC	GGCAACAATATCCAC TTTACCAG

Gene cloning, plasmid construction, and transient transfection

To elevate MEF2C expression, PCR was first used to amplify its complete coding sequence from cDNA derived from A2780 cells using dedicated cloning primers detailed in **Table 1**. Although MEF2C produces multiple transcript isoforms with minor variations, the primers were strategically positioned to amplify the segment spanning exons 4-12. This approach encompassed the core coding region shared among the predominant functional isoforms, while excluding untranslated regions.

Following amplification, the DNA product was purified using a GEL/PCR purification kit (Favorgen, Taiwan) before being inserted into a TA cloning vector. The insert was then released by digestion and ligated into the pcDNA3.1 mammalian expression plasmid with T4 DNA ligase (Thermo Scientific, USA) per the manufacturer's guidelines. Competent DH5- α *E. coli* cells were transformed with the ligation product. Ampicillin-resistant colonies were picked from LB-agar plates and screened by colony-PCR. Final verification of the correct MEF2C insert in the recombinant plasmid was achieved by Sanger sequencing.

Subsequently, A2780cp cells were transiently transfected with either the control empty pcDNA3.1 vector (mock control) or the constructed pcDNA3.1-MEF2C plasmid (overexpression) using Lipofectamine 3000 (Invitrogen, USA) exactly as instructed by the manufacturer. Cells were harvested 48 hours after transfection for further analysis.

MTT assay

A2780 and A2780cp cells were trypsinized, counted, and seeded at 5×10^3 cells per well into 96-well culture plates, then incubated at 37 °C under 5% CO₂ conditions. Twenty-four hours later, varying doses of cisplatin (0, 20, 35, 50, 65, and 80 μ M; Sobhan Oncology, Iran) were introduced. Viability was determined by the MTT method: each well received 10 μ L of a 5 mg/mL solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide. After 4 hours at 37 °C, the resulting formazan crystals were dissolved using 100 μ L DMSO (Sigma, UK). After 20 minutes at room temperature, absorbance was measured at 570 nm using a microplate ELISA reader (Thermo Fisher Scientific, USA). Experiments included three independent biological replicates.

In parallel experiments, A2780cp cells were transfected with either a mock control vector or an MEF2C overexpression plasmid. After 24 hours, these cells were exposed to cisplatin concentrations of 0, 35, 50, or 65 μ M. Viability was assessed using the identical MTT procedure. IC₅₀ values were computed with GraphPad Prism software (version 10.3.1).

Immunoblotting assay

Proteins were isolated from A2780cp cells that had undergone transient transfection with either mock or MEF2C-overexpression constructs. The extracted lysates were subjected to SDS-PAGE separation, transferred to PVDF membranes, and immunoblotted using primary antibodies against: MEF-2C (G-5) (sc-518152), cleaved caspase-3 p11 (H176)-R (sc-22171-R), Nur77 (C-5) (sc-365113), caspase-9 (Human Specific), β -Actin (2A3): sc-517582, and GAPDH (6C5) (sc-32233). After washes, the membranes were treated with HRP-conjugated secondary antibodies, including m-IgG κ BP-HRP (sc-516102) and mouse anti-rabbit IgG-HRP (sc-2357). Visualization of protein bands was performed with an ECL chemiluminescence detection system (Millipore, Billerica, MA, USA), and densitometric quantification was carried out using ImageJ software.

PI-Annexin V apoptosis assay

After transfection, A2780cp cells were exposed to 10 μ g/ml cisplatin or PBS vehicle. Apoptosis evaluation followed the manufacturer's protocol for the FITC-Annexin V Apoptosis Detection Kit with PI (BioLegend). Staining was performed with FITC-Annexin V and propidium iodide, after which samples were acquired on a FACS Calibur flow cytometer.

Analysis of the collected data was conducted using FlowJo software (version 10).

Visualization

Volcano plots of differentially expressed genes, bar graphs summarizing statistical outcomes, and dot plots of MTT results were generated using GraphPad Prism (version 10.3.1). Flow cytometry apoptosis dot plots were generated in FlowJo software (version 10).

Statistical analysis

Data analysis was performed using GraphPad Prism (version 10.3.1) and R (version 4.4.0). All quantitative results are reported as mean $\pm$ standard deviation (SD). Nonparametric t-tests (one- or two-sample, depending on the experiment) were used to assess statistical differences. A threshold of $P < 0.05$ was applied to determine significance throughout the study.

Results and Discussion

Identification of differentially expressed genes between cisplatin-sensitive and -resistant A2780 cells

Three separate RNA-seq datasets (GSE98230, GSE173201, GSE176218) were analyzed to identify genes differentially expressed between the cisplatin-sensitive A2780 and the cisplatin-resistant A2780cp ovarian cancer cell lines. Volcano plot representations highlighted pronounced and consistent downregulation of MEF2C transcripts in the resistant cells across all datasets (**Figure 1a**). Supporting analyses, including principal component analysis (PCA) and MA plots, verified strong separation between groups and confirmed dataset quality. Overlap analysis of the DEGs identified across the three datasets yielded 2,399 shared genes: 1,520 downregulated and 879 upregulated in A2780cp cells relative to A2780, using thresholds of $|\log_2FC| > 1$ and adjusted P-value < 0.05 (**Figures 1b and 1c**). MEF2C showed significant suppression in the resistant line across all three datasets (**Figure 1d**), with a mean $\log_2$ fold change of -9.97 , underscoring its strong association with the resistant state (**Table 2**).

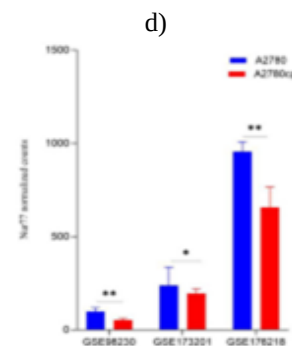
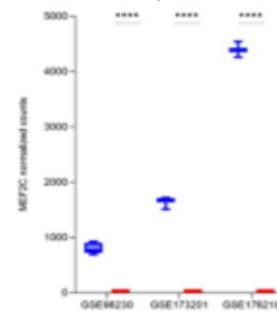
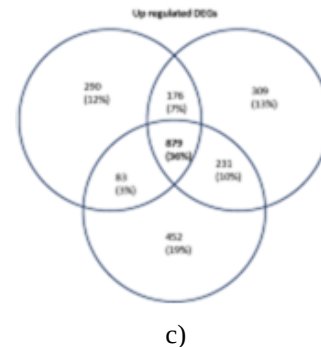
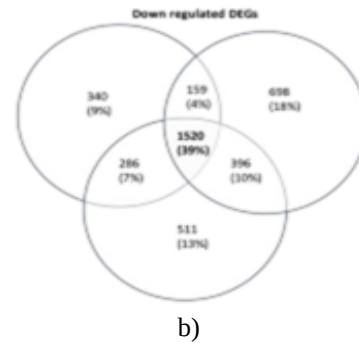
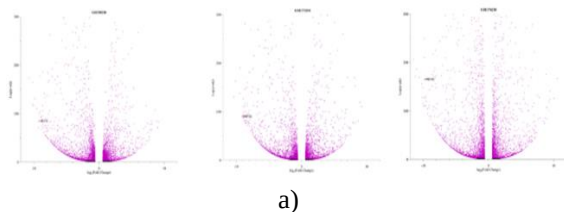


Figure 1. Differentially Expressed Genes between cisplatin-sensitive and resistant A2780 cells

(a) Volcano plots illustrate the overall distribution of differentially expressed genes across the three RNA-seq datasets. In each plot, MEF2C is prominently marked as a gene with markedly reduced expression in the resistant cells. (b-c) Venn diagrams depict the overlap among the

three DEG lists from the independent datasets, identifying 2399 genes that are commonly altered. Of these, 1520 genes show significant downregulation in cisplatin-resistant cells compared with the sensitive parental cells. In parallel, 879 genes exhibit significant upregulation in the A2780cp-resistant line relative to the A2780-sensitive line (adjusted P-value < 0.05). (d) Box plots show the expression levels of MEF2C derived from normalized HTSeq-count data across the three RNA-seq datasets. MEF2C transcript abundance is substantially lower in the cisplatin-resistant A2780cp cells than in the sensitive A2780 cells, and (e) Box plots display the expression profile of Nur77 using the same normalized HTSeq-count values from the three RNA-seq datasets. Nur77 also shows significantly lower counts in the A2780cp-resistant group than in sensitive A2780 cells (Blue bars correspond to the A2780 sensitive cell line, and red bars represent the A2780cp-resistant cell line in panels (d) and (e)).

Table 2. List of DEGs that are downregulated in A2780cp versus A2780 cells (top 10)

No	Gene name	P-adj	Log ₂ FC	Status in platinum-based drug resistance in cancer
1	<i>DLX6-AS1</i>	8.6532E-175	-11.30227422	Reported by Du <i>et al.</i> [26] and Zhao <i>et al.</i> [27]
2	<i>COL6A3</i>	6.4957E-132	-10.65462052	Reported by Park <i>et al.</i> [28]
3	<i>XIST</i>	2.2502E-121	-10.49513217	Reported by Hua <i>et al.</i> [29] and Zheng <i>et al.</i> [30]
4	<i>LIN28A</i>	8.2268E-118	-10.17279508	Reported by Zhang <i>et al.</i> [31]
5	<i>ARHGAP28</i>	1.414E-132	-9.974865732	Not reported
6	<i>BST2</i>	6.50445E-92	-9.61081939	Reported by Kuang <i>et al.</i> [32]
7	<i>MEST</i>	6.41829E-72	-9.476348237	Reported by Ruan <i>et al.</i> [33]
8	<i>MEF2C</i>	4.72118E-86	-9.413938049	Not reported
9	<i>MAB21L1</i>	1.1284E-101	-9.379412467	Not reported
10	<i>SCN9A</i>	1.69624E-95	-9.264144563	Reported by Palugulla <i>et al.</i> [34]

Data analysis suggested a relation between the MEF2C transcription factor and Nur77

Bioinformatics examination of the RNA-seq profiles obtained from GSE98230, GSE173201, and GSE176218 indicated clear suppression of both MEF2C and Nur77

transcripts in the cisplatin-resistant A2780cp ovarian cancer cells when compared with the sensitive A2780 parental line (**Figures 1d and 1e**). In addition, we searched the JASPAR open-access database of transcription factor binding profiles to assess whether MEF2C directly influences Nur77 expression. This search identified a large number of potential MEF2C recognition sequences across the human genome. Altogether, 879 candidate binding sites were detected, including one high-scoring motif (score = 113.213) situated within the promoter area of the Nur77 gene. The strength of this predicted interaction points to a likely direct regulatory relationship.

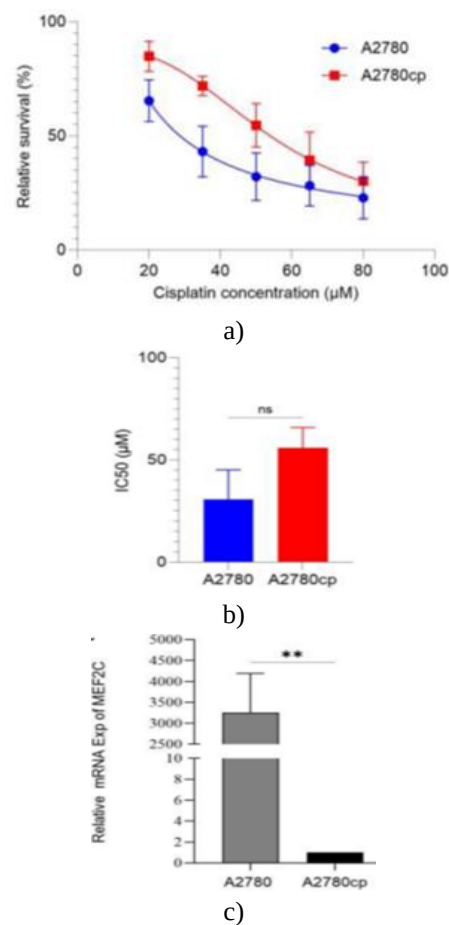


Figure 2. Differential cisplatin sensitivity and the comparison of MEF2C expression levels between A2780 and A2780cp cell lines

(a) Results from the MTT assay establish the cisplatin-sensitive character of A2780 cells alongside the resistant nature of A2780cp cells, (b) Bar chart illustrates the IC50 values obtained for both A2780 and A2780cp lines, and (c) RT-qPCR analysis highlights the variation in MEF2C

mRNA abundance between the A2780 and A2780cp cell lines (P-value < 0.05).

Experimental validation of MEF2C downregulation in cisplatin-resistant A2780cp cells

MTT testing was initially carried out to validate the contrasting cisplatin responses of the A2780 and A2780cp lines. Data indicated considerably reduced cell survival in the sensitive A2780 population compared with the resistant A2780cp cells when exposed to matching cisplatin doses (**Figure 2a**). Derived IC₅₀ figures were 30.72 μ M for A2780 cells versus 55.97 μ M for A2780cp cells, clearly demonstrating heightened resistance in the A2780cp variant (**Figure 2b**). RT-qPCR testing subsequently confirmed pronounced suppression of MEF2C mRNA in the resistant A2780cp cells, matching the patterns observed in the RNA-seq results (**Figure 2c**).

MEF2C overexpression enhances cisplatin sensitivity in A2780cp cells

The influence of MEF2C restoration on cisplatin responsiveness was examined by introducing either a MEF2C-expressing plasmid or a control empty vector into A2780cp cells. RT-qPCR analysis verified effective upregulation of MEF2C transcripts in the overexpression condition (**Figure 3a**). After transfection, cells were treated with cisplatin at 0, 35, 50, and 65 μ M, followed by viability assessment using an MTT assay. Cells with elevated MEF2C showed notably decreased viability relative to mock controls at all drug levels examined, with greater divergence appearing at the higher concentrations (**Figure 3b**). The resulting IC₅₀ value dropped from roughly 52.64 μ M in the mock-transfected group to 43.60 μ M upon MEF2C overexpression (**Figure 3c**), a change of clear biological relevance. Collectively, the results indicate that MEF2C upregulation can enhance cisplatin sensitivity and mitigate resistance in A2780cp cells.

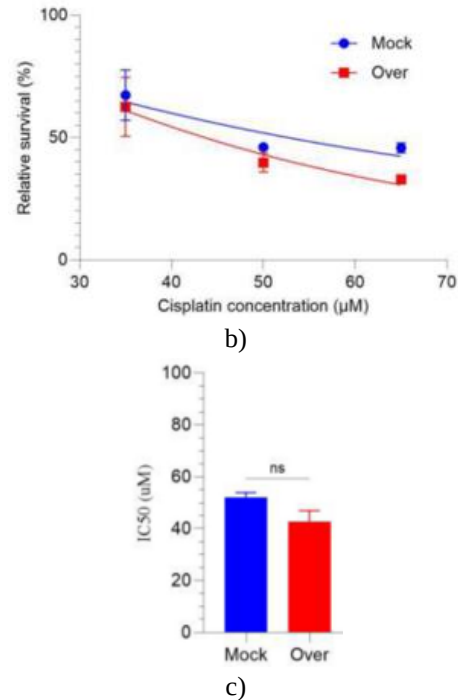
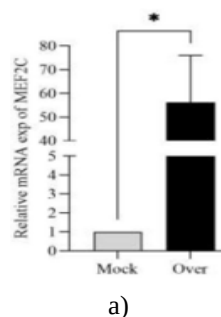


Figure 3. MEF2C overexpression effect on the IC₅₀ rate in A2780cp cells

(a) Successful elevation of MEF2C mRNA levels after transfection was verified through RT-qPCR, (b) MTT assay plots demonstrate reduced cell viability in A2780cp cells that overexpress MEF2C when exposed to cisplatin, and (c) Bar chart highlights the decrease in cisplatin IC₅₀ value resulting from MEF2C overexpression in the A2780cp line.

MEF2C overexpression induces apoptosis and increases Nur77 protein expression

Further experiments were conducted to clarify the pathway through which MEF2C restores cisplatin sensitivity in resistant A2780cp cells. Western blot assays assessed alterations in apoptosis-related proteins along with Nur77 abundance following MEF2C overexpression. Findings showed clear increases in the active forms of both cleaved caspase-3 and cleaved caspase-9, pointing to enhanced mitochondrial apoptotic signaling (**Figures 4a and 4b**). Nur77 protein levels were also substantially higher in cells with elevated MEF2C, reinforcing its contribution to apoptosis promotion in this context (**Figure 4b**).

For normalization, GAPDH was selected as the reference for MEF2C and cleaved caspase-3 quantification. At the same time, β -Actin served as a control for Nur77 and

cleaved caspase-9 to prevent band overlap issues arising from their comparable molecular sizes.

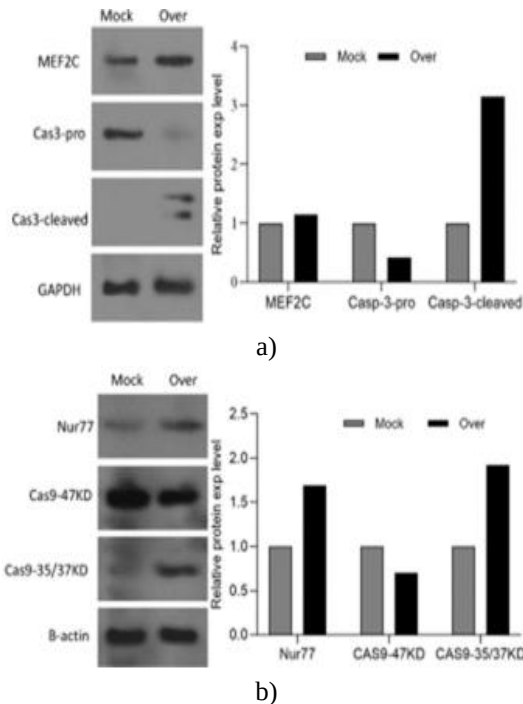


Figure 4. MEF2C overexpression effect on caspase activity and Nur77 protein level in A2780cp Cells

(a) Representative Western blot images along with densitometric quantification confirm higher MEF2C protein amounts in transfected A2780cp cells. These blots also show reduced pro-caspase-3 and elevated cleaved caspase-3 levels, indicating enhanced apoptosis induction after MEF2C overexpression. GAPDH acted as the internal loading control for this set of gels. (b) Corresponding Western blot images and quantitative analysis reveal upregulated Nur77 protein in A2780cp cells expressing additional MEF2C. The data also indicate reduced full-length 47 kD caspase-9 protein together with increased cleaved 35/37 kD caspase-9 fragments. Such changes demonstrate engagement of the intrinsic apoptosis pathway and help explain how MEF2C modulates cisplatin resistance in ovarian cancer. β -Actin served as the loading control in this panel. All grouped Western blot images were generated from multiple gels that were run simultaneously under identical conditions.

MEF2C overexpression enhances apoptosis rate in A2780cp cells

Flow cytometry analysis with Annexin V and PI double staining was utilized to examine how MEF2C overexpression affects cell death in A2780cp cells. Cells were first transfected with either a MEF2C-encoding plasmid or a control empty vector, then treated with 10 μ M cisplatin or PBS. Under PBS-only conditions, cell viability showed a small decline from 74.2% in mock controls to 67.5% in MEF2C-overexpressing cells, reflecting a limited direct apoptotic influence of MEF2C (**Figure 5a**). In the presence of cisplatin, viability decreased more dramatically, falling from 42.3 percent in the mock-transfected population to 28.8% in cells with elevated MEF2C (**Figure 5b**). These results highlight that MEF2C overexpression strongly sensitizes A2780cp cells to cisplatin-driven apoptosis.

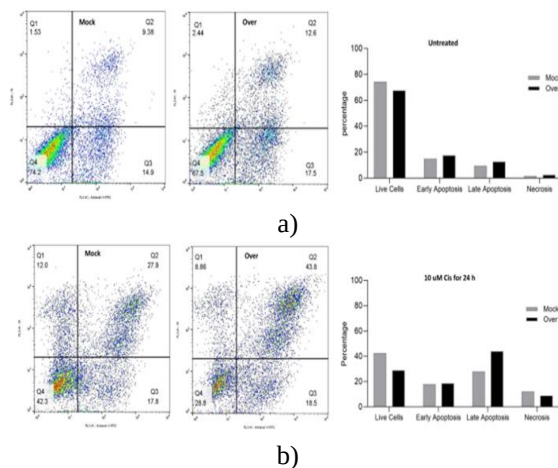


Figure 5. MEF2C overexpression enhances apoptosis rate in A2780cp cells, as validated by flow-cytometry assay

(a) A2780cp cells transfected with either an MEF2C overexpression vector or mock control were treated with 10 μ M PBS. Cell survival decreased modestly by 6–7% in the MEF2C-overexpressing group (Mock: Q1 1.53% — Q2 9.38% — Q3 14.9% — Q4 74.2%; Over: Q1 2.44% — Q2 12.6% — Q3 17.5% — Q4 67.5%), (b) A2780cp cells transfected with either an MEF2C overexpression vector or mock control were treated with 10 μ M cisplatin. Combined MEF2C overexpression and cisplatin exposure led to elevated rates of both early and late apoptosis, resulting in a 13.5% further reduction in cell survival (Mock: Q1 12% — Q2 27.9% — Q3 17.8% — Q4 42.3%; Over: Q1 8.86% — Q2 43.8% — Q3 18.5% — Q4 28.8%). (Q1, Q2, Q3, and Q4 correspond to necrosis, late apoptosis, early apoptosis, and viable cells, respectively).

Ovarian cancer continues to rank among the most deadly cancers of the female reproductive system, maintaining elevated death rates even with improvements in detection and treatment options. Platinum agents such as cisplatin form the cornerstone of initial therapy; yet acquired resistance frequently undermines treatment success and drives unfavorable prognoses [35, 36]. Elucidating the underlying molecular processes responsible for this resistance is therefore essential for designing better targeted and individualized interventions.

In the present study, MEF2C was identified as an important gene associated with cisplatin resistance in A2780 ovarian cancer cells. Transcriptomic profiling demonstrated clear downregulation of MEF2C in the resistant A2780cp subline compared with the parental sensitive cells, a result subsequently validated through RT-qPCR. These observations suggest that MEF2C contributes to determining cellular responsiveness to cisplatin.

A broad transcriptomic comparison between sensitive and resistant cells revealed an intricate network of transcription factors with varied activity. Within this network, MEF2C stood out as a notable component. This aligns with earlier work by Szenajch *et al.* [23], who observed reduced MEF2C expression in ovarian cancer cells that had developed cross-resistance to paclitaxel and cisplatin, further implicating the factor in drug responsiveness. Members of the MEF2 transcription factor family are recognized for playing dual roles in cancer, functioning as either oncogenes or tumor suppressors depending on cellular context and protein partners. For example, Li *et al.* [21] reported that MEF2D, a closely related family member, exerts oncogenic effects and contributes to cisplatin resistance in ovarian cancer cells. In contrast to MEF2D's tumor-promoting activity, our data indicate that MEF2C may function in an opposing manner, acting in a tumor-suppressive capacity by enhancing cisplatin sensitivity. Earlier research has established that MEF2C can trigger apoptosis in various immune cells, including T lymphocytes and macrophages, by regulating activation-induced cell death. This process involves upregulation of the nuclear receptor Nur77, which promotes cell death through mitochondrial routes [37]. Our computational analysis identified potential MEF2C binding motifs in the Nur77 promoter, suggesting direct regulation in the context of cisplatin resistance. Nur77 can relocate to mitochondria, where it converts Bcl-2 into a pro-apoptotic factor, thereby triggering cytochrome c release

and subsequent caspase activation [18, 19]. In agreement with this model, our Western blot experiments showed that MEF2C overexpression in A2780cp cells boosted Nur77 protein abundance and increased cleavage of both caspase-3 and caspase-9, consistent with stimulation of the intrinsic apoptotic cascade [38].

Complementary functional tests reinforced this pathway. MTT viability assays demonstrated that MEF2C overexpression significantly reduced the IC₅₀ for cisplatin, indicating improved drug sensitivity. Flow cytometry apoptosis assays similarly revealed higher proportions of apoptotic cells, especially when MEF2C overexpression was paired with cisplatin exposure. These combined results indicate that MEF2C re-sensitization operates largely through the MEF2C–Nur77 signaling axis to augment cisplatin-triggered cell death.

In summary, this study positions MEF2C as a factor capable of reversing cisplatin resistance in A2780 ovarian cancer cells. By stimulating Nur77 and activating downstream apoptotic effectors, elevated MEF2C expression restores susceptibility to cisplatin-mediated killing. The data underscore the promise of modulating the MEF2C–Nur77 pathway to combat platinum resistance in ovarian cancer (**Figure 6**).

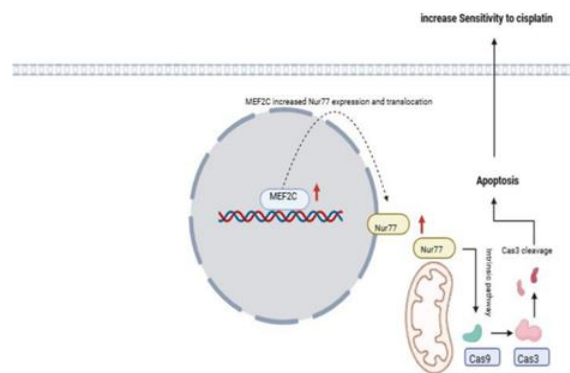


Figure 6. Graphical abstract showing the role of MEF2C in drug resistance in ovarian cancer A2780 cell lines

MEF2C overexpression in A2780cp cells elevates Nur77 levels and promotes its nuclear-to-mitochondrial translocation. Once in the mitochondria, Nur77 triggers the intrinsic apoptotic cascade, leading to caspase-9 and -3 cleavage, which ultimately drives cell apoptosis. This cascade restores cisplatin sensitivity in the resistant cells. Although the current findings are encouraging, several limitations should be acknowledged. Additional work is required to fully map the downstream targets regulated

by MEF2C and to determine whether the observed mechanisms function in living organisms. Techniques such as ChIP-seq promoter analysis would strengthen confidence in the direct regulatory connection between MEF2C and Nur77 in cisplatin-resistant A2780 cells. Future research employing animal models and patient-derived clinical specimens from individuals with cisplatin-resistant ovarian cancer will be important to confirm the clinical applicability of the MEF2C–Nur77 pathway.

Conclusion

Overall, this study shows that the transcription factor MEF2C is suppressed in cisplatin-resistant A2780cp ovarian cancer cells, and that restoring its expression resensitizes these cells to cisplatin by enhancing caspase-3 activation and increasing apoptosis. The results position MEF2C as a pro-apoptotic regulator that likely operates via the MEF2C–Nur77 signaling axis to stimulate the intrinsic apoptotic route. A deeper understanding of this regulatory network could prove valuable for devising novel approaches to circumvent chemoresistance. However, more comprehensive investigations are necessary to define the exact molecular details of MEF2C-driven apoptosis and to verify its therapeutic potential using patient samples and in vivo systems.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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