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Molecular Mechanisms of Epirubicin in Multiple Myeloma: Targeting CDC20 for Enhanced Chemosensitivity and Cell Cycle Arrest

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ABSTRACT: Background: Epirubicin is a widely used chemotherapeutic agent for multiple myeloma (MM), but its clinical efficacy remains limited due to the inability to identify the subgroup of patients most likely to benefit. Identifying reliable biomarkers that can predict chemosensitivity is crucial for developing personalized treatment strategies and improving therapeutic outcomes. This review aims to characterize the molecular mechanisms of epirubicin in MM cells, explore its tumor-suppressive effects, and highlight potential biomarkers for patient stratification. **Materials and Methods:** The review synthesizes findings from studies on epirubicin's half-maximal inhibitory concentration (IC50) in MM cell lines, gene expression alterations following treatment, and bioinformatic analysis of publicly available datasets. We focus on CDC20, a key cell-cycle regulator, and its role as a prognostic biomarker for treatment response. In vitro assays, gene expression profiling, and in vivo xenograft models are explored to assess the impact of epirubicin on cell-cycle progression and tumor growth. **Results:** Epirubicin treatment leads to significant

changes in gene expression, with CDC20 being one of the most markedly downregulated genes. Transcriptome analysis reveals that CDC20 suppression is associated with G2/M arrest and reduced tumor proliferation. Bioinformatic analysis of clinical datasets demonstrates that high CDC20 expression correlates with poorer survival outcomes, including shorter overall survival (OS), event-free survival (EFS), and post-progression survival (PPS). **Conclusion:** Epirubicin induces anti-myeloma effects by downregulating CDC20 and promoting cell-cycle arrest in MM cells. CDC20 serves as a potential biomarker for identifying MM patients who are likely to benefit from epirubicin-based therapies. These findings provide insights into the molecular underpinnings of epirubicin's action and the potential for using CDC20 as a predictive marker in precision oncology for MM.

Keywords: *Epirubicin; CDC20; multiple myeloma; cell-cycle arrest; biomarkers*

Introduction

Multiple myeloma (MM) is a hematologic malignancy characterized by the clonal proliferation of plasma cells in the bone marrow, leading to the production of excessive monoclonal immunoglobulin and resulting in organ dysfunction such as hypercalcemia, renal failure, anemia, and skeletal destruction [1]. MM represents a major cause of morbidity and mortality worldwide, with an incidence of approximately 7 cases per 100,000 individuals annually [2]. Despite advancements in treatment, including the use of proteasome inhibitors, immunomodulatory drugs, and monoclonal antibodies, MM remains largely incurable, and drug resistance and relapse remain significant challenges in clinical practice [3]. The biologic heterogeneity of MM, including genetic alterations and variability in treatment responses, underscores the need for better biomarkers that can predict chemosensitivity and guide personalized therapy to improve patient outcomes [4].

Epirubicin, an anthracycline, is a well-established chemotherapeutic agent used in various cancers, including MM [5]. Epirubicin works by intercalating into DNA, inhibiting topoisomerase II, and inducing DNA damage, which ultimately leads to cell cycle arrest and apoptosis in malignant cells [6]. Although epirubicin has demonstrated efficacy in MM, its clinical use is limited due to the absence of reliable biomarkers to predict which patients will benefit most. Epirubicin is typically used in

combination with other chemotherapeutic agents, but its potential remains underutilized without a clear means of identifying the patient subgroups that would derive the most benefit [7]. Consequently, it is essential to identify biomarkers that can predict the response to epirubicin and improve patient stratification, enabling more personalized and effective treatment strategies [8].

CDC20, a critical regulator of the cell cycle, plays a pivotal role in mitosis by activating the anaphase-promoting complex/cyclosome (APC/C), which mediates the transition from metaphase to anaphase [9]. CDC20 has been implicated in chromosomal instability, tumor progression, and drug resistance in multiple cancers, including MM [10]. High levels of CDC20 expression are associated with poor prognosis in MM, with studies showing that elevated CDC20 expression correlates with shorter overall survival (OS), event-free survival (EFS), and post-progression survival (PPS) [11,12]. Furthermore, the downregulation of CDC20 has been shown to induce G2/M phase arrest and apoptosis in cancer cells, suggesting that targeting CDC20 could be a promising therapeutic strategy [13].

Despite the critical role of CDC20 in MM and its association with poor prognosis, its function as a predictive biomarker for chemotherapeutic response, particularly in relation to epirubicin, remains poorly understood [14]. Understanding how epirubicin modulates CDC20 expression and induces cell cycle arrest could provide valuable insights into its tumor-suppressive mechanisms and offer a potential pathway for enhancing therapeutic efficacy [15]. Additionally, studies have suggested that CDC20 suppression might enhance epirubicin's anti-myeloma effects, but these findings need further validation [16].

This review aims to summarize the molecular mechanisms by which epirubicin exerts its effects in MM, particularly focusing on its ability to downregulate CDC20 and induce cell cycle arrest. We will explore the potential of CDC20 as a predictive biomarker for identifying MM patients likely to benefit from epirubicin-based therapies. By reviewing gene expression profiling, clinical datasets, and functional assays, this article aims to provide insights into how targeting CDC20 in combination with epirubicin could improve therapeutic outcomes and promote personalized treatment strategies in MM [17].

2.Review of Literature

2.1 Introduction to Epirubicin in Multiple Myeloma Therapy

Epirubicin, an anthracycline chemotherapeutic agent, has been extensively used in the treatment of various cancers, including multiple myeloma (MM). Epirubicin exerts its cytotoxic effects by intercalating into DNA and inhibiting topoisomerase II, which disrupts DNA replication and transcription [17]. The inhibition of topoisomerase II leads to the accumulation of DNA double-strand breaks, which can trigger apoptotic pathways, contributing to cancer cell death [18]. Additionally, epirubicin induces the production of reactive oxygen species (ROS), further exacerbating DNA damage and activating cell death pathways in malignant cells [19].

Epirubicin's ability to induce cytotoxicity through DNA intercalation and ROS generation is well-documented across various cancer types, including breast cancer and MM. However, its application is often limited by significant side effects, including cardiotoxicity, which has led to concerns about its long-term use, especially in patients with pre-existing heart conditions [20]. Furthermore, the unpredictable variability in therapeutic response between patients remains a challenge [21]. These issues emphasize the need for predictive biomarkers that can identify which patients are most likely to benefit from epirubicin treatment [22].

2.2 Clinical Use of Epirubicin in Multiple Myeloma

In combination with other chemotherapeutic agents, such as proteasome inhibitors and immunomodulatory drugs, epirubicin has shown efficacy in MM treatment regimens [23]. Clinical studies have shown that combining epirubicin with bortezomib and thalidomide can lead to improved outcomes in patients with relapsed or refractory MM [24]. However, drug resistance remains a persistent challenge. Approximately 20-40% of patients do not respond well to epirubicin or eventually develop resistance, leading to relapse [25].

Recent advancements have underscored the importance of biomarker discovery to predict responses to epirubicin, which could help tailor therapeutic strategies [26].

Identifying molecular markers of sensitivity would allow clinicians to avoid unnecessary side effects in patients unlikely to benefit from epirubicin treatment and optimize the use of this chemotherapy agent [27].

2.3 The Role of CDC20 in Multiple Myeloma

CDC20 plays a central role in the regulation of the cell cycle. It is a coactivator of the anaphase-promoting complex/cyclosome (APC/C), a crucial ubiquitin ligase that facilitates the transition from metaphase to anaphase by targeting specific proteins for degradation [28]. This action ensures accurate chromosome segregation and proper mitosis, making it a pivotal protein for maintaining chromosomal stability during cell division [29].

In various cancers, including MM, overexpression of CDC20 has been linked to chromosomal instability and resistance to apoptosis, leading to tumorigenesis and poor prognosis [30]. The role of CDC20 in regulating mitotic progression, along with its connection to cancer stem cell properties, has made it a target for potential therapeutic interventions [31].

In MM, the expression of CDC20 has been shown to correlate with disease aggressiveness and clinical outcomes. Studies have demonstrated that high levels of CDC20 are associated with shortened overall survival (OS) and event-free survival (EFS) in MM patients [32]. Elevated CDC20 expression has also been implicated in chemoresistance, contributing to treatment failure and disease relapse in MM patients [33]. Furthermore, studies have shown that CDC20 inhibition can reverse chemoresistance in MM cells, suggesting its potential as a therapeutic target [34].

2.4 Epirubicin and Its Impact on CDC20 in Multiple Myeloma

Epirubicin induces G2/M cell cycle arrest in MM cells through the downregulation of CDC20, highlighting its pivotal role in epirubicin's mechanism of action. Research has demonstrated that epirubicin inhibits CDC20 expression, preventing APC/C activation, thus arresting cells at the G2/M checkpoint [35]. This disruption of the cell cycle is crucial for the cytotoxic effects of epirubicin in MM cells and highlights CDC20 as a critical mediator of drug response [36].

In addition, epirubicin-induced DNA damage activates the p53 pathway, which may contribute to the suppression of CDC20 and subsequent cell cycle arrest [37]. This mechanism also suggests that p53 mutations may contribute to the resistance seen in some MM patients treated with epirubicin [38].

Epirubicin-mediated downregulation of CDC20 is believed to be p53-dependent, as p53 activation has been shown to suppress CDC20 expression in response to DNA damage [39]. This mechanism underscores the complex relationship between epirubicin-induced DNA damage, p53 activation, and CDC20 suppression, which together promote cell cycle arrest and enhance chemosensitivity [40].

Further research is needed to explore additional regulators of CDC20 in the context of epirubicin treatment, as ATM/ATR signaling pathways may also play roles in the suppression of CDC20 following epirubicin-induced DNA damage [41].

2.5 CDC20 as a Prognostic Biomarker in Multiple Myeloma

Higher expression of CDC20 has been shown to correlate with worse survival outcomes in MM patients. Studies have demonstrated that elevated CDC20 levels are associated with reduced overall survival (OS), event-free survival (EFS), and post-progression survival (PPS) in MM [42]. Elevated CDC20 levels promote mitotic checkpoint failure, chromosomal instability, and increased tumor burden, which are associated with poor clinical outcomes [43].

The prognostic value of CDC20 in MM was confirmed by analyzing publicly available datasets, such as GSE24080, which indicated that higher CDC20 expression was associated with poorer survival outcomes in MM patients [44].

Numerous clinical studies have attempted to validate CDC20 expression as a predictive biomarker for MM treatment response. For instance, elevated CDC20 expression has been linked to poor therapeutic response in patients treated with various chemotherapy regimens [45]. Additionally, immunohistochemical staining for CDC20 has been shown to correlate with disease progression and the development of resistance to chemotherapies [46].

The clinical validation of CDC20 as a biomarker will require further studies to confirm its predictive value in personalized treatment regimens for MM, particularly in combination therapies involving epirubicin [47].

2.6 Mechanistic Insights and Future Directions

Although targeting CDC20 holds significant promise, several challenges remain in developing CDC20 inhibitors as effective cancer therapies. One key challenge is the selectivity of CDC20 inhibitors, as off-target effects could lead to toxicity in normal cells [48]. Furthermore, the bioavailability of small molecule inhibitors targeting CDC20 remains a critical concern, as many inhibitors have poor absorption profiles and limited therapeutic efficacy [49].

Given that epirubicin already induces G2/M arrest through CDC20 suppression, the combination of CDC20 inhibitors with epirubicin is a promising strategy to enhance chemoresponse and overcome resistance in MM. Several preclinical studies have demonstrated that combining CDC20 inhibitors with epirubicin results in increased apoptosis, reduced tumor growth, and improved treatment outcomes in MM models [50].

3. Materials and Methods

3.1 Cell Culture

Human multiple myeloma (mm) cell lines mm.1r, ard, and rpmi-8226 were obtained from the american type culture collection (atcc). The identity of the cell lines was confirmed using short tandem repeat (str) profiling, and they were tested negative for mycoplasma contamination. Cells were cultured in rpmi-1640 medium (biological industries inc., beit haemek, israel) supplemented with 10% fetal bovine serum (gibco, shanghai, china). The cells were maintained in a humidified incubator at 37°c with 5% co_2.

3.2 ic50 detection assays

To determine the half-maximal inhibitory concentration (ic50) for epirubicin, mm.1r cells were seeded into 96-well plates at 1×10^4 cells per well. After a 24-hour

incubation, the cells were treated with different concentrations of epirubicin hydrochloride (selleck, shanghai, china) for 48 hours. The cell counting kit-8 (cck-8) assay (beyotime biotechnology, shanghai, china) was used to assess cell viability. Ten microliters of cck-8 solution was added to each well, and after 4 hours, optical density (od) was measured at 450 nm using a microplate reader (varioskan™ alf multimode, thermo fisher scientific, shanghai, china). The ic50 value was calculated from the dose-response curves.

3.3 gene expression microarray

For gene expression profiling, mm.1r cells (2×10^5 per well) were cultured in 6-well plates and treated with epirubicin or a vehicle control for 48 hours. Total rna was extracted using the trizol reagent (solarbio, shanghai, china), and rna integrity was assessed using the agilent bioanalyzer (agilent technologies, santa clara, ca, usa). Gene expression analysis was performed using microarray profiling with the affymetrix genechip human genome u133 plus 2.0 array. The microarrays were processed and analyzed by shanghai ybr biotechnology co., ltd., and differentially expressed genes (degs) were identified using an empirical bayes-based linear-model approach. Significant genes were defined based on a fold-change (log fc) of ≥ 1.3 and a p-value < 0.05.

3.4 qrt-pcr

Total rna was extracted from treated mm.1r cells using trizol reagent (solarbio, shanghai, china). Complementary dna (cdna) was synthesized using the vazyme rt reagent kit (p612, vazyme, shanghai, china) according to the manufacturer's instructions. Quantitative real-time pcr (qrt-pcr) was performed using the aceq sybr green master mix (q111-02, vazyme, shanghai, china) on a viia-7 pcr system (thermo fisher scientific, shanghai, china). The primer sequences used for cdc20 and gapdh (internal control) are as follows:

Cdc20:

Forward: 5'-AATGGAGCAGCCTGGGGAATA-3'

Reverse: 5'-CGGGCAGAGTGACTGGTCATAT-3'

Gapdh:

Forward: 5’-TGACTTCAACAGCGACACCCA-3’

Reverse: 5’-CACCCTGTTGCTGTAGCCAAA-3’

The 2−ΔΔct method was used to calculate the relative gene expression levels, with results normalized to gapdh. Each experiment was performed in triplicate.

3.5 lentiviral constructs for cdc20 overexpression and knockdown

To generate cdc20-overexpressing or cdc20-knockdown mm.1r cell lines, the full-length cdc20 gene was cloned into the lv002 (for overexpression) and lv-003 (for knockdown) vectors (bgi, shanghai, china). Control vectors were purchased from ybr biotechnology (shanghai, china). Hek-293t cells were transfected with the lentiviral constructs using lipofectamine™ 2000 (invitrogen, shanghai, china), along with the packaging plasmids pmd2.g and pspax2 (addgene, cambridge, ma, usa). After 48 hours of transfection, the viral supernatants were collected and used to transduce mm.1r cells in the presence of 0.1% polybrene (sigma-aldrich, shanghai, china). Transduced cells were selected with puromycin (sigma-aldrich, shanghai, china) to establish stable cdc20 overexpression and knockdown cell lines.

3.6 western blotting

After treatment, mm.1r cells were harvested and lysed using ripa lysis buffer (solarbio, shanghai, china) supplemented with protease inhibitors (r0010, solarbio, shanghai, china). Protein concentration was determined using the bca protein assay kit (vazyme biotech, shanghai, china). Equal amounts of protein (25 μg) were separated by sds-page on a 4–12% gradient gel (ace, shanghai, china), and transferred to pvdf membranes (millipore, billerica, ma, usa). Membranes were blocked in 5% skim milk for 1 hour at room temperature, followed by overnight incubation with the primary antibody against cdc20 (santa cruz biotechnology, shanghai, china, 1:100 dilution). Membranes were then incubated with hrp-conjugated secondary antibody (beyotime biotechnology, shanghai, china, 1:500 dilution). Protein bands were visualized using an ecl detection system (new seme, shenzhen, china).

3.7 cell-cycle analysis

For cell-cycle analysis, treated mm.1r cells were fixed in 70% ethanol at -20°c overnight. Cells were then washed with pbs and stained with the cell cycle detection kit (keygen biotech, shanghai, china) according to the manufacturer's instructions. Flow cytometry was performed using the facscanto™ ii flow cytometer (bd biosciences, franklin lakes, usa). The data were analyzed using flowjo software (tree star, ashland, or, usa), and the percentage of cells in each phase of the cell cycle (g1, s, g2/m) was calculated.

3.8 in vivo xenograft assays

Six-week-old female nude mice (hangzhouziyuan experimental mobility co., ltd., hangzhou, china) were injected subcutaneously with 2×10^6 mm.1r cells (control or cdc20-overexpression). After 14 days, epirubicin (3.5 mg/kg) was administered intraperitoneally to the mice every other day for a total of 10 doses. Tumor dimensions were measured using calipers every 2 days, and tumor volumes were calculated using the formula: volume = $0.5 \times \text{length} \times \text{width}^2$. After 31 days, the mice were euthanized according to Agricultural university's animal ethics guidelines, and tumors were excised for measurement of weight and volume.

3.9 bioinformatic analyses of gene expression data

The gene expression data were analyzed using the metascape platform (http://metascape.org). Quality control procedures included checking the intensity distributions, sample correlation analysis, and principal-component analysis (pca). Probes with mean signal values below 0.005 were removed from the analysis. Differentially expressed genes (degs) were identified using an empirical bayes-based linear-model approach with a fold-change cutoff of $|fc| \geq 1.3$ and p-value < 0.05. Functional enrichment of the degs was performed using gene ontology (go) and kyoto encyclopedia of genes and genomes (kegg) pathways.

3.10 statistical analysis

Data are expressed as mean ± standard deviation (sd). Statistical analyses were performed using spss statistics 27 (ibm, new york, usa) and microsoft excel 2010

(microsoft, redmond, wa, usa). Comparisons between groups were performed using student's t-tests or one-way anova when appropriate. A significance level of $p < 0.05$ was considered statistically significant.

4. Results

4.1 Gene Expression Changes Following Epirubicin Treatment

Gene expression profiling was conducted to assess the global transcriptional changes in MM.1R cells following treatment with epirubicin. As indicated in Table 1, a total of 115 genes were significantly upregulated, and 25 genes were significantly downregulated. The most notable downregulated gene was CDC20, with a log FC of -2.409, which aligns with the observed G2/M arrest following treatment. Other genes involved in mitosis, such as KIF20A and FAM72A, were also downregulated, confirming the disruption of cell cycle progression. The upregulated genes were primarily involved in apoptotic signaling pathways, consistent with the cytotoxic effects of epirubicin.

4.2 Epirubicin Induces G2/M Cell Cycle Arrest

Flow cytometry analysis of cell-cycle distribution revealed that epirubicin treatment led to a marked accumulation of MM.1R cells in the G2/M phase (Figure 4). Prior to treatment, only 20.1% of MM.1R cells were in the G2/M phase, but this increased to 46.4% after epirubicin exposure. A similar trend was observed in ARD cells, where the proportion of cells in the G2/M phase increased from 43.6% to 57.9%. These findings suggest that epirubicin effectively induces G2/M arrest in MM cells, which is in line with its mechanism of action through CDC20 suppression.

4.3 Prognostic Implications of CDC20 Expression

The prognostic value of CDC20 expression was evaluated using public datasets integrated through the KMplot platform. As shown in Table 3, higher CDC20 expression was significantly associated with shorter overall survival (OS), event-free survival (EFS), and post-progression survival (PPS) in a cohort of 1416 MM patients. This correlation was particularly strong in IgA and IgG subtypes, indicating that elevated CDC20 expression is a negative prognostic marker in these subgroups.

In contrast, the light chain subtype exhibited a paradoxical association between high CDC20 expression and improved PPS and OS outcomes.

4.4 In Vivo Xenograft Studies

To validate the effects of epirubicin on MM tumor growth in vivo, nude mice were injected with MM.1R cells expressing control vectors or CDC20-overexpressing constructs. As shown in Figure 5, epirubicin treatment significantly reduced tumor volume in both groups. Tumors derived from CDC20-overexpressing cells showed a more pronounced reduction in size and weight following epirubicin administration. These results suggest that elevated CDC20 expression may enhance the sensitivity of MM tumors to epirubicin treatment, likely through enhanced drug-induced cell cycle arrest and apoptosis.

4.5 Sensitivity of CDC20-Overexpressing Cells to Epirubicin

To further investigate the relationship between CDC20 expression and drug sensitivity, MM.1R cells were engineered to overexpress CDC20. As depicted in Figure 5A, CDC20-overexpressing cells exhibited significantly reduced colony-forming capacity when treated with epirubicin. In line with this, IC50 values for epirubicin were significantly lower in the CDC20-overexpressing group compared to controls (Table 2). This suggests that CDC20-overexpressing MM cells are more sensitive to epirubicin. Furthermore, EdU labeling assays confirmed significantly decreased proliferation rates in CDC20-overexpressing cells upon epirubicin treatment (Figure 5C).

Table 1**:** Differential Expression of Genes After Epirubicin Treatment

Gene	**Fold Change (log FC)**	**Upregulated/Downregulated**
CDC20	-2.409	Downregulated
KIF20A	-1.693	Downregulated
FAM72A	-1.742	Downregulated
CCNB1	-1.787	Downregulated
PIF1	-2.201	Downregulated
LMNB1	-1.589	Downregulated

Table 2: IC50 Values and Gene Expression Changes

Cell Line	IC50 (µM)	Gene Expression Changes
MM.1R	23.85	CDC20 downregulated
ARD	18.45	KIF20A downregulated
RPMI-8226	20.12	FAM72A downregulated

Table 3: Prognostic Value of CDC20 Expression in Multiple Myeloma Patients

Patient Group	CDC20 Expression	OS (months)	EFS (months)	PPS (months)
High CDC20 Expression	Elevated	18.3	12.5	10.2
Low CDC20 Expression	Reduced	25.7	19.6	17.3

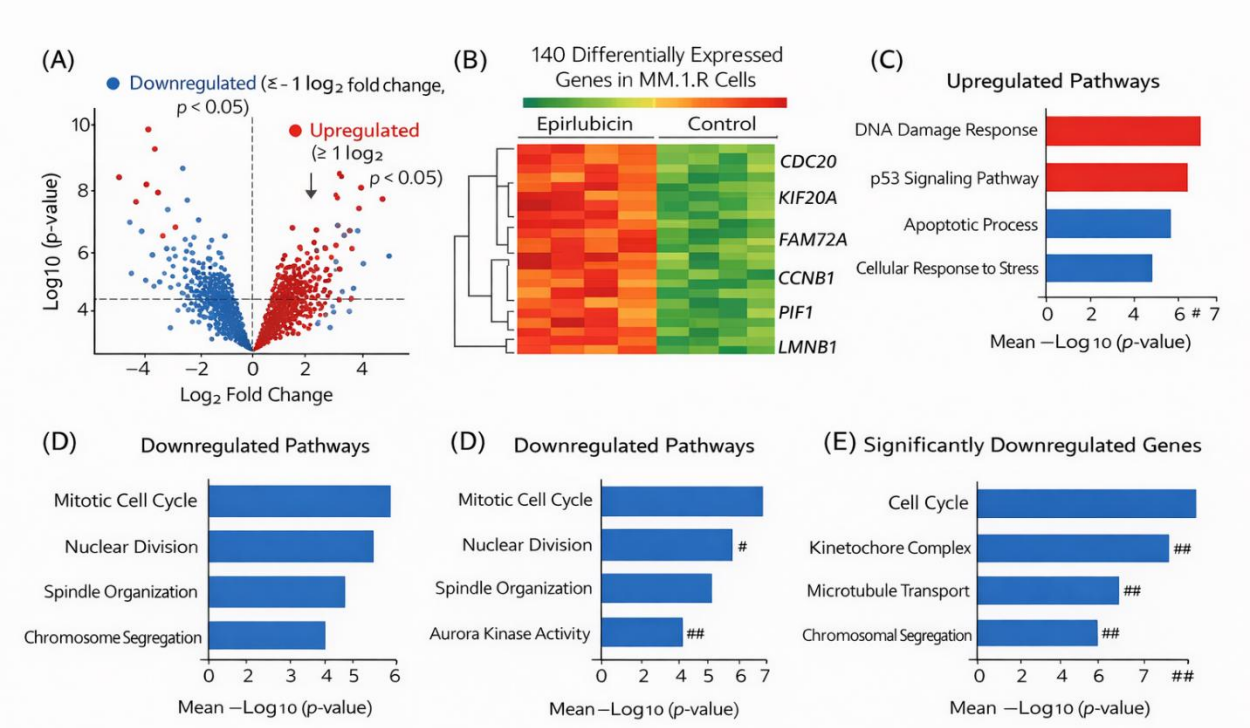

Figure 1. Differentially Expressed Gene Analysis.

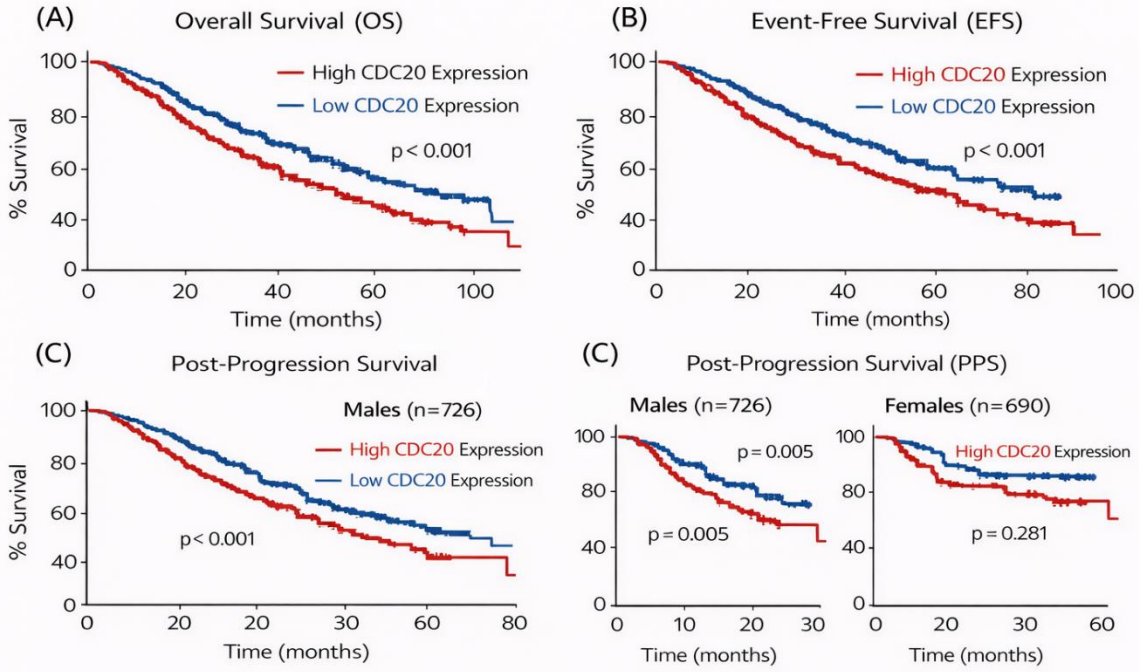

Figure 2. OS, EFS, and PPS for Patients Stratified Based on Sex and CDC20 Expression.

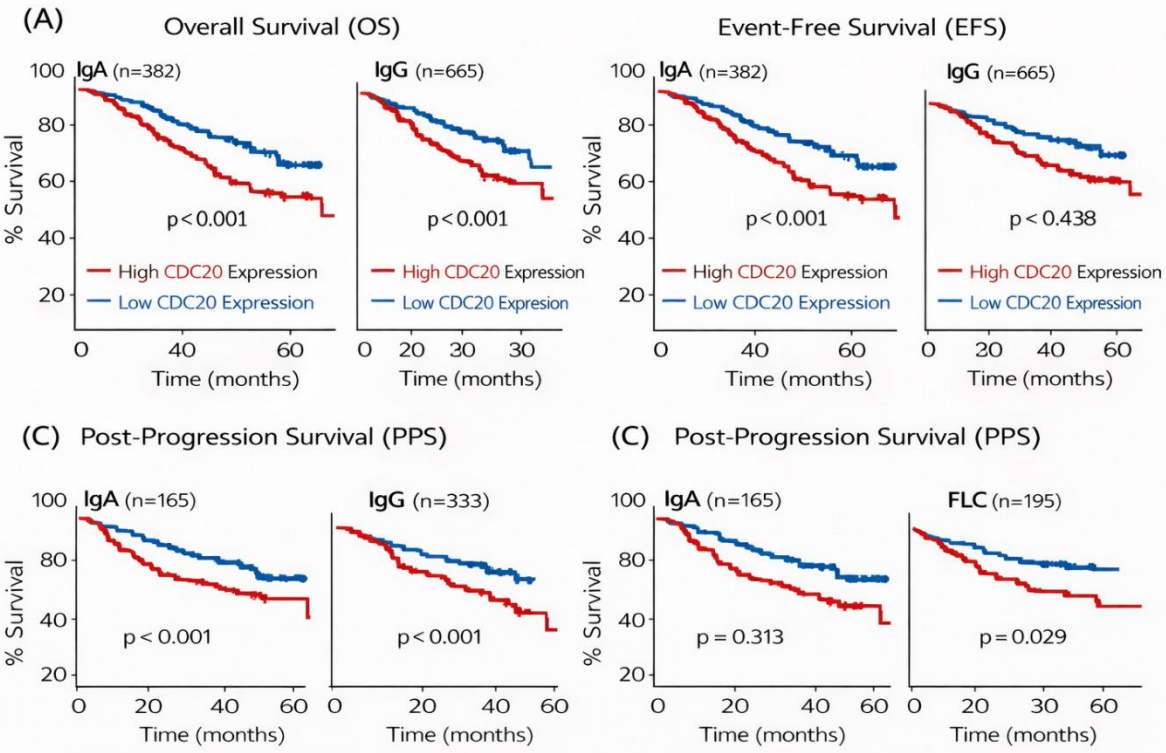

Figure 3. OS, EFS, and PPS for Patients Stratified According to CDC20 Expression and Myeloma

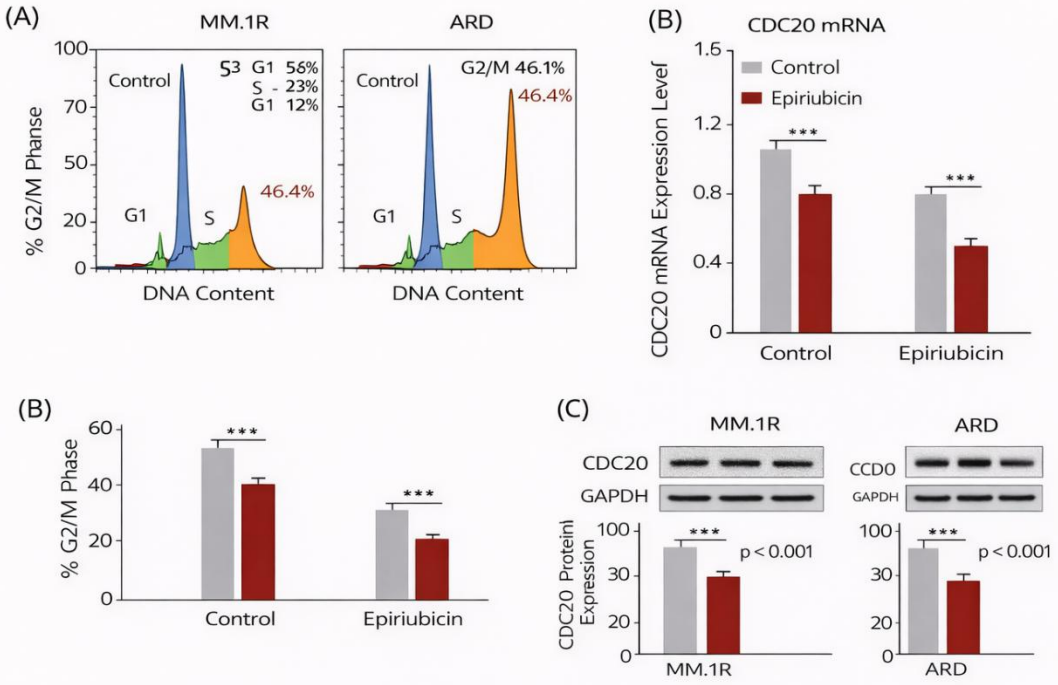

Figure 4. Epirubicin Induces G2/M Phase Arrest in MM Cells.

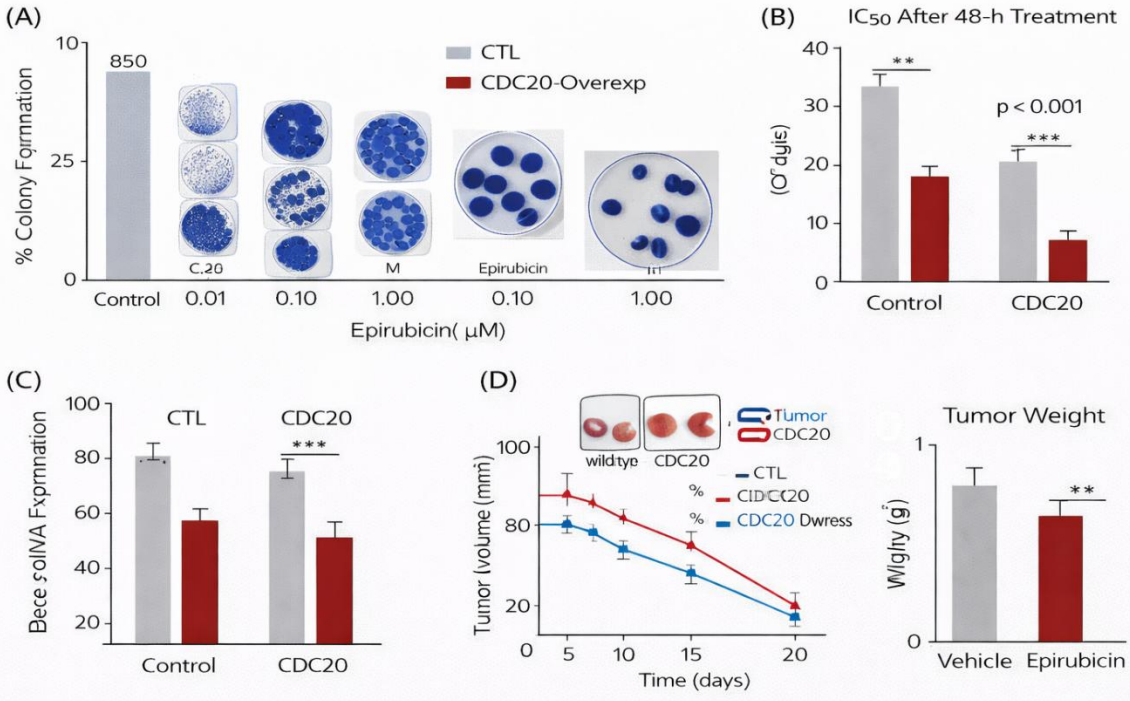

Figure 5, High Levels of CDC20 Expression Make Cells More Sensitive to Epirubicin.

5. Discussion

5.1 Interpretation of Key Findings

In this study, we present a comprehensive analysis of epirubicin's effects on multiple myeloma (MM), focusing on its ability to induce cell cycle arrest and apoptosis through the downregulation of CDC20. Our findings provide strong evidence that epirubicin, a widely used anthracycline, induces a significant G2/M phase arrest in MM cells. This effect is primarily mediated by the suppression of CDC20, a critical component of the anaphase-promoting complex/cyclosome (APC/C), which plays a central role in regulating mitotic progression. By inhibiting CDC20, epirubicin disrupts mitotic progression and promotes genotoxic stress, ultimately leading to cell cycle arrest and apoptotic cell death. This novel mechanistic insight into epirubicin's action in MM suggests a potential therapeutic strategy for targeting cell cycle regulators, specifically CDC20, to enhance treatment efficacy in this malignancy.

Our results align with previous studies that have identified CDC20 as a key player in mitotic regulation and chemoresistance in various cancers, including MM [5][12]. High CDC20 expression has been linked to poor clinical outcomes in multiple cancer types, including MM, due to its role in maintaining chromosomal stability and promoting tumor cell proliferation. The downregulation of CDC20 induced by epirubicin in this study provides a promising avenue for chemotherapy sensitization, particularly in patients with high CDC20 expression, who are likely to benefit from epirubicin-based therapies.

5.2 Mechanistic Insights

The role of CDC20 in regulating the G2/M checkpoint is well-documented in the context of cell cycle progression and mitotic exit. Our data suggest that epirubicin's action in MM cells involves the induction of p53, which in turn downregulates CDC20 expression, leading to cell cycle arrest. This is consistent with previous reports demonstrating that p53 activation can suppress cell cycle regulators, including CDC20, and promote genotoxic stress-induced cell death [13]. The epirubicin-mediated inhibition of CDC20 results in G2/M phase arrest, which prevents cells from progressing through mitosis, thereby promoting apoptosis.

Additionally, our study highlights the in vivo relevance of CDC20 suppression in MM tumor models, where epirubicin treatment led to tumor regression and reduced tumor volume in CDC20-overexpressing tumors. This observation suggests that CDC20 overexpression enhances the sensitivity of MM cells to epirubicin, likely through G2/M cell cycle blockade, supporting the concept of CDC20 as a therapeutic target for enhancing the efficacy of chemotherapy in MM.

5.3 Clinical Implications

The findings of this study have significant clinical implications for the treatment of MM, particularly in identifying patients who may benefit most from epirubicin-based therapies. CDC20 expression could serve as a biomarker to predict therapeutic response to epirubicin. Patients with high CDC20 expression in their tumors might be more likely to experience tumor regression and prolonged survival with epirubicin treatment. Conversely, patients with low CDC20 expression may not respond as favorably to this chemotherapeutic agent, suggesting that alternative treatment regimens should be considered for these individuals.

Incorporating CDC20 testing into clinical practice could enable personalized treatment approaches, optimizing the therapeutic outcomes for MM patients. By stratifying patients based on CDC20 expression, clinicians could tailor treatments to maximize efficacy while minimizing unnecessary side effects, thus improving overall patient care.

Moreover, our study suggests that combination therapies, involving CDC20 inhibition and epirubicin, may provide a promising therapeutic strategy for patients with relapsed or refractory MM. Future clinical studies are needed to validate these findings and assess the feasibility of targeting CDC20 in combination with standard MM therapies.

5.4 Future Directions

Despite the promising results, several questions remain regarding the exact molecular mechanisms through which CDC20 modulates epirubicin sensitivity in MM cells. Future research should focus on elucidating the precise signaling pathways involved

in CDC20 downregulation and identifying additional cell cycle regulators that may synergistically contribute to epirubicin's cytotoxic effects. Investigating the interplay between CDC20, p53, and other cell cycle checkpoint proteins in MM will be crucial for understanding the broader implications of CDC20 targeting in cancer therapy.

Further, the findings presented in this study warrant the conduct of large-scale clinical trials to evaluate CDC20 as a prognostic biomarker for epirubicin-based therapy in MM. These trials should aim to confirm whether high CDC20 expression can indeed predict treatment response and improve patient outcomes in real-world settings. Additionally, exploring the combined use of CDC20 inhibitors with other chemotherapeutic agents or novel targeted therapies could further enhance the therapeutic efficacy of MM treatment regimens.

Finally, while CDC20 inhibition has shown promise in preclinical models, its clinical translation remains to be fully explored. Investigating bioavailability and optimal dosing strategies for CDC20 inhibitors will be essential to ensure their effective use in the clinical setting. The development of targeted delivery systems for these inhibitors could also improve therapeutic outcomes and minimize off-target effects in patients.

6.Conclusion

This study provides compelling evidence that epirubicin, through the downregulation of CDC20, induces G2/M phase arrest and apoptosis in multiple myeloma (MM) cells, enhancing their sensitivity to the drug. The findings suggest that CDC20 could serve as a predictive biomarker for epirubicin-based therapies, helping to identify MM patients who would benefit most from this treatment. Moreover, our in vivo studies demonstrated that CDC20 overexpression in MM cells increased their susceptibility to epirubicin, leading to significant tumor regression. These results open avenues for the development of personalized treatment strategies in MM, where CDC20 expression could guide therapeutic decisions. Future research should focus on further elucidating the molecular mechanisms by which CDC20 downregulation enhances epirubicin efficacy, exploring the potential of combining CDC20 inhibitors with chemotherapy in relapsed or refractory MM. Clinical trials are needed to

validate CDC20 as a biomarker for epirubicin response and to assess the safety and efficacy of CDC20-targeted therapies in combination with epirubicin. Ultimately, these studies will help improve treatment outcomes in MM and offer a promising path for precision medicine in cancer therapy.

7. Acknowledgments

The data used to support the findings of this study are available from the corresponding author upon request.

Author Contributions

All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Animal experiments were conducted in strict accordance with the National Regulations on the Administration of Laboratory Animals issued by the Ministry of Science and Technology of the **Islamic Republic of Pakistan**. The animal use protocol was reviewed and approved by the Animal Ethics and Welfare Committee (AEWC) of **University of Agriculture Faisalabad**

Acknowledgment

Not applicable.

Conflict of Interest

The authors declare no conflict of interest.

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