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Research Article

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CUDC-101 as a dual-target inhibitor of EGFR and HDAC enhances the anti-myeloma effects of bortezomib by regulating G2/M cell cycle arrest

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Abstract: CUDC-101, an effective and multi-target inhibitor of epidermal growth factor receptor (EGFR), histone deacetylase (HDAC), and human epidermal growth factor receptor 2 (HER2), has been reported to inhibit many kinds of cancers, such as acute promyelocytic leukemia and non-Hodgkin's lymphoma. However, no studies have yet investigated whether CUDC-101 is effective against myeloma. Herein, we proved that CUDC-101 effectively inhibits the proliferation of multiple myeloma (MM) cell lines and induces cell apoptosis in a time- and dose-dependent manner. Moreover, CUDC-101 markedly blocked the signaling pathway of EGFR/phosphoinositide-3-kinase (PI3K) and HDAC, and regulated the cell cycle G2/M arrest. Moreover, we revealed through in vivo experiment that CUDC-101 is a potent anti-myeloma drug. Bortezomib is one of the important drugs in MM treatment, and we investigated whether CUDC-101 has a synergistic or additive effect with bortezomib. The results showed that this drug combination had a synergistic anti-myeloma effect by inducing G2/M phase blockade. Collectively, our findings revealed that CUDC-101 could act on its own or in conjunction with bortezomib, which provides insights into exploring new strategies for MM treatment.

Key words: CUDC-101; Multiple myeloma; Bortezomib; Epidermal growth factor receptor (EGFR); Cell cycle

1 Introduction

Multiple myeloma (MM) is a monoclonal plasma cell malignant proliferative tumor that has become the second most common hematological tumor worldwide, with the main clinical manifestations of bone destruction, hypercalcemia, anemia, and renal failure (van de Donk et al., 2021). Thus far, proteasome inhibitors, immunomodulators, and monoclonal antibodies are first-choice treatment options for MM; however, during the course of treatment, some patients still develop drug resistance, leading to disease progression (Anderson, 2016; Joshua et al., 2019). Thus, it is imperative to make a dramatic breakthrough by finding innovative ways for treatment.

CUDC-101, a multi-target inhibitor, can directly interrupt the histone deacetylase (HDAC), epidermal growth factor receptor (EGFR), and human epidermal growth factor receptor 2 (HER2) signaling pathways (Wang et al., 2013). Recently, CUDC-101 has been reported to be an effective anti-tumor agent, which leads to apoptosis by inhibiting cell migration and proliferation (Bass et al., 2021). Besides, the combination of CUDC-101 with a variety of conventional therapeutic agents has produced a synergistic effect on tumor killing, such as with gemcitabine (Ji et al., 2018; Li et al., 2021), arsenic trioxide (Zhang et al., 2020), or carfilzomib (Zhang et al., 2016). However, to date, CUDC-101 has not been used to treat myeloma.

In this study, we demonstrated that CUDC-101 has strong anti-MM effect by regulating the cell cycle

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G2/M phase arrest, providing a crucial theoretical basis for MM therapy.

2 Results

2.1 Inhibition of the proliferation of MM cells by CUDC-101

CUDC-101 is a small-molecule inhibitor targeting EGFR, HDAC, and HER2 to exert anti-tumor effects. Hence, we first analyzed the relationship between EGFR expression and MM, and the data showed that high expression levels of EGFR were associated with short overall survival in MM patients in the Multiple Myeloma Research Foundation (MMRF) CoMMpass database, suggesting that EGFR is a significant prognostic indicator in MM (Fig. 1a). Next, to explore the anti-myeloma cell proliferation effect of CUDC-101, six human myeloma cell lines (AMO1, ARP-1, CAG, L363, LP-1, and OPM2) were exposed to different concentrations of CUDC-101 for 24 or 48 h. The results of cell proliferation assay showed that CUDC-101 could significantly decrease the viability among these cell lines in a time- and dose-dependent manner (Figs. 1b and 1c). Next, to further study the role of CUDC-101 in MM cells, we chose the ARP-1 and CAG cells and detected the apoptosis of MM cells after treatment with CUDC-101 for 24 h using the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) assay. As shown in Fig. 1d, CUDC-101 markedly induced the apoptosis of MM cells in a dose-dependent manner. Collectively, these results indicated that CUDC-101 has the potential to inhibit MM cell proliferation.

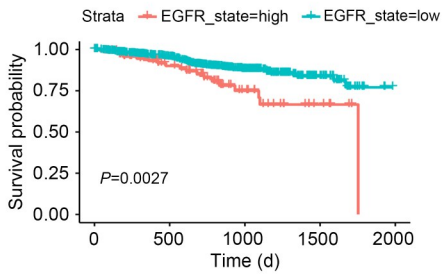
2.2 Induction of apoptosis of MM cell lines and primary MM cells by CUDC-101

In order to further investigate the role of CUDC-101 on MM cells, the apoptosis of MM cells was detected by cytometry after treatment with various concentrations of CUDC-101 for 24 or 48 h. Figs. 2a and 2b show that CUDC-101 obviously induced apoptosis in a time- and dose-dependent manner in ARP-1 and CAG cell lines. Meanwhile, cleaved poly(ADP-ribose) polymerase (PARP), cleaved caspase-3, cleaved caspase-9, and B-cell lymphoma-2 (BCL-2)-associated X protein (BAX) levels were higher, and BCL-XL levels were lower in the CUDC-101 group compared to the control group (Figs. 2c and 2d). Next, to determine whether CUDC-101 induced apoptosis in primary MM samples, we isolated cluster of differentiation 138-positive ($CD138^+$) cells from MM patients and treated them with CUDC-101 or vehicle for 24 h. Consistent with the results for cell lines, CUDC-101 could lead to the apoptosis of $CD138^+$ plasma cells (Fig. 2e). In addition, we also obtained peripheral blood mononuclear cells (PBMCs) from healthy donors, and after 24 h of treatment with various concentrations of CUDC-101, the results showed that the cytotoxicity of CUDC-101 to PBMCs did not increase with the increase in concentration (Fig. 2f). Overall, these findings proved that CUDC-101 can promote the apoptosis of MM cells with limited damage caused to normal human cells.

2.3 Effects of CUDC-101 on EGFR/PI3K and HDAC signaling pathways and cell cycle arrest in MM cells

In order to further explore the molecular mechanism of CUDC-101 anti-myeloma effect, we characterized the alterations in the EGFR/phosphoinositide-3-kinase (PI3K)/extracellular signal-regulated kinase (ERK) and HDAC signaling pathways. The MM cell lines ARP-1 and CAG were exposed to CUDC-101 for 24 h followed by western blot. CUDC-101 significantly inhibited the protein levels of phosphorylated EGFR, PI3K, phosphorylated protein kinase B (AKT), phosphorylated mammalian target of rapamycin (mTOR), and phosphorylated ERK (Fig. 3a). Meanwhile, the protein levels of HDAC3, HDAC4, and HDAC7 were also decreased (Fig. 3b). In the MMRF database, MM patients with high expression of HDAC3, HDAC4, and HDAC7 had shorter overall survival (OS) time (Fig. S1a). International Staging System (ISS) was concluded by analyzing 10750 untreated MM patients provided by 17 institutions, which had good guiding significance for the therapeutic schedule and prognosis of MM patients. The results of MMRF database showed that HDAC3 was higher in ISS stage III (high risk) than in ISS stages I (low risk) and II (standard risk), but there was no difference between ISS stages I and II. Furthermore, HDAC4 expression was different only between ISS stages I and III, but not in stage II compared to I and III. There was no statistical difference in HDAC7 expression among the three groups (Fig. S1b). This result suggested that CUDC-101

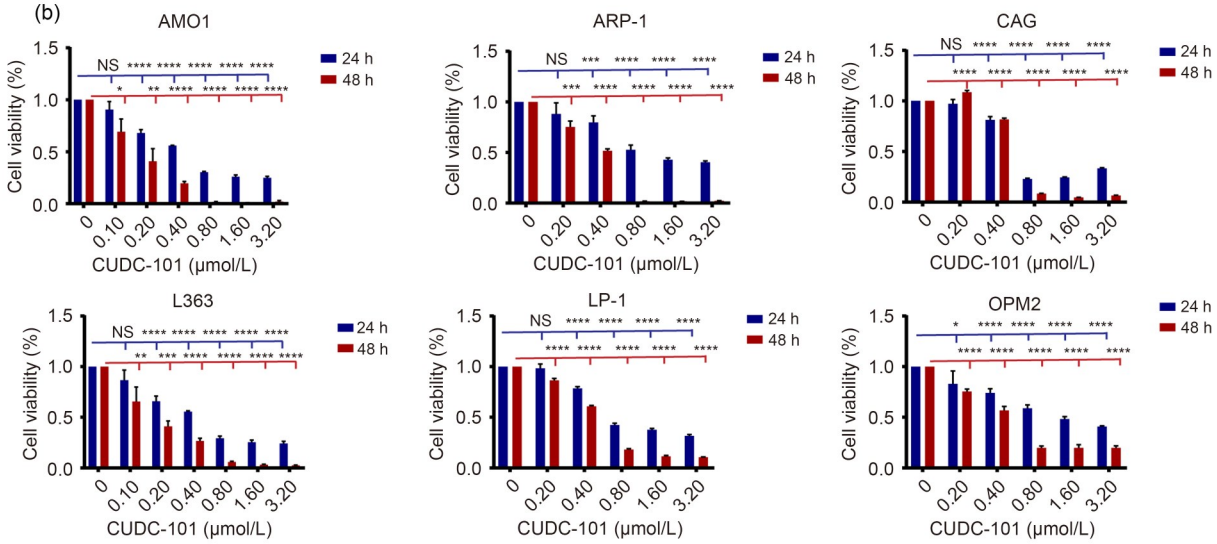
(a)



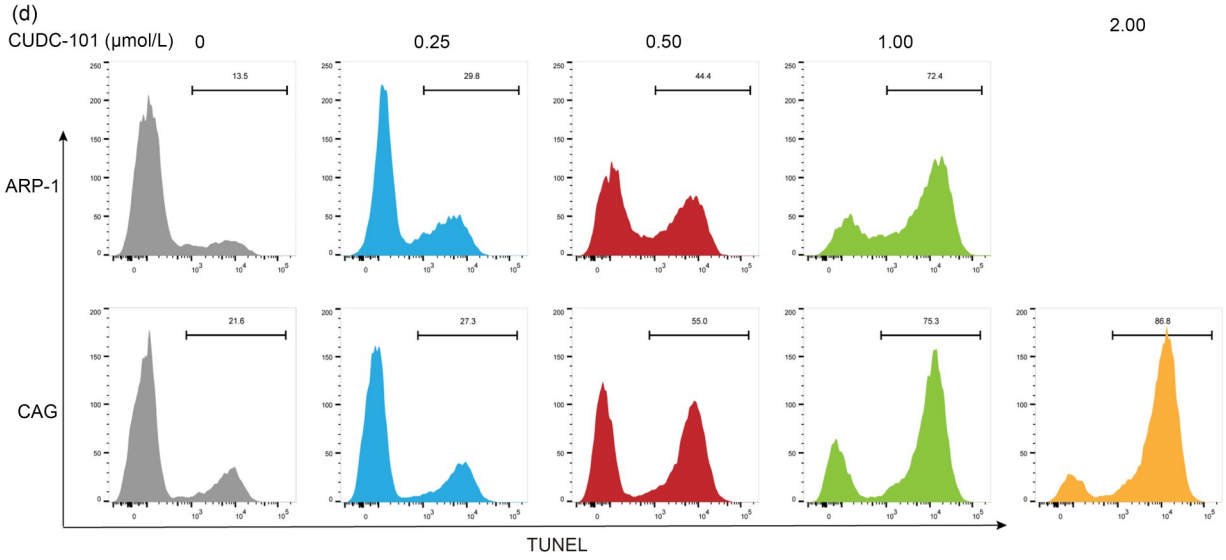
(c)

Cell line	IC_{50} (μmol/L)	
	24 h	48 h
AMO1	0.48	0.19
ARP-1	0.40	0.41
CAG	0.84	0.46
L363	0.47	0.18
LP-1	0.48	0.45
OPM2	1.46	0.49

(b)



(d)



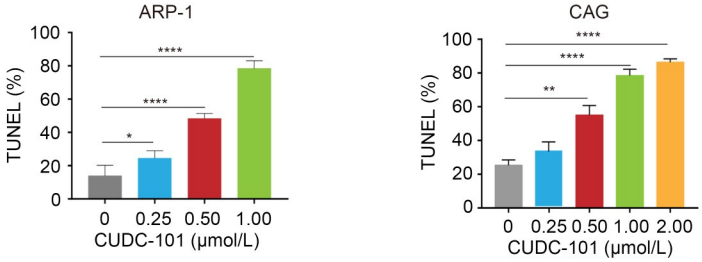


Fig. 1 Inhibition of the proliferation of MM cells by CUDC-101. (a) The overall survival analysis of patients with MM in the EGFR high-level and EGFR low-level groups from the MMRF CoMMpass database. (b) Cell proliferation assay to detect the inhibitory effects of CUDC-101 on six myeloma cell lines after 24 or 48 h. (c) The IC_{50} of CUDC-101 in the treatment of six MM cell lines. (d) TUNEL assay to detect apoptosis by flow cytometry after treatment with CUDC-101 or vehicle after incubation for 24 h. Representative flow cytometry analysis shows the apoptosis-inducing effect of CUDC-101 in ARP-1 and CAG cells. The results are summarized using data from at least three independent experiments. All values are represented as mean±SD. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001. NS: not significant; MM: multiple myeloma; EGFR: epidermal growth factor receptor; MMRF: Multiple Myeloma Research Foundation; IC_{50}: half-maximal inhibitory concentration; TUNEL: terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; SD: standard deviation.

could inhibit several signaling pathways including EGFR and HDAC.

In previous studies, CUDC-101 could induce cell cycle arrest, resulting in G1 or G2/M arrest; however, whether CUDC-101 can regulate the cycle progression of MM cells has not been reported. In the present research, MM cells were first cultured with serum starvation for 24 h to synchronize cycles and then exposed to CUDC-101 for 24 h to detect the cell cycle changes. The low concentration of CUDC-101 did not affect the cell cycle, but high concentrations could cause sub-G1 phase (considered to be a result of DNA fragmentation to cell early apoptosis) and G2 phase arrest (Figs. 3c and 3d). The above changes were also verified by western blot; the expression of cyclin-dependent kinase inhibitor 1A (P21) and cyclin-dependent kinase inhibitor 1B (P27) increased, while the expression of cell division cycle protein 2 (CDC2) and cyclin B1 decreased in the MM cells after treatment with CUDC-101 (Fig. 3e). Taken together, these results demonstrated that CUDC-101 can cause DNA damage and cell cycle arrest.

2.4 Inhibition of the growth of MM xenografts in vivo by CUDC-101

A MM xenograft mouse model was used to verify the anti-MM cell effect of CUDC-101. The MM cell line ARP-1 (2×10^6 cells per mouse) was subcutaneously injected to non-obese diabetic-severe combined immunodeficiency (NOD-SCID) mice, and treated with vehicle control or CUDC-101 (30 mg/kg, daily). The tumor size was detected daily. As expected, compared with the vehicle group, CUDC-101 could significantly inhibit tumor growth (Figs. 4a–4c). Meanwhile, the tumor weight was obviously lower in the CUDC-101 treatment group (Fig. 4d). There was no statistically significant difference in body weight between the two groups (Fig. 4e). Furthermore, immunohistochemistry of xenografts could detect the presence of cleaved PARP and cleaved caspase-3. Fig. 4f shows that the cleaved PARP and cleaved caspase-3 expression levels were higher in the CUDC-101 group. To sum up, these data illustrated that CUDC-101 has a significant anti-tumor effect in vivo.

2.5 Synergistical effects of CUDC-101 and bortezomib on cellular proliferation and cell apoptosis in MM cells

In order to further establish the ability of CUDC-101 to trigger MM cell apoptosis, we next treated MM cells with CUDC-101 in combination with bortezomib to determine whether CUDC-101 and bortezomib have a synergistic anti-tumor effect. The combination index (CI) of CUDC-101 and bortezomib was detected by cell counting kit-8 (CCK-8) experiment. As shown in Figs. 5a and 5b, CUDC-101 and bortezomib showed a combined effect at low concentrations, and CI<1. Meanwhile, compared with the CUDC-101 or bortezomib group, the combination group presented a significant apoptosis of MM cells by flow cytometry (Fig. 5c). Moreover, the apoptosis-related molecular cleaved caspase-3 and PARP were significantly elevated in the combination group (Figs. 5d and 5e). Although bortezomib is one of the preferred agents for MM treatment, the harmful effect of bortezomib on peripheral nerves should not be ignored. In this study, we found that compared with the effect of a high concentration of bortezomib, low concentration of CUDC-101 combined with bortezomib resulted in a higher apoptosis of MM cells but weaker killing effect on human neuroblastoma SH-SY5Y. CCK-8 assay further verified this effect (Fig. S2). Altogether, these results suggested that CUDC-101 combined with bortezomib has a synergistic anti-myeloma effect, and the effect on nerve cells is weaker than that on MM cells.

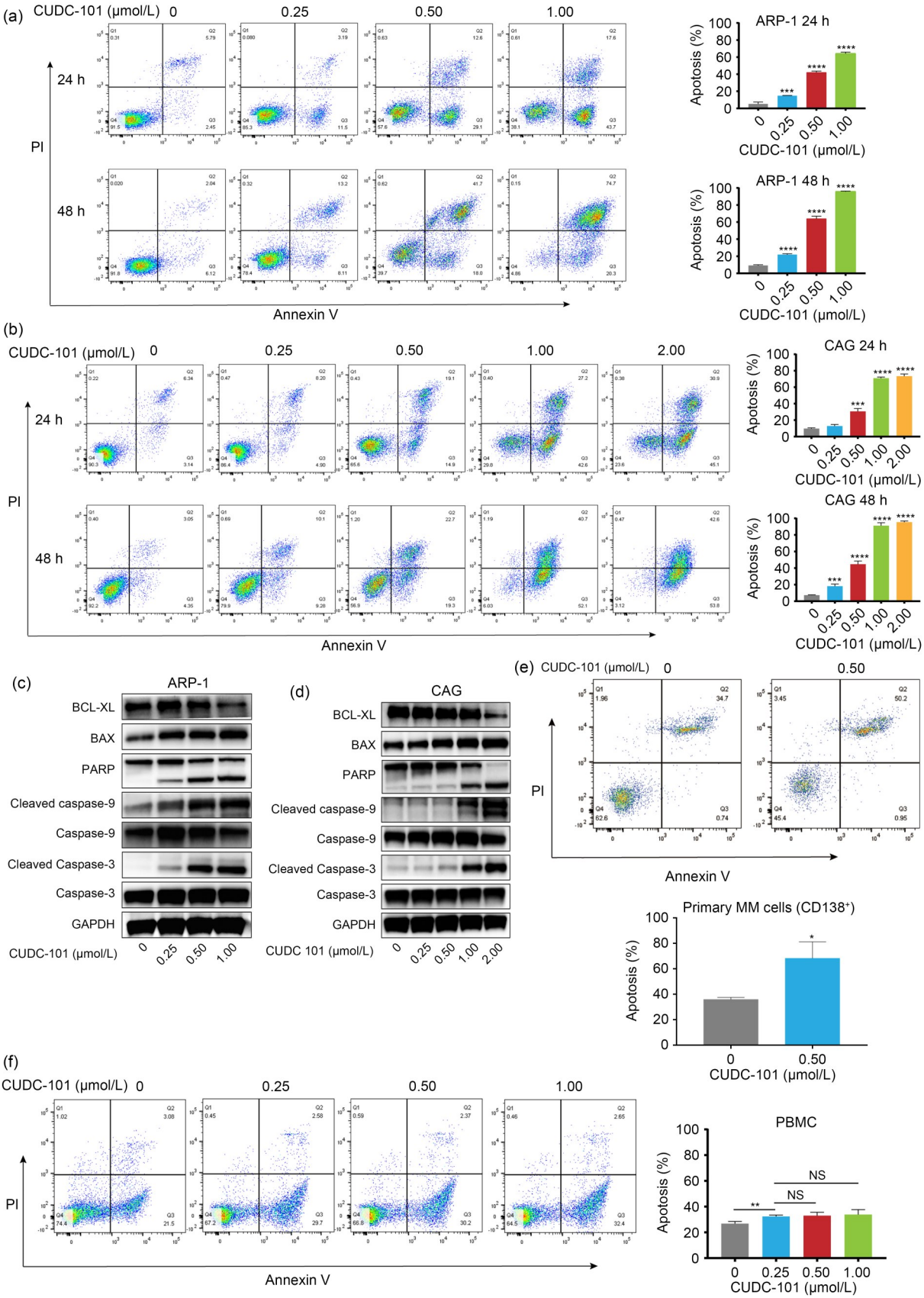

(a)
CUDC-101 (μmol/L) 0 0.25 0.50 1.00
24 h
48 h
PI
Annexin V
ARP-1 24 h
ARP-1 48 h
Apotosis (%)
CUDC-101 (μmol/L)
(b)
CUDC-101 (μmol/L) 0 0.25 0.50 1.00 2.00
CAG 24 h
CAG 48 h
(c)
ARP-1
BCL-XL
BAX
PARP
Cleaved caspase-9
Caspase-9
Cleaved Caspase-3
Caspase-3
GAPDH
(d)
CAG
(e)
Primary MM cells (CD138+)
(f)
PBMC
NS

Fig. 2 Induction of apoptosis in MM cell lines and primary MM cells by CUDC-101. (a, b) ARP-1 (a) and CAG (b) cells were incubated with vehicle or CUDC-101 for 24 and 48 h, and then flow cytometry was performed to analyze the apoptosis of MM cell lines. (c, d) Western blot analyses of apoptosis-related proteins in ARP-1 (c) and CAG (d) cells incubated with vehicle or various concentrations of CUDC-101. (e) $CD138^+$ plasma cells from MM patients (n=3) were incubated with vehicle or CUDC-101 (0.50 μmol/L) for 24 h and analyzed for apoptosis by flow cytometry. (f) PBMCs from healthy donors (n=3) were incubated with vehicle or CUDC-101 for 24 h and analyzed for apoptosis by flow cytometry. All data are represented as mean±SD of at least three independent experiments. * P<0.05, ** P<0.01, *** P<0.001, **** P<0.0001 vs. vehicle. MM: multiple myeloma; PBMCs: peripheral blood mononuclear cells; SD: standard deviation; PI: propidium iodide; BCL-XL: B-cell lymphoma XL; BAX: B-cell lymphoma-2 (BCL-2)-associated X protein; PARP: poly(ADP-ribose) polymerase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; $CD138^+$: cluster of differentiation 138-positive; NS: not significant.

2.6 Synergistical inhibitory effect of CUDC-101 and bortezomib on cellular proliferation through G2/M cell cycle arrest

Studies have found that bortezomib can induce G2/M phase arrest in MM cells. We wondered whether the synergistic effect of CUDC-101 and bortezomib is through the induction of G2/M cycle arrest. Therefore, we treated MM cells with single drugs or their combination for 24 h, and flow cytometry was used to analyze the cell cycle. Bortezomib combined with CUDC-101 obviously induced sub-G1 phase and G2/M arrest compared with either single group (Figs. 6a and 6b). Meanwhile, we found that low concentration of CUDC-101 combined bortezomib could enhance the expression of P21 and P27, but had no effect on cyclin B1 or phosphorylated CDC2 (pCDC2). However, when CUDC-101 and bortezomib concentrations were increased, the expression of cyclin B1 and pCDC2 decreased. These results were consistent with the above minor effect of CUDC-101 on G2/M in MM cells (Figs. 6c and 6d), indicating that CUDC-101 and bortezomib had a synergistic anti-MM effect through the regulation of G2/M phase arrest.

3 Discussion

MM is a highly heterogeneous disease. Bortezomib, as a first-generation proteasome inhibitor, has been reported to greatly improve the treatment effect and disease prognosis of MM, and extend the OS (Kumar et al., 2017). However, some patients will still show disease relapse and progression after bortezomib treatment, and the peripheral neuropathy caused by bortezomib can seriously affect life quality (Argyriou et al., 2008; Wallington-Beddoe et al., 2018). Therefore, there is a prominent clinical demand to explore new MM treatment drugs or to reduce the side effects of bortezomib treatment. In recent years, an increasing number of small-molecule inhibitors have been shown to have powerful anti-myeloma effects, acting as single agents or combined drugs to promote the apoptosis of MM cells. For example, the HDAC inhibitor chidamide can significantly induce the apoptosis of MM by inducing G1 phase arrest (He et al., 2018). In addition, the Wee1 kinase inhibitor adavosertib has an anti-myeloma effect in combination with bortezomib (Liang et al., 2020). In a further example, the Bcl-2 inhibitor venetoclax combined with bortezomib and dexamethasone significantly increased the objective response rate (ORR) and treatment depth in relapsed and refractory multiple myeloma (RRMM) (Kumar et al., 2020). All of these studies indicate that small-molecule inhibitors have significant potential in the treatment of MM.

As the first small molecule designed to multi-target and inhibit EGFR, HDAC, and HER2, CUDC-101 has exhibited potent anti-tumor effects in various tumors, including breast cancer (Zhou et al., 2021), non-small cell lung cancer (NSCLC) (Wang et al., 2013), and non-Hodgkin's lymphoma (Li et al., 2021). The finding that CUDC-101 can be safely used in advanced solid tumors at a maximum dose of 275 mg/m^2 also sets the foundation for its clinical application in other tumor types (Shimizu et al., 2014). CUDC-101 was shown to inhibit tumor cell proliferation and induce apoptosis, not only by blocking EGFR and HDAC signaling, but also indirectly affecting other signaling networks, such as mesenchymal-epithelial transition (MET)- and AKT-mediated signaling, causing cell cycle arrest and caspase-dependent apoptosis.

The EGFR signaling pathway plays an important role in tumorigenesis and drug resistance; therefore, targeting EGFR has also become an effective treatment strategy for various tumors, such as breast cancer, NSCLC, and colon cancer (Jiang et al., 2012; Wu and Shih, 2018; Harbeck et al., 2019). In fact, EGFR is also highly expressed in MM cells rather than normal

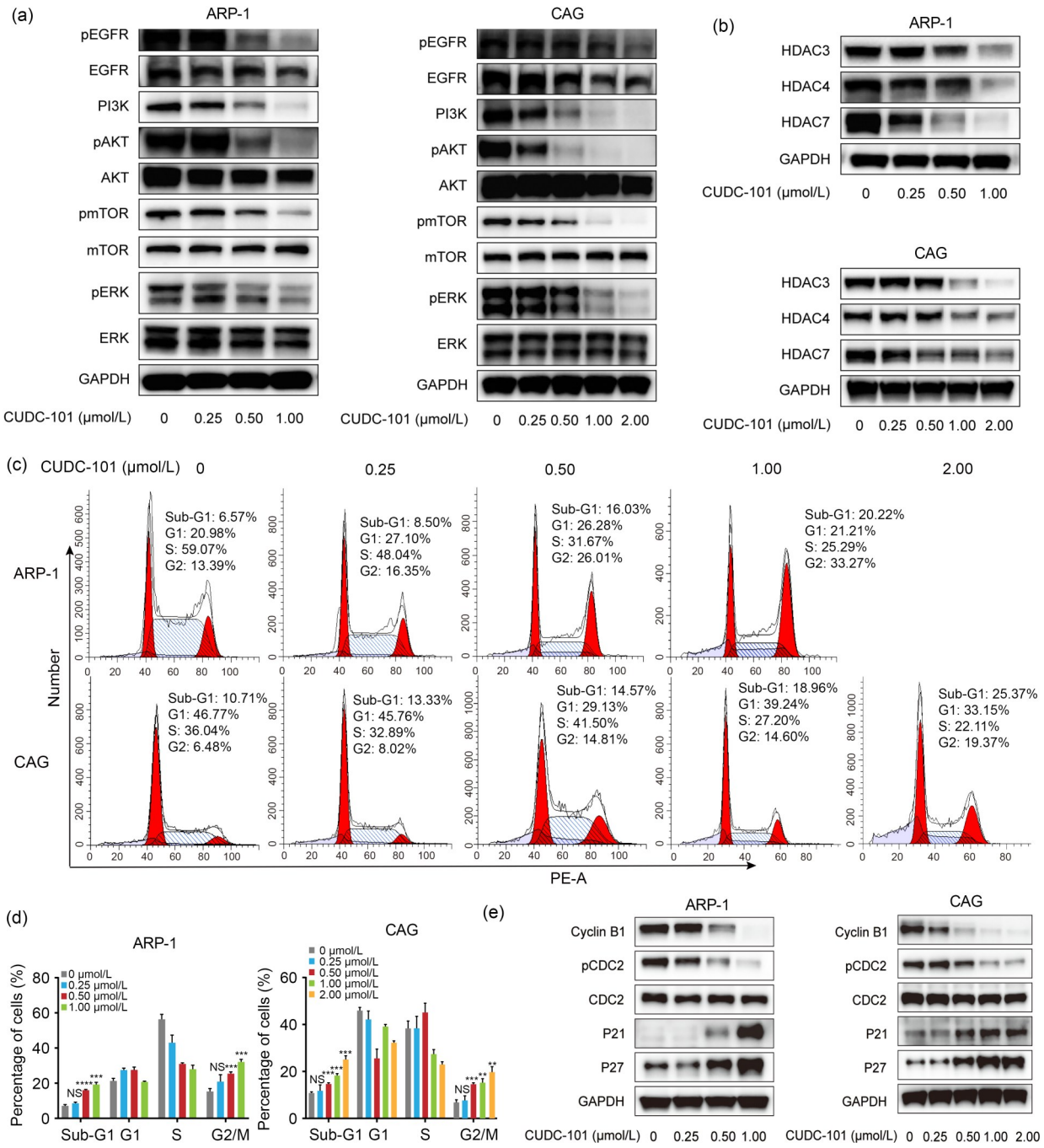


Fig. 3 Effects of CUDC-101 on EGFR/PI3K and HDAC signaling pathways and cell cycle arrest in MM cells. (a) ARP-1 and CAG cells were exposed to a series of concentrations of CUDC-101, and western blot was used to detect the expression of EGFR signaling pathway proteins pEGFR, PI3K, pAKT, pmTOR, and pERK. (b) ARP-1 and CAG cells were exposed to a series of concentrations of CUDC-101, and western blot was used to detect the expression of HDAC proteins (HDAC3, HDAC4, and HDAC7). (c, d) ARP-1 and CAG cells were exposed to a series of concentrations of CUDC-101. Flow cytometry was performed to analyze the cell cycle. (e) Western blot was used to detect the expression of cell cycle-relate proteins cyclin B1, CDC2, pCDC2, P21, and P27. All data are represented as mean±SD of at least three independent experiments. ** P<0.01, *** P<0.001, **** P<0.0001 vs. vehicle. NS: not significant; EGFR: epidermal growth factor receptor; PI3K: phosphoinositide-3-kinase; HDAC: histone deacetylase; MM: multiple myeloma; pEGFR: phosphorylated EGFR; pAKT: phosphorylated protein kinase B; pERK: phosphorylated extracellular signal-regulated kinase; pmTOR: phosphorylated mammalian target of rapamycin; pCDC2: phosphorylated cell division cycle protein 2; SD: standard deviation; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.

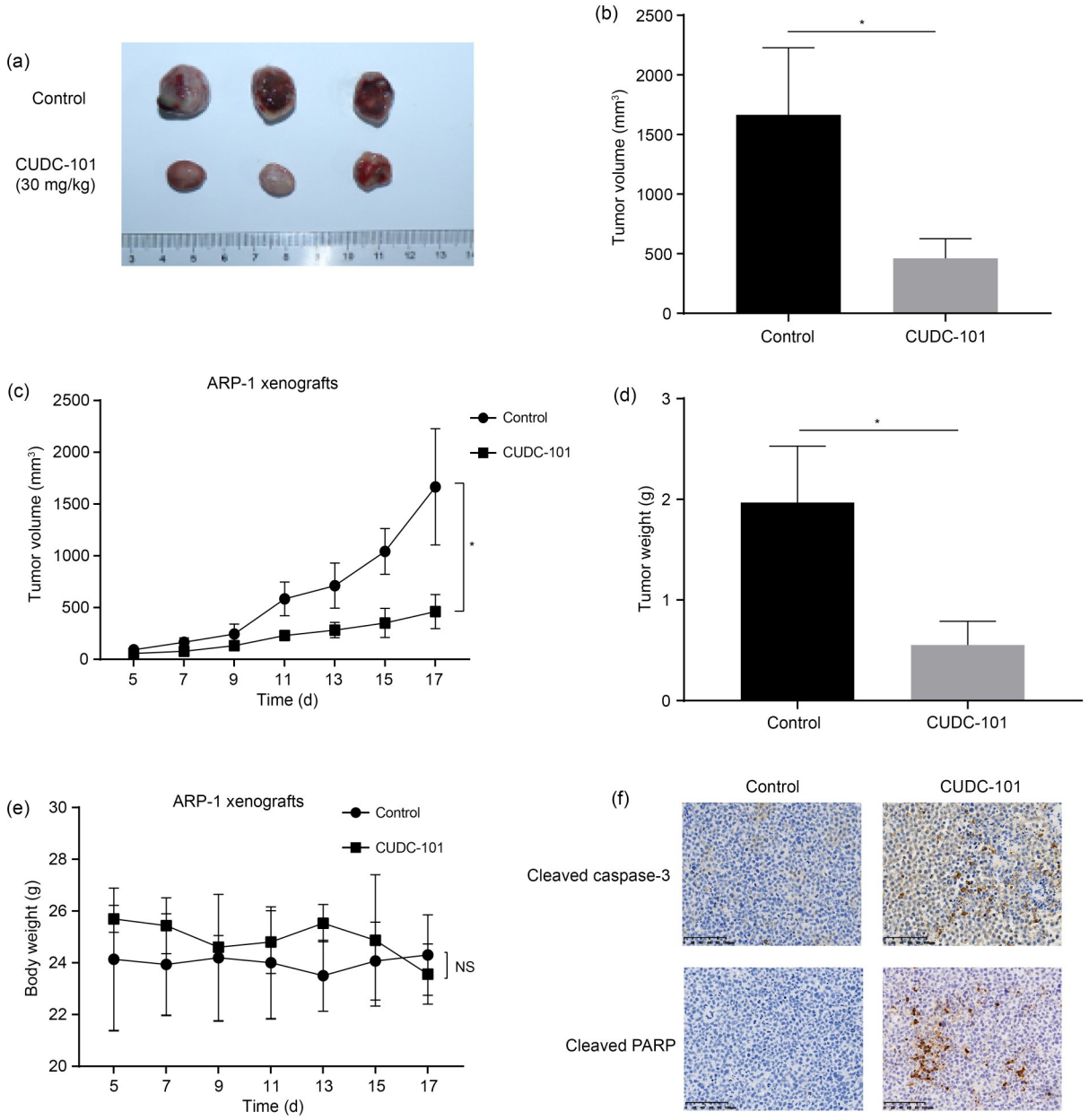
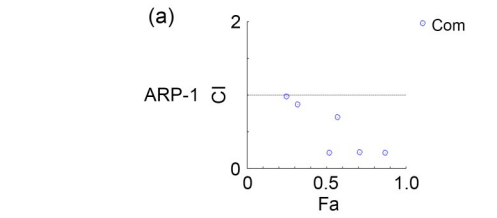
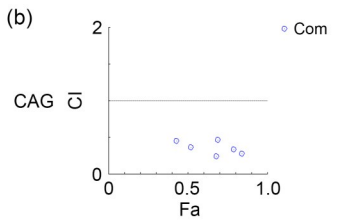


Fig. 4 Inhibition of the growth of MM xenografts in vivo by CUDC-101. (a, b) Subcutaneous MM xenograft in the vehicle and CUDC-101 treatment (30 mg/kg, daily) groups. (c, d) Tumor volume (c) and tumor weight (d) of the xenografts in the vehicle and CUDC-101 treatment groups. (e) The mouse body weights in the two groups were measured in the xenograft model. (f) Immunohistochemistry staining was performed to detect the cleaved caspase-3 and cleaved PARP levels in a MM xenograft model (scale bar=100 μm). All data are represented as mean±SD (n=3). * P<0.05. NS: not significant; MM: multiple myeloma; PARP: poly(ADP-ribose) polymerase; SD: standard deviation.

plasma cells (Mahtouk et al., 2005). Our study has also shown that MM patients with higher EGFR have shorter OS. Luo et al. (2021) found that activated leukocyte cell adhesion molecule (ALCAM) could retard the development of myeloma through inhibiting the mitogen-activated extracellular signal-regulated kinase (MEK)/ERK, PI3K/AKT, and Hedgehog pathways by interacting with EGFR. In another study, an anti-EGFR antibody cetuximab was applicable for RRMM patients with Durie-Salmon stage II or III. Compared with the single reagent, it had a better effect in anti-MM when combined with dexamethasone (von Tresckow et al., 2014). Our study has found that CUDC-101 could significantly decrease the levels of HDAC3, 4,



CUDC-101 (μmol/L)	Bortezomib (nmol/L)	CI
0.125	2.5	0.985
0.250	2.5	0.872
0.500	2.5	0.701
0.125	5.0	0.211
0.250	5.0	0.222
0.500	5.0	0.211



CUDC-101 (μmol/L)	Bortezomib (nmol/L)	CI
0.125	2.5	0.454
0.250	2.5	0.365
0.500	2.5	0.241
0.125	5.0	0.468
0.250	5.0	0.340
0.500	5.0	0.278

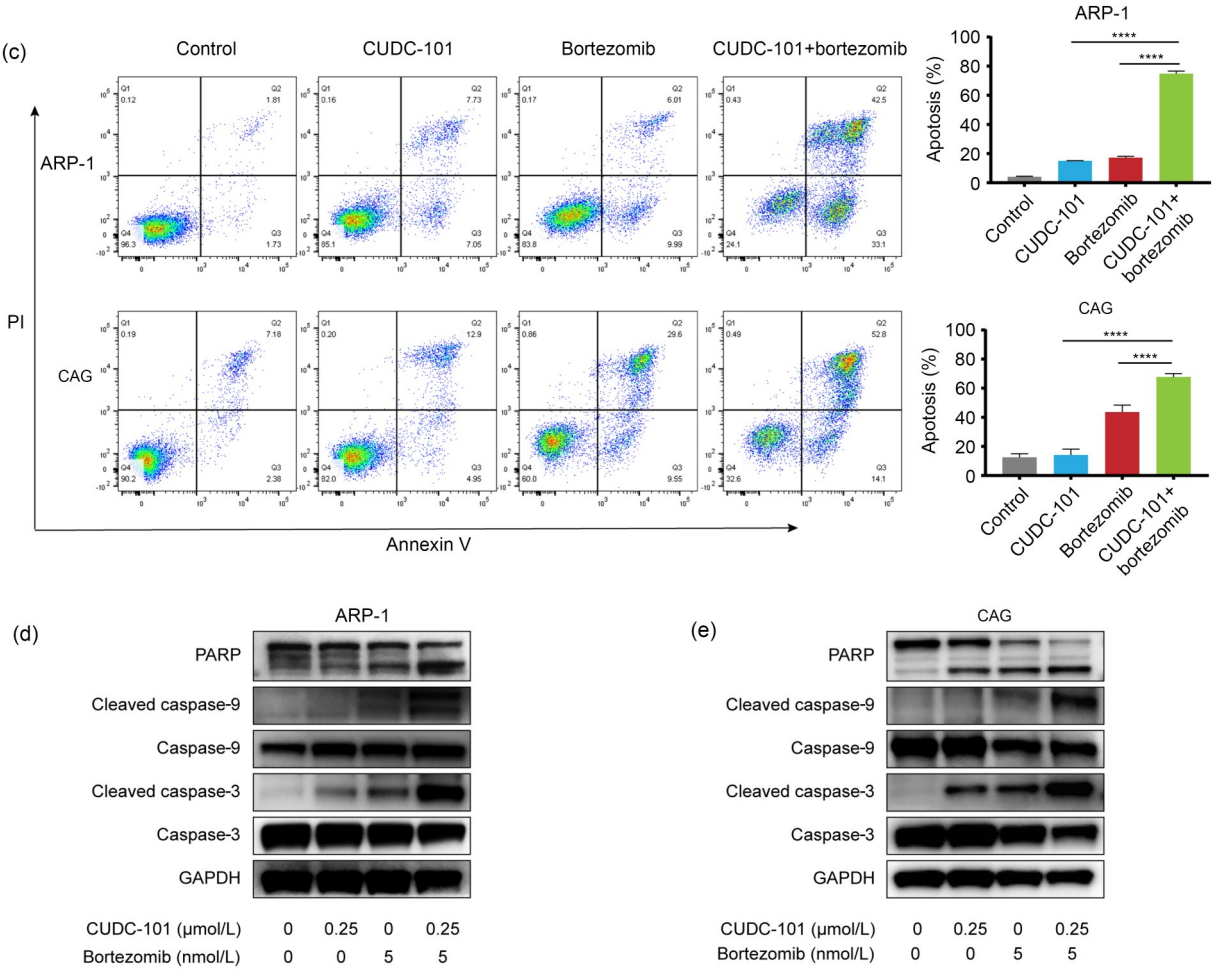


Fig. 5 Synergistical inhibitory effects of CUDC-101 and bortezomib on cellular proliferation in MM cells. (a, b) ARP-1 (a) and CAG (b) MM cells were treated with the indicated concentrations of CUDC-101 and bortezomib for 24 h. The Chou-Talalay method was used to calculate the CI values. CI<1, =1, and >1 mean synergistic, additive, and antagonistic effects, respectively. (c) MM cells were treated with CUDC-101 (0.25 μmol/L) or/and bortezomib (5 nmol/L) for 24 h, and flow cytometry analysis was carried out to measure apoptosis. (d, e) ARP-1 (d) and CAG (e) cells were analyzed for the expression of apoptosis-related proteins after CUDC-101 (0.25 μmol/L) or/and bortezomib (5 nmol/L) treatments. All data are represented as mean±SD of at least three independent experiments. **** P<0.0001. MM: multiple myeloma; CI: combination index; Fa: fraction affected; SD: standard deviation; PI: propidium iodide; PARP: poly(ADP-ribose) polymerase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; Com: combination group.

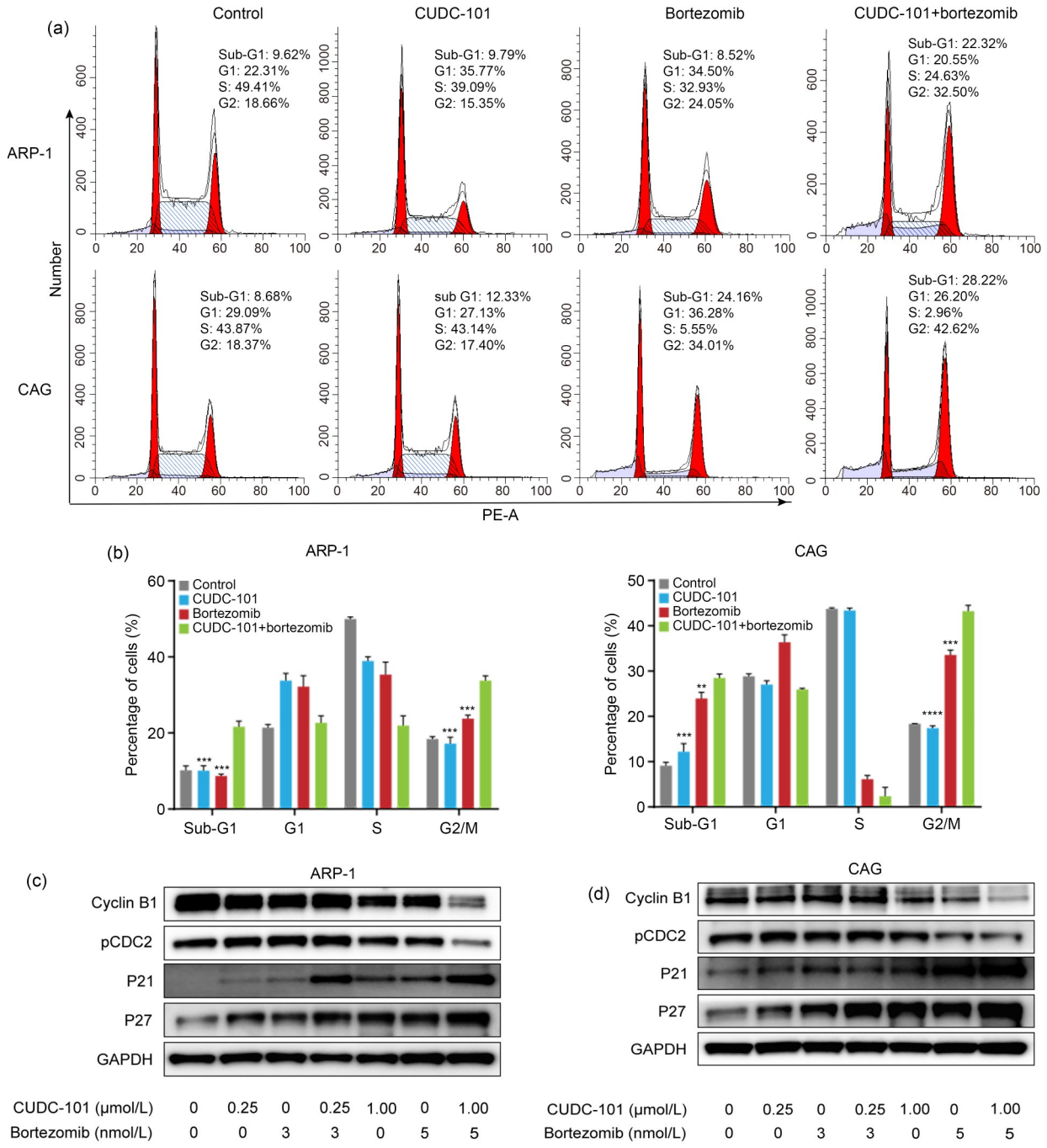


Fig. 6 Synergistical inhibitory effects of CUDC-101 and bortezomib on cellular proliferation through G2/M cell cycle arrest in MM cells. (a, b) Cell cycle analysis in ARP-1 and CAG cells treated with CUDC-101 (0.25 μmol/L) or/and bortezomib (3 nmol/L). (c, d) ARP-1 (c) and CAG (d) cells were analyzed for the expression of cell cycle-related proteins cyclin B1, pCDC2, P21, and P27 after different concentrations of CUDC-101 (0.25 and 1.00 μmol/L) or/and bortezomib (3 and 5 nmol/L) treatments. All data are represented as mean±SD of at least three independent experiments. ** *P*<0.01, * *P*<0.001, vs. control. MM: multiple myeloma; pCDC2: phosphorylated cell division cycle protein 2; SD: standard deviation; PE: phycoerythrin; GAPDH: glyceraldehyde-3-phosphate dehydrogenase.**

and 7. Interestingly, the MMRF database supports shorter OS time for MM patients with high expression levels of HDAC3, 4, and 7. According to the study by Minami et al. (2014), HDAC3 knockdown or inhibition by BG45 (HDAC3 selective inhibitor) could significantly block the MM growth, while BG45

had a synergistic effect with bortezomib in MM treatment. Another study found that a decreased level of HDAC4 could impair MM survival through autophagy pathways (Kikuchi et al., 2015). HDAC4 has also been reported to maintain MM survival by reducing the levels of Bcl-2-like 11 (BIM) and Bcl-2-modifying factor (BMF) (Vallabhapurapu et al., 2015). However, there have been few relevant studies on HDAC7 in MM, prompting further exploration of this topic. Nonetheless, all the above studies prove that EGFR, HDAC3, and HDAC4 could serve as biomarkers or targets for MM therapeutic strategy.

Cell cycle arrest is like a double-edged sword during the process of DNA damage in cells. On the one hand, when the genome becomes damaged, this will trigger a quick check response, which results in the cell cycle process slowing down or halting altogether to give time for cell repair. After passing the arrest phase, cells can re-enter the normal cell cycle, thus reducing the probability of tumorigenesis (Li and Yuan, 2021; Matthews et al., 2022). On the other hand, when the cell cycle progression is uncontrolled, the DNA damage checkpoint becomes abnormal, cells cannot repair damage for the genome and begin to indefinitely proliferate, which may eventually lead to tumorigenesis. Numerous studies have proved that the G1/S and G2/M phases are the two most common cell cycle arrest checkpoints. A variety of drugs or molecules block or promote tumorigenesis by interfering with the cell cycle. For example, many small molecules that target cyclin-dependent kinase inhibitors (CDKIs) can block the G1/S or G2/M phase of cancer cells, such as breast and lung cancers, thus playing an anti-tumor effect (Zhang et al., 2021). Certainly, many proteins could also modulate the cell cycle. For instance, Huang et al. (2022) found that after bortezomib treatment, neuronally expressed developmentally downregulated 4 (NEDD4)-like E3 ubiquitin protein ligase (NEDD4L) protein knockdown could significantly increase the G2/M phase ratio, enhancing the tolerance of MM cells to bortezomib and protecting these cells. These results all indicate that a better understanding of cell cycle control in cancer may create new treatment opportunities in the future.

Through this study, we found the potential efficacy of CUDC-101 for MM treatment and the underlying mechanisms. Our results indicated that CUDC-101 can trigger cell cycle arrest such that it effectively inhibits proliferation and induces apoptosis of MM cell lines and primary $CD138^+$ MM cells by the inhibition of EGFR/PI3K and HDAC signaling pathways. In the MM xenografts model, CUDC-101 also showed a significant inhibitory effect on tumor growth. Further investigation revealed the pivotal anti-tumor mechanism of CUDC-101 is the G2/M phase arrest, which involves the upregulation of P21 and P27. Additionally, CUDC-101 used alone or in combination with bortezomib can be both effective treatment options for MM. All these results suggest CUDC-101 as an incredibly potent drug in MM treatment.

Nonetheless, this study still has some shortcomings. First, the expression of EGFR in MM is low; therefore, whether the effect of CUDC-101 on MM is mainly through the HDAC pathway or the EGFR pathway needs further investigation. Second, the mechanisms of action of bortezomib are complex in MM treatment, and thus other pathways may be involved in the effect of CUDC-101 in synergy with bortezomib. Third, whether CUDC-101 and MM first-line therapy agents (lenalidomide, daratumumab, dexamethasone, etc.) have relevant combined effects should also be further studied.

In conclusion, this study has confirmed CUDC-101 as an effective drug in MM treatment, whether as a single agent or in combination with bortezomib, and thus provides the foundation for a new strategy in future MM therapies.

Materials and methods

The full material and methods are provided in the electronic supplementary material of this paper.

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Author contributions

Wen CAO, Li YANG, and Zhen CAI initiated and designed the study. Wen CAO and Shunnan YAO performed the majority of the experiments. Wen CAO wrote the manuscript. Anqi LI, Haoguang CHEN, Liqin CAO, and Jinna ZHANG performed the research and analyzed the data. Yifan HOU, Zhenfeng DAI, Jing CHEN, and Xi HUANG collected primary

samples for the study. Enfan ZHANG, Li YANG, and Zhen CAI supervised the experiments. All authors have read and approved the final manuscript, and therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Wen CAO, Shunnan YAO, Anqi LI, Haoguang CHEN, Enfan ZHANG, Liqin CAO, Jinna ZHANG, Yifan HOU, Zhenfeng DAI, Jing CHEN, Xi HUANG, Li YANG, and Zhen CAI declare that they have no conflict of interest.

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (the Research Ethics Committee of the First Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China) (No. IIT20220720A) and with the Helsinki Declaration of 1975, as revised in 2013. Informed consent was obtained from all patients for being included in the study. Additional informed consent was obtained from all patients for which identifying information is included in this article. All institutional and national guidelines for the care and use of laboratory animals were followed.

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Supplementary information

Materials and methods; Figs. S1 and S2