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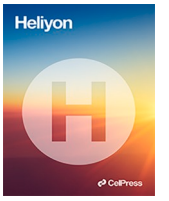

Research article

Capivasertib reverses chemotherapy-induced esophageal cancer resistance via inhibiting Akt-associated Mcl-1 upregulation

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ABSTRACT

The development of resistance to chemotherapy in esophageal cancer represents a significant challenge in cancer treatment. Therefore, our study aimed to identify effective therapeutic strategies by examining the molecules involved in this chemoresistance. We consistently observed an increase in the expression of Mcl-1 in cells exposed to both short and long-term treatment with cisplatin, a drug commonly used in esophageal cancer therapy. Functional analysis showed that Mcl-1 regulates esophageal cancer cell response to cisplatin treatment. Notably, this upregulation of Mcl-1 was not dependent on eukaryotic initiation factor 4E (eIF4E). Instead, it was associated with increased stability due to the activation of Akt. Capivasertib, a potent pan-Akt kinase drug, significantly decreased Mcl-1 level via inhibiting Akt signaling pathway in chemo-resistant cells. In addition, capivasertib not only decreased the viability of chemo-resistant esophageal cancer cells but also synergistically enhanced the effects of cisplatin. In multiple mouse models, representing both chemo-resistant and chemo-sensitive esophageal cancer, capivasertib administered at non-toxic doses demonstrated remarkable efficacy. It significantly extended the overall survival of the mice. Our research underscores the pivotal role of Akt-associated Mcl-1 upregulation in the development of chemo-resistance in esophageal cancer cells. Furthermore, it highlights the potential of capivasertib to reverse this resistance mechanism.

1. Introduction

Esophageal cancer is one of the most prevalent and lethal cancers worldwide, and the incidence has been increasing [1]. Despite its widespread impact, treatment approaches for esophageal cancer have seen minimal innovation over the past three decades, and the survival rate remains stagnant at less than 20 % [2]. In the advanced stages of esophageal cancer, particularly for patients with metastasis and recurrence, conventional chemotherapy is the only treatment option with limited efficacy [3]. Treating advanced esophageal cancer presents a myriad of challenges, stemming from the limitations of current treatment options, treatment toxicity, the development of chemoresistance, and the intricate nature of tumor biology [4]. The mechanisms underlying chemoresistance in esophageal cancer include the dysregulation of drug metabolism, impairment of apoptosis pathways, the presence of cancer stem cells, interactions with tumor-associated stromal cells, alterations in energy metabolism, and modifications to the tumor microenvironment [5]. Novel strategies are needed to circumvent or counteract chemoresistance mechanisms, thereby enhancing treatment efficacy and

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improving patient prognosis.

The hyperactivation of the PI3K/AKT/mTOR pathway is closely linked to the development of resistance to various forms of treatment, such as chemotherapy and targeted therapy, as well as the progression of cancer [5]. In a variety of cancer types, including breast cancer [6], colorectal cancer [7], lung cancer [8], gastric cancer [9] and esophageal cancer [10], the PIK3CA gene is frequently mutated or amplified. Which leads to PI3K activation and serves as a key determinant of standard anticancer therapy [11]. Among the components of this pathway, AKT stands out as the principal signal transduction protein responsible for phosphorylating a range of substrates and downstream effectors. Over the past few decades, significant progress has been made in cancer treatment through the approval of numerous inhibitors associated with the PI3K/AKT/mTOR signaling cascade [12]. Capivasertib, an orally bioavailable, small and potent ATP-competitive Akt kinase inhibitor, has recently approved for the treatment of breast cancer [13]. Mcl-1, an anti-apoptotic protein, holds a critical role in the survival of cancer cells, and accumulating evidence underscores its connection with treatment resistance and unfavorable prognosis in cancer [14]. Research findings suggest that Akt activation serves as an effective mechanism for preventing the decline in Mcl-1 expression through translational and posttranslational regulatory pathways [15,16]. We hypothesized that Akt activation induces an upregulation of Mcl-1 levels, resulting in chemoresistance in esophageal cancer cells.

In this study, our objective was to investigate the involvement of Mcl-1 and Akt in chemotherapy resistance within esophageal cancer cells, and to evaluate the potential of the Akt inhibitor, capivasertib, to reverse this resistance. Our findings reveal that Mcl-1 is upregulated in esophageal cancer cells in response to cisplatin, and this upregulation is intricately linked to the activation of Akt. Importantly, our research underscores the effectiveness of capivasertib in reversing chemoresistance in esophageal cancer. Furthermore, we demonstrate that capivasertib administration significantly prolongs the overall survival of mice. Our study sheds light on the mechanisms driving chemotherapy resistance in esophageal cancer, particularly involving Mcl-1 and Akt. The efficacy of the capivasertib in reversing this resistance highlights a potential therapeutic strategy to improve patient clinical outcomes.

2. Materials and methods

2.1. Cell lines, generation of chemo-resistant cells, compounds and experimental design

Human esophageal carcinoma cell lines, namely KYSE-70, OE33, and FLO-1 (obtained from Sigma), were cultured in RPMI 1640 medium supplemented with 2 mM glutamine and 10 % fetal bovine serum. KYSE-70-R, a cisplatin-resistant cell line, was developed from the parental KYSE-70 cells (KYSE-70-P) through extended exposure to cisplatin. The initiation dose of cisplatin was 0.5 nM, gradually increasing with each subsequent dose set at 75 % of the prior one until stable proliferation was observed. KYSE-70-R cells were maintained in culture medium containing 25 μM cisplatin. Capivasertib and cisplatin were procured from Selleckchm, reconstituted in dimethyl sulfoxide (DMSO) and dimethylformamide (DMF), respectively, and stored at −20°C as aliquots. Flow chart of experimental design was shown in Supplementary Fig. 1.

2.2. Cell viability and combination index (CI) analyses

Cells were initially plated at a density of 10,000 cells per well in a 96-well plate. The following day, various concentrations of the drug were added. After a 3-day incubation period, cell viability was assessed using the CellTiter-Glo® luminescent cell viability assay by following the manufacturer's protocol from Promega). The IC_{50} of single drug was firstly determined using Prism 5 in single arm experiments. For combination studies, the cells were exposed to increasing doses of a single drug or to a combination of drugs at a constant-ratio concentration. The CI, reflecting growth inhibition percentages ranging from 0 % to 100 %, was calculated using CalcuSyn immediately after inputting the data of doses and their corresponding effects.

2.3. Denaturing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot (WB) analyses

A total of 10^6 cells were lysed with a 4 % SDS solution, and the quantification of total protein content was carried out employing the bicinchoninic acid protein assay kit from Thermo Scientific. Subsequently, proteins were separated through denaturing SDS-PAGE and subjected to WB analysis following the standard protocol. Antibodies for the WB analysis were sourced from Cell Signaling.

2.4. Transfection

A total of 10^5 cells were initially seeded into a 12-well plate. To achieve specific knockdown of eIF4E and Mcl-1, the cells were transfected with eIF4E siRNA (100 nM, sourced from Sigma) or Mcl-1 siRNA (100 nM, from Sigma) using Lipofectamine TM 2000 and OptiMEM (Invitrogen), following the manufacturer's prescribed protocol.

2.5. Real-time PCR

The extraction of total RNA and subsequent cDNA synthesis followed the guidelines provided by the manufacturer, utilizing TRIzol (Invitrogen) and the iScript cDNA Synthesis Kit (Bio-rad). To detect the human MCL-1 RNA signal, quantitative real-time PCR was performed with the cDNA serving as the template on the CFX96 Touch System (Bio-Rad). The primer sequences for MCL-1 were as follows: the forward primer -5′-TGA AAT CGT TGT CTC GAG TGA TG-3′, and the reverse primer -5′-TCA CAA TCG CCC CAG TTT-3'.

2.6. Esophageal carcinoma xenograft and immunohistochemistry in severe combined immunodeficiency (SCID) mice

The animal experiments underwent approval from the Institutional Animal Care and Use Committee of Tongji Medical College (IACUC number 20200616). Athymic mice, aged six weeks, were purchased from Biocytogen Inc and were accommodated in a controlled, pathogen-free habitat. The establishment of xenograft mouse models for chemo-sensitive and chemo-resistant esophageal carcinoma followed the same methodology as delineated in our prior study [17]. Briefly, tumor cells (KYSE-70-R, OC33, and FLO-1) were subcutaneously injected into the mice's flanks. When the tumor volume, calculated as length x width x width/2, reached approximately 200 mm^3, the mice were allocated randomly into distinct treatment groups. Details regarding drug doses, administration routes, and treatment duration were explicitly described in the Figure legends. The tumor volume was closely monitored throughout the treatment regimen. For immunohistochemistry, mice were humanely euthanized and tumors were dissected for sectioning. Tumor section slides were fixed using 4 % paraformaldehyde from Sigma. Antigen retrieval was achieved through citric acid, followed by immunostaining with *p*-Akt and p-S6 antibodies, as well as secondary antibodies. The sections were counterstained with hematoxylin (Sigma).

2.7. Statistical analyses

The data is presented as the mean and standard deviation. To assess differences between two groups, statistical analyses were conducted through a one-way analysis of variance (ANOVA) followed by an unpaired Student's t-test. A significance level of $p < 0.05$ was established as the threshold for statistical significance.

3. Results

3.1. Mcl-1 is consistently upregulated in esophageal cancer cells exposed to chemotherapy regardless of eIF4E

In a previous study, we established the involvement of eIF4E in the progression of esophageal cancer [17]. Building on this, we delved into the question of whether eIF4E plays a role in the emergence of chemoresistance in esophageal cancer cells. To explore this,

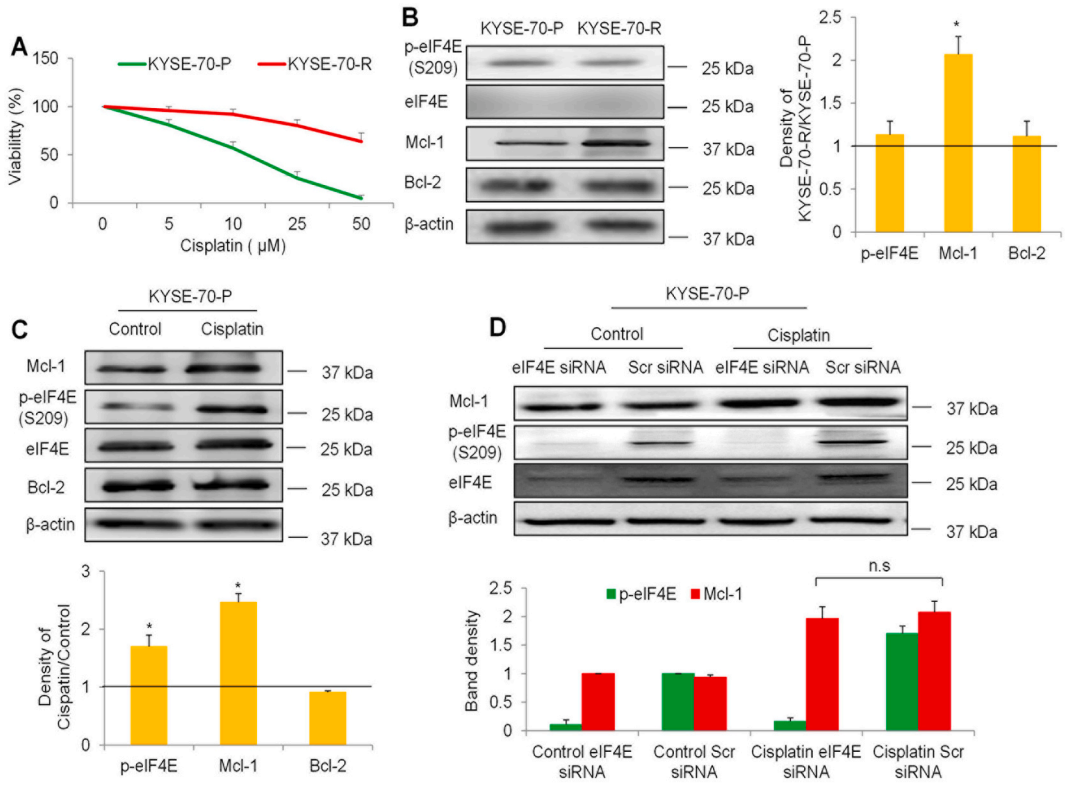

Fig. 1. Mcl-1 is upregulated in chemo-resistant esophageal carcinoma cells regardless of eIF4E. (A) Viability of chemo-sensitive (KYSE-70-P) and chemo-resistant (KYSE-70-R) cells in the presence of cisplatin. (B) Immunoblotting of Mcl-1, *p*-eIF4E, eIF4E and Bcl-2 in KYSE-70-P and KYSE-70-R cells. (C) Immunoblotting of Mcl-1, *p*-eIF4E, eIF4E and Bcl-2 and their quantificaiton in KYSE-70-P after cisplatin (10 μM) treatment. (D) Immunoblotting of Mcl-1, *p*-eIF4E and eIF4E and their quantification in eIF4E-depleted KYSE-70-P cells in the absence or presence of cisplatin (10 μM). *$p < 0.05$, compared to control.

we generated a cisplatin-resistant esophageal cancer cell line known as KYSE-70-R and compared its responsiveness to cisplatin with that of the parental cell line, KYSE-70-P. Our findings revealed a significant increase in resistance to cisplatin in KYSE-70-R when compared to KYSE-70-P (Fig. 1A). Specifically, the IC_{50} of cisplatin in the resistant cells was at least five times higher than that of the parental cells (Supplementary Table 1). Interestingly, our immunoblotting analysis did not reveal any significant differences in the levels of phosphorylated and total eIF4E between the parental and resistant cells (Fig. 1B and Supplementary S2). In contrast, we observed a two-fold increase in the expression of Mcl-1, an anti-apoptotic protein, in the resistant cells when compared to the parental cells. However, there was no significant alteration in another anti-apoptotic protein, Bcl-2. Notably, RNA sequencing expression analysis of 182 esophageal cancer and 286 normal esophageal samples did not show significant difference on Mcl-1 transcriptional level between normal and tumor (Supplementary Fig. 3).

Subsequently, we subjected KYSE-70-P cells to cisplatin exposure and conducted immunoblotting analysis to assess the expression of eIF4E, Mcl-1, and Bcl-2. We observed a noteworthy increase in the levels of Mcl-1 and phosphorylated eIF4E after a 24-h treatment with cisplatin. In contrast, Bcl-2 expression remained unaltered (Fig. 1C and Supplementary Fig. 4). To further investigate the connection between eIF4E and Mcl-1, we depleted eIF4E in KYSE-70-P cells prior to administering cisplatin treatment. Our findings indicated that cisplatin led to a significant augmentation of Mcl-1 levels in eIF4E-depleted cells (Fig. 1D and Supplementary Fig. 5), suggesting that this upregulation of Mcl-1 is independent of eIF4E.

3.2. *Mcl-1 contributes to esophageal cancer cell chemo-resistance*

We delved into the impact of Mcl-1 on the functional characteristics of esophageal cancer cells in response to chemotherapy, employing both gain-of-function and loss-of-function approaches. We showed that esophageal cancer cells exhibiting Mcl-1 overexpression displayed notably reduced sensitivity to cisplatin, resulting in a less significant decrease in cell viability when compared to control cells (Fig. 2A and B and Supplementary Fig. 6). Conversely, Mcl-1 depletion substantially augmented the inhibitory effects of cisplatin on the viability of esophageal cancer cells (Fig. 2C and D and Supplementary Fig. 7). In addition, Mcl-1 depletion significantly decreased viability in chemo-resistant cells (Fig. 2E and F and Supplementary Fig. 8). These suggest that Mcl-1 plays a pivotal role in

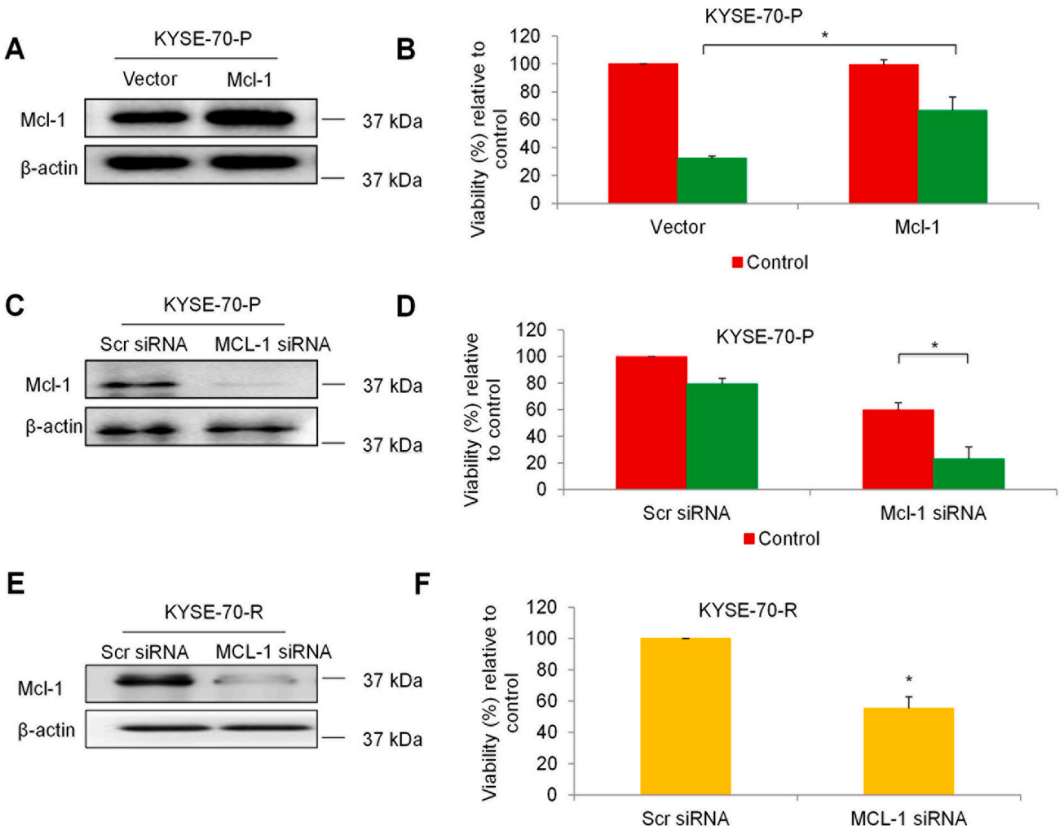

Fig. 2. The effects of Mcl-1 upregulation and downregulation in chemo-sensitive and chemo-resistant esophageal carcinoma cells. (A) Immunoblotting of Mcl-1 in KYSE-70-P cells after transfecting with Mcl-1-overexpressing plasmid. (B) Overexpression of Mcl-1 significantly alleviated the inhibitory effect of cisplatin in KYSE-70-P cell viability. (C) Immunoblotting of Mcl-1 in KYSE-70-R cells after transfecting with MCL-1 siRNA. (D) Depletion of Mcl-1 significantly enhanced the inhibitory effect of cisplatin in KYSE-70-P cell viability. (E) Immunoblotting of Mcl-1 in KYSE-70-R cells after transfecting with MCL-1 siRNA. (F) Depletion of Mcl-1 significantly decreased KYSE-70-R cell viability. *$P < 0.05$, compared to vector or Scr siRNA.

the response of esophageal cancer cells to chemotherapy, and inhibiting Mcl-1 activity proves effective in reversing chemoresistance.

3.3. *Mcl-1 upregulation in chemo-resistant esophageal cancer cells is mediated by increased stability and can be reversed by capivasertib*

The regulation of Mcl-1 expression is a multifaceted process involving both transcriptional and posttranslational mechanisms. To investigate these aspects, we conducted an examination of MCL-1 mRNA levels using quantitative PCR. We did not find any significant alterations in the mRNA levels within KYSE-70-P and KYSE-70-R cells (Fig. 3A). Phosphorylation of Mcl-1 at T163 contributes to its stability, while phosphorylation at S159 triggers ubiquitination and subsequent proteasomal degradation of Mcl-1 [18]. Our immunoblotting analysis unveiled distinct patterns in KYSE-70-R cells when compared to their parental counterparts, showing increased levels of Mcl-1, elevated *p*-Mcl-1 at T163, and reduced *p*-Mcl-1 at S159 (Fig. 3B and Supplementary Fig. 9), suggesting that Mcl-1 is upregulated due to enhanced stability and decreased degradation in chemo-resistant cells. Studies have shown that Akt upregulates Mcl-1 through multiple mechanisms, including activation of transcriptional factor CREB [19] and glucose metabolism [15]. In addition, AKT activation form a positive feedback loop in the process of MCL-1 protein synthesis [16]. Consistently, we observed increased *p*-Akt and p-S6 in KYSE-70-R cells (Fig. 3B and Supplementary Fig. 9), suggesting activation of Akt signaling pathway in chemo-resistant esophageal cancer cells. In line with previous studies [20,21], we further demonstrated that capivasertib induced an increase in Akt phosphorylation, but caused a decrease in Akt kinase activity, as substantiated by the diminished phosphorylation levels of Akt substrates, namely S6 and GSK3β, in KYSE-70-R cells (Fig. 3C and Supplementary Fig. 10). Notably, following capivasertib treatment, a significant reduction in the Mcl-1 protein levels was observed in KYSE-70-R cells.

3.4. *Capivasertib is effective in decreasing viability of chemo-resistant esophageal cancer cells and acts synergistically with cisplatin*

We evaluated the potential of capivasertib in reversing chemoresistance in esophageal cancer cells. We showed that capivasertib at low micromolar concentrations significantly decreased chemo-resistant cell viability in a dose-dependent manner (Fig. 4A). Furthermore, we employed Chou and Talalay's methodology [22] to investigate the combined effects of capivasertib with cisplatin in chemo-sensitive cells. The resulting combination index (CI) theorem of Chou-Talalay offers quantitative definition for additive effect

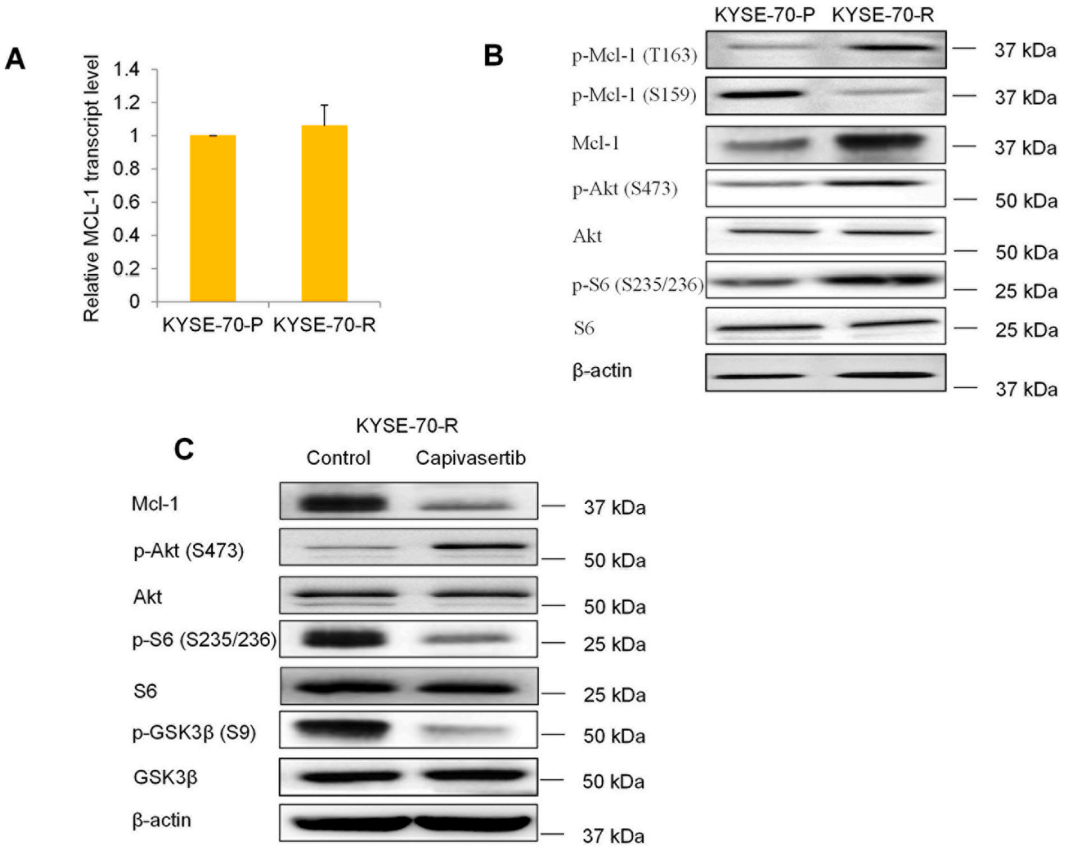

Fig. 3. Mcl-1 degradation is decreased and Akt signaling is increased in chemo-resistant esophageal carcinoma cells. (A) Transcriptional levels of MCL-1 in KYSE-70-P and KYSE-70-R cells. (B) Immunoblotting of *p*-Mcl-1 (T163), *p*-Mcl-1 (S159), Mcl-1, *p*-Akt (S473), Akt, p-S6(S235/236) and S6 in KYSE-70-P and KYSE-70-R cells. (C) Immunoblotting of Mcl-1, *p*-Akt (S473), Akt, p-S6(S235/236), S6, *p*-GSK3β (S9) and GSK3β in KYSE-70-R cells in the absence and presence of capivasertib (1 μM).

(CI = 1), synergism (CI < 1), and antagonism (CI > 1) in drug combinations. As demonstrated in the CI plot, we observed CI values consistently below 1 across the entire spectrum of affected fraction, ranging from 0 % to 100 %, in KYSE-70 cells (Fig. 4B). This result demonstrates that the combination of capivasertib and cisplatin is synergistic. OE33 and FLO-1 cell lines, commonly employed to simulate primary and metastatic esophageal cancer *in vitro*, exhibit diverse cellular origins and genetic profiles [23,24]. To confirm the combinatory effects of capivasertib and cisplatin, we performed combination studies on OE33 and FLO-1 cell lines. Notably, the synergy between capivasertib and cisplatin is not limited to KYSE-70 cells; OE33 and FLO-1 cells responded in a similar manner (Fig. 4C and D). In addition, the CI50 values of capivasertib and paclitaxel were also below 1 in all tested esophageal cancer cell lines (Supplementary Fig. 11).

3.5. Capivasertib displays therapeutic efficacy in esophageal cancer mouse models

We finally evaluated the translational potential of capivasertib in multiple esophageal cancer mouse models. Athymic mice were inoculated with KYSE-70-R cells to establish chemo-resistant esophageal cancer cell model. Mice exhibited good tolerance to 50 mg/kg capivasertib, as evidenced by the absence of weight loss or any discernible abnormal appearance (data not shown). Capivasertib effectively inhibited the growth of chemo-resistant esophageal cancer, resulting in a 50 % reduction in tumor size after 20 days treatment (Fig. 5A). However, tumors in the capivasertib group resumed growth and reached a size comparable to that of the control group after an additional 20 days. Immunohistochemistry analysis of tumor sections demonstrated that capivasertib led to a decrease in *p*-GSK3β and p-S6 *in vivo* (Fig. 5B and Supplementary Fig. 12).

In chemo-sensitive esophageal cancer models established using OE-33 and FLO-1 cells, both capivasertib and cisplatin, when administered as single drugs at doses that modestly inhibited tumor growth, exhibited remarkable effectiveness when used in combination. This combination therapy resulted in a consistent and substantial decrease in tumor growth throughout the treatment duration (Fig. 5C and D). Notably, even after 55 days of treatment, the tumor size in the combination group reached only 50 % and 40 % of the size of control tumors in the OE33 and FLO-1 models, respectively.

Moreover, in the case of mice subjected to capivasertib treatment, there was a noteworthy increase in their overall survival times when compared to the control group. The median survival time was 45 days for the treated mice in contrast to 25 days for the control

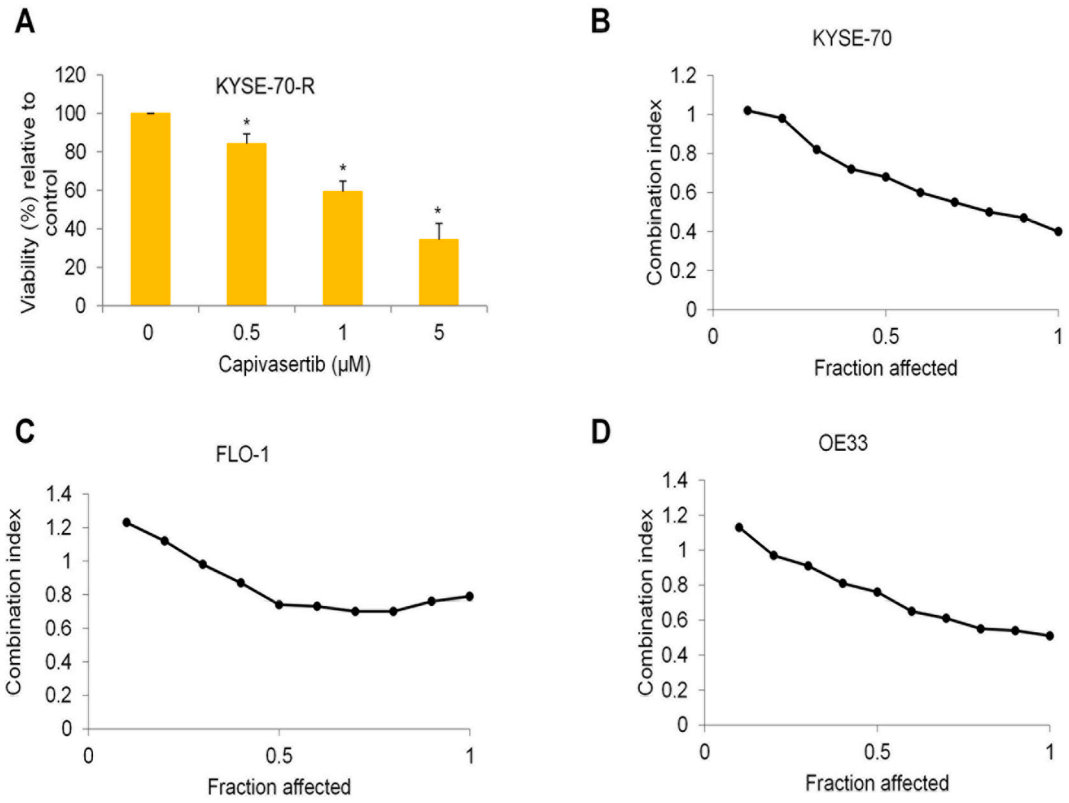

Fig. 4. Capivasertib is active against chemo-resistant esophageal carcinoma cells and acts synergistically with cisplatin in chemo-sensitive esophageal carcinoma cells. (A) Capivasertib at 0.5 μM, 1 μM, and 5 μM significantly decreases viability of KYSE-70-R cells. (B to D) Isobologram analysis of combination index (CI) values in KYSE-70, FLO-1 and OE33 cells. Fraction affected (Fa) versus combination index plots were generated using the method of Chou and Talalay to determine the extent of synergy. A CI < 0.9 indicates synergism, while CI > 1.1 indicates antagonism between the two combined drugs. *, $p < 0.05$, compared to control.

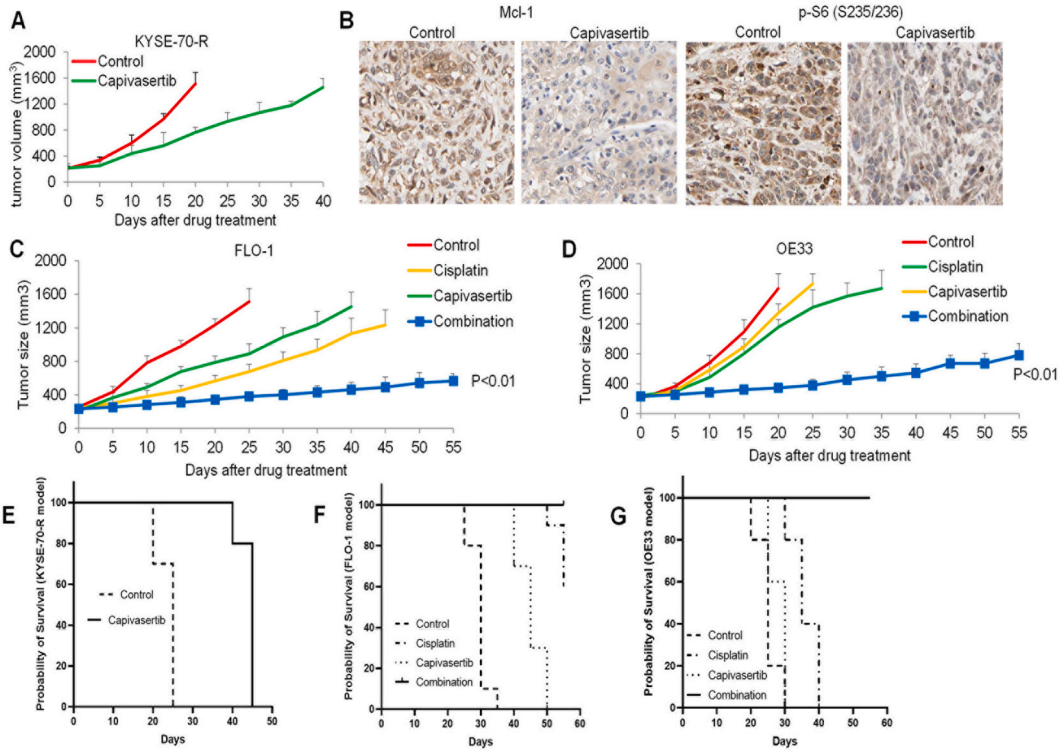

Fig. 5. The *in vivo* efficacy of capivasertib alone and with cisplatin in esophageal carcinoma in mice. (A) Capivasertib significantly decreased KYSE-70-R growth in mice. Capivasertib at 50 mg/kg was administrated daily by oral gavage. (B) The levels of phospho-Akt and phospho-S6 in subcutaneous tumors receiving vehicle control and capivasertib determined by IHC. The representative IHC images were shown. Each group consisted of 6 mice, and the experiment was conducted twice. (C) Combination of capivasertib and cisplatin resulted in a significant better efficacy than single drug alone in inhibiting OE33 tumor growth in mice. Capivasertib at 20 mg/kg was administrated daily by oral gavage. Cisplatin at 0.5 mg/kg was administrated by intraperitoneal injection twice weekly. (D) Combination of capivasertib and cisplatin resulted in a significant better efficacy than single drug alone in inhibiting FLO-1 tumor growth in mice. Capivasertib at 30 mg/kg was administrated daily by oral gavage. Cisplatin at 0.75 mg/kg was administrated by intraperitoneal injection twice weekly. Each group consisted of 10 mice, and the experiment was conducted once. (E to G) Kaplan-Meier curves representing the survival probability of each treatment group in KYSE-70-R, FLO-1 and OE33 xenograft models. Each group consisted of 10 mice, and the experiment was conducted once.

group (Fig. 5E). Additionally, combination in both the OE33 and FLO-1 models exhibited a significant extension in overall survival for the mice in comparison to the control group (Fig. 5F and G).

4. Discussion

The underlying molecular mechanisms driving drug resistance in advanced esophageal cancer remain largely elusive, posing significant challenges to improving clinical outcomes. Our study addresses this gap by uncovering novel insights into the role of Mcl-1 in chemotherapy resistance and identifying capivasertib as a promising therapeutic agent to overcome resistance in esophageal cancer. Our prior investigation pinpointed eIF4E, a vital translational factor, especially significant for tumor cells [25], as a promoter of growth and survival in esophageal cancer cells [17]. Building upon this foundation, the present study reveals a previously unrecognized link between Mcl-1 upregulation and chemotherapy resistance in esophageal cancer cells. Furthermore, we demonstrate that this resistance can be reversed through the use of the Akt inhibitor capivasertib. These findings hold great promise for the advancement of treatment options for esophageal cancer that are resistant to chemotherapy.

Elevated MCL-1 expression has been observed in a wide range of human malignancies, including hematological malignancies and solid tumors. Importantly, high levels of MCL-1 expression have been associated with aggressive tumor phenotypes, resistance to chemotherapy, and poor clinical outcomes in many cancer types [14]. Recent research has highlighted the contribution of Mcl-1 upregulation to drug resistance in solid cancers. For instance, Mcl-1 upregulation alleviates the apoptosis induced by the BET bromodomain inhibitor JQ1 in breast cancer [26]. Mcl-1 upregulation is also a defining feature of resistance to venetoclax in small cell lung cancer [27]. In cases of leukemia, Mcl-1 overexpression results in resistance to venetoclax and azacytidine [28]. In line with these previous findings, our study reveals that Mcl-1 is upregulated in esophageal cancer cells, both under short-term and prolonged exposure conditions. Furthermore, our functional analysis demonstrates that Mcl-1 plays a crucial role in regulating the response of esophageal cancer cells to cisplatin. While a small number of MCL-1 inhibitors have entered clinical trials for cancer treatment, none

have yet received clinical approval [29]. In our efforts to identify drugs capable of mitigating Mcl-1 upregulation, we analyzed the underlying mechanisms responsible for this upregulation in esophageal cancer cells.

Although Mcl-1 is the downstream target of eIF4E-regulated protein synthesis [30], our research indicates that Mcl-1 upregulation is not dependent on eIF4E, as it remains elevated in cells even after eIF4E depletion. A recent study has shown that Mcl-1 protein stability increases in radiation-resistant nasopharyngeal carcinoma cells compared to their radiation-sensitive counterparts, a phenomenon attributed to the regulation of reactive oxygen species (ROS) and Akt signaling. This is because repeated exposure to ionizing radiation results in a sustained elevation of ROS production, promoting continuous activation of AKT signaling [16]. The Akt/Mcl-1 signaling pathway is well-documented for its role in conveying antiapoptotic signals, whether in chronic lymphocytic leukemia B cells or in mediating resistance to Bcl-2/PARP inhibitors [15,31,32]. Consistent with prior studies, our research demonstrates that Mcl-1 upregulation primarily occurs at the post-translational level, where activated Akt enhances stability and reduces the ubiquitination of Mcl-1. This finding aligns with the common observation that PI3K/Akt activation is a prevalent occurrence in various human cancers and a major driver of resistance to chemotherapy [11]. While the PI3K/Akt signaling pathway is widely recognized for its role in promoting cancer cell survival and resistance to treatment [33,34], the mechanisms governing its regulation in esophageal cancer remain not well known. Research has suggested that long non-coding RNA PCAT5 and the transcription factor HOXC10 may serve as activators of Akt in esophageal cancer [35,36].

A significant finding of this study is our identification of capivasertib, an Akt inhibitor that is also an FDA-approved anti-breast cancer medication with well-established pharmacological characteristics [13,37], as a promising therapeutic agent for overcoming chemotherapy resistance in esophageal cancer. Through targeting the Akt signaling pathway, capivasertib effectively reduces Mcl-1 levels, mitigating the mechanisms driving resistance to conventional chemotherapeutic agents like cisplatin. Importantly, our findings demonstrate that capivasertib not only decreases the viability of chemo-resistant esophageal cancer cells but also synergistically enhances the effects of cisplatin, significantly inhibiting tumor growth and extending overall survival in preclinical mouse models. While our preclinical mouse model provides valuable insights into the therapeutic efficacy of capivasertib in overcoming chemotherapy resistance, it's essential to recognize the limitations of mouse models in predicting clinical outcomes in human patients. Further validation in clinically relevant models, such as patient-derived xenografts, is warranted.

Furthermore, our combination index analysis clearly indicated that capivasertib synergizes with cisplatin. Importantly, the combined treatment of capivasertib and cisplatin exhibited significantly enhanced efficacy in inhibiting esophageal cancer growth in mice and extending overall survival. This finding aligns with previous reports that have emphasized the potent combinatory effects of capivasertib with anti-cancer agents in overcoming Akt-related resistance in various other cancers. For instance, when combined with irradiation, capivasertib substantially reduced the size of oral squamous cell carcinoma in an orthotopic mouse model [21]. The combination of capivasertib with SERD fulvestrant has proven effective in preclinical models of palbociclib-resistant estrogen receptor-positive breast cancer [30]. Given that capivasertib is already an approved drug for breast cancer treatment, our findings hold significant promise for expediting clinical trials aimed at repurposing capivasertib for the treatment of advanced esophageal cancer. However, factors such as potential side effects of capivasertib (eg, diarrhea, decreased lymphocytes and hyperglycemia), patient selection criteria, and treatment regimen optimization will need to be carefully considered in future clinical investigations.

While it's important to note that Akt/Mcl-1 may not represent the exclusive mechanism responsible for chemotherapy resistance, our findings unequivocally illustrate that Akt activation plays a critical role in preserving Mcl-1 stability, and inhibiting this pathway is an effective approach to counteract chemo-resistance in esophageal cancer (Fig. 6). In summary, our research strongly suggests that capivasertib has the potential to be a valuable treatment option for patients in advanced stages of esophageal cancer.

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Data availability statement

Data will be made available upon reasonable request the corresponding author:

CRediT authorship contribution statement

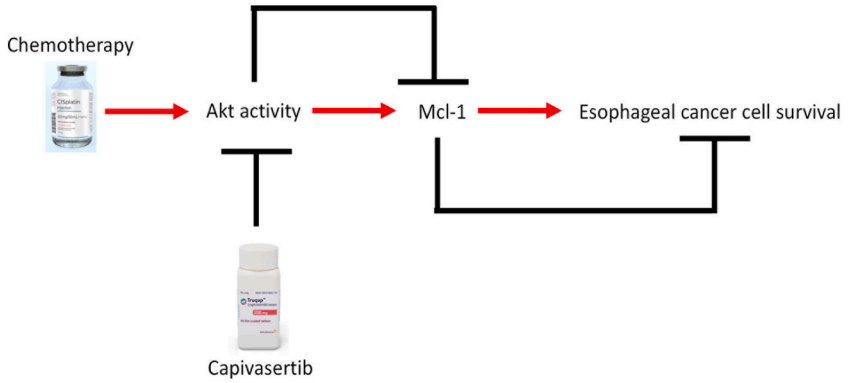
Jindan Kai: Writing – original draft, Investigation, Formal analysis, Data curation. **Kai Kang:** Writing – original draft, Investigation, Data curation. **Zhixiao Jiang:** Writing – review & editing, Formal analysis. **Fei Xiong:** Writing – review & editing, Investigation. **Sheng Wang:** Writing – review & editing, Supervision, Funding acquisition, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33567.



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References

[1] S. Lander, E. Lander, M.K. Gibson, Esophageal cancer: overview, risk factors, and reasons for the rise, Curr. Gastroenterol. Rep. (2023), https://doi.org/10.1007/s11894-023-00899-0.

[2] Y. Liu, Perioperative immunotherapy for esophageal squamous cell carcinoma: now and future, World J. Gastroenterol. 29 (34) (2023) 5020–5037, https://doi.org/10.3748/wjg.v29.i34.5020.

[3] J.A. Weiss, S. Jain, Neoadjuvant and adjuvant therapy in esophageal cancer, J. Gastrointest. Oncol. 14 (4) (2023) 1927–1932, https://doi.org/10.21037/jgo-22-735.

[4] W. Zhang, M. Zhu, Y. Xiang, Y. Sun, S. Li, J. Cai, et al., Current and future perspectives in unresectable locally advanced esophageal squamous cell cancer (Review), Oncol. Rep. 51 (5) (2024), https://doi.org/10.3892/or.2024.8724.

[5] M. Lohan-Codeco, M.L. Barambo-Wagner, L.E. Nasciutti, L.F. Ribeiro Pinto, N. Meireles Da Costa, A. Palumbo Jr., Molecular mechanisms associated with chemoresistance in esophageal cancer, Cell. Mol. Life Sci. 79 (2) (2022) 116, https://doi.org/10.1007/s00018-022-04131-6.

[6] B.L.V. de Godoy, M.G. Moschetta-Pinheiro, L.G.A. Chuffa, N.F. Ponde, R.J. Reiter, J. Colombo, et al., Synergistic actions of Alpelisib and Melatonin in breast cancer cell lines with PIK3CA gene mutation, Life Sci. 324 (2023) 121708, https://doi.org/10.1016/j.lfs.2023.121708.

[7] I.A. Voutsadakis, KRAS mutated colorectal cancers with or without PIK3CA mutations: clinical and molecular profiles inform current and future therapeutics, Crit. Rev. Oncol. Hematol. 186 (2023) 103987, https://doi.org/10.1016/j.critrevonc.2023.103987.

[8] S. Daher, A. Zer, R. Tschernichovsky, R. Yacobi, I. Barshack, S. Tsabari, et al., Driver mutation characteristics of phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) in advanced non-small cell lung cancer, Lung Cancer 178 (2023) 229–236, https://doi.org/10.1016/j.lungcan.2023.02.023.

[9] J. Yao, Q. You, X. Zhang, Y. Zhang, J. Xu, X. Zhao, et al., PIK3CA somatic mutations as potential biomarker for immunotherapy in elder or TP53 mutated gastric cancer patients, Clin. Genet. 103 (2) (2023) 200–208, https://doi.org/10.1111/cge.14260.

[10] D.C. Lin, J.J. Hao, Y. Nagata, L. Xu, L. Shang, X. Meng, et al., Genomic and molecular characterization of esophageal squamous cell carcinoma, Nat. Genet. 46 (5) (2014) 467–473, https://doi.org/10.1038/ng.2935.

[11] A. Glaviano, A.S.C. Foo, H.Y. Lam, K.C.H. Yap, W. Jacot, R.H. Jones, et al., PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer, Mol. Cancer 22 (1) (2023) 138, https://doi.org/10.1186/s12943-023-01827-6.

[12] X. Huang, L. You, E. Nepovimova, M. Psotka, D. Malinak, M. Valko, et al., Inhibitors of phosphoinositide 3-kinase (PI3K) and phosphoinositide 3-kinase-related protein kinase family (PIKK), J. Enzym. Inhib. Med. Chem. 38 (1) (2023) 2237209, https://doi.org/10.1080/14756366.2023.2237209.

[13] A. Andrikopoulou, S. Chatzinikolaou, E. Panourgias, M. Kaparelou, M. Liontos, M.A. Dimopoulos, et al., The emerging role of capivasertib in breast cancer, Breast 63 (2022) 157–167, https://doi.org/10.1016/j.breast.2022.03.018.

[14] A.H. Wei, A.W. Roberts, A. Spencer, A.S. Rosenberg, D. Siegel, R.B. Walter, et al., Targeting MCL-1 in hematologic malignancies: rationale and progress, Blood Rev. 44 (2020) 100672, https://doi.org/10.1016/j.blre.2020.100672.

[15] J.L. Coloff, A.N. Macintyre, A.G. Nichols, T. Liu, C.A. Gallo, D.R. Plas, et al., Akt-dependent glucose metabolism promotes Mcl-1 synthesis to maintain cell survival and resistance to Bcl-2 inhibition, Cancer Res. 71 (15) (2011) 5204–5213, https://doi.org/10.1158/0008-5472.CAN-10-4531.

[16] Y.Y. Liang, F.Y. Niu, A.A. Xu, L.L. Jiang, C.S. Liu, H.P. Liang, et al., Increased MCL-1 synthesis promotes irradiation-induced nasopharyngeal carcinoma radioresistance via regulation of the ROS/AKT loop, Cell Death Dis. 13 (2) (2022) 131, https://doi.org/10.1038/s41419-022-04551-z.

[17] J. Kai, Y. Wang, F. Xiong, S. Wang, Genetic and pharmacological inhibition of eIF4E effectively targets esophageal cancer cells and augments 5-FU's efficacy, J. Thorac. Dis. 10 (7) (2018) 3983–3991, https://doi.org/10.21037/jtd.2018.06.43.

[18] M. Sancho, D. Leiva, E. Lucendo, M. Orzaez, Understanding MCL1: from cellular function and regulation to pharmacological inhibition, FEBS J. 289 (20) (2022) 6209–6234, https://doi.org/10.1111/febs.16136.

[19] J.M. Wang, J.R. Chao, W. Chen, M.L. Kuo, J.J. Yen, H.F. Yang-Yen, The antiapoptotic gene mcl-1 is up-regulated by the phosphatidylinositol 3-kinase/Akt signaling pathway through a transcription factor complex containing CREB, Mol. Cell Biol. 19 (9) (1999) 6195–6206, https://doi.org/10.1128/MCB.19.9.6195.

[20] L. Lang, C. Shay, X. Zhao, Y. Xiong, X. Wang, Y. Teng, Simultaneously inactivating Src and AKT by saracatinib/capivasertib co-delivery nanoparticles to improve the efficacy of anti-Src therapy in head and neck squamous cell carcinoma, J. Hematol. Oncol. 12 (1) (2019) 132, https://doi.org/10.1186/s13045-019-0827-1.

[21] L. Lang, T. Lam, A. Chen, C. Jensen, L. Duncan, F.C. Kong, et al., Circumventing AKT-associated radioresistance in oral cancer by novel nanoparticle-encapsulated capivasertib, Cells 9 (3) (2020), https://doi.org/10.3390/cells9030533.

[22] T.C. Chou, Drug combination studies and their synergy quantification using the Chou-Talalay method, Cancer Res. 70 (2) (2010) 440–446, https://doi.org/10.1158/0008-5472.CAN-09-1947.

[23] D.S. Liu, S.J. Hoefnagel, O.M. Fisher, K.K. Krishnadath, K.G. Montgomery, R.A. Busuttil, et al., Novel metastatic models of esophageal adenocarcinoma derived from FLO-1 cells highlight the importance of E-cadherin in cancer metastasis, Oncotarget 7 (50) (2016) 83342–83358, https://doi.org/10.18632/oncotarget.13391.

[24] Y. Shimada, M. Imamura, T. Wagata, N. Yamaguchi, T. Tobe, Characterization of 21 newly established esophageal cancer cell lines, Cancer 69 (2) (1992) 277–284, https://doi.org/10.1002/1097-0142(19920115)69:2<277::aid-cncr2820690202>3.0.co;2-c.

[25] M. Bhat, N. Robichaud, L. Hulea, N. Sonenberg, J. Pelletier, I. Topisirovic, Targeting the translation machinery in cancer, Nat. Rev. Drug Discov. 14 (4) (2015) 261–278, https://doi.org/10.1038/nrd4505.

[26] X. Wang, X. Wei, Y. Cao, P. Xing, Mcl-1 inhibition overcomes BET inhibitor resistance induced by low FBW7 expression in breast cancer, J. Cell Mol. Med. 26 (5) (2022) 1672–1683, https://doi.org/10.1111/jcmm.17210.

[27] Z. Valko, Z. Megyesfalvi, A. Schwendenwein, C. Lang, S. Paku, N. Barany, et al., Dual targeting of BCL-2 and MCL-1 in the presence of BAX breaks venetoclax resistance in human small cell lung cancer, Br. J. Cancer 128 (10) (2023) 1850–1861, https://doi.org/10.1038/s41416-023-02219-9.

[28] J.L. Carter, Y. Su, X. Qiao, J. Zhao, G. Wang, M. Howard, et al., Acquired resistance to venetoclax plus azacitidine in acute myeloid leukemia: in vitro models and mechanisms, Biochem. Pharmacol. 216 (2023) 115759, https://doi.org/10.1016/j.bcp.2023.115759.
[29] H. Wang, M. Guo, H. Wei, Y. Chen, Targeting MCL-1 in cancer: current status and perspectives, J. Hematol. Oncol. 14 (1) (2021) 67, https://doi.org/10.1186/s13045-021-01079-1.
[30] H.G. Wendel, R.L. Silva, A. Malina, J.R. Mills, H. Zhu, T. Ueda, et al., Dissecting eIF4E action in tumorigenesis, Genes Dev. 21 (24) (2007) 3232–3237, https://doi.org/10.1101/gad.1604407.
[31] P.G. Longo, L. Laurenti, S. Gobessi, S. Sica, G. Leone, D.G. Efremov, The Akt/Mcl-1 pathway plays a prominent role in mediating antiapoptotic signals downstream of the B-cell receptor in chronic lymphocytic leukemia B cells, Blood 111 (2) (2008) 846–855, https://doi.org/10.1182/blood-2007-05-089037.
[32] A.R. Mattoo, A. Joun, J.M. Jessup, Repurposing of mTOR complex inhibitors attenuates MCL-1 and sensitizes to PARP inhibition, Mol. Cancer Res. 17 (1) (2019) 42–53, https://doi.org/10.1158/1541-7786.MCR-18-0650.
[33] X. Wang, W. Jiang, Y. Du, D. Zhu, J. Zhang, C. Fang, et al., Targeting feedback activation of signaling transduction pathways to overcome drug resistance in cancer, Drug Resist. Updates 65 (2022) 100884, https://doi.org/10.1016/j.drup.2022.100884.
[34] Y. Hu, Y. Xu, T. Zhang, Q. Han, L. Li, M. Liu, et al., Cisplatin-activated ERbeta/DCAF8 positive feedback loop induces chemoresistance in non-small cell lung cancer via PTEN/Akt axis, Drug Resist. Updates 71 (2023) 101014, https://doi.org/10.1016/j.drup.2023.101014.
[35] H. Liu, H. Yin, T. Yang, J. Lin, T. Sun, Long non-coding RNA PCAT5 regulates the progression of esophageal squamous cell carcinoma via miR-4295/PHF20, Heliyon 9 (12) (2023) e22086, https://doi.org/10.1016/j.heliyon.2023.e22086.
[36] X. He, H. Wang, R. Wang, Y. Li, S. Li, X. Cao, et al., HOXC10 promotes esophageal squamous cell carcinoma progression by targeting FOXA3 and indicates poor survival outcome, Heliyon 9 (10) (2023) e21056, https://doi.org/10.1016/j.heliyon.2023.e21056.
[37] R. Bartsch, E. Bergen, Asco 2018: highlights in HER2-positive metastatic breast cancer, Memo 11 (4) (2018) 280–283, https://doi.org/10.1007/s12254-018-0441-x.