SPANDIDOS PUBLICATIONS

MOLECULAR MEDICINE REPORTS 21: 1399-1407, 2020

Anti-proliferative effect of cardamonin on mTOR inhibitor-resistant cancer cells

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Received July 5, 2019; Accepted December 4, 2019

DOI: 10.3892/mmr.2019.10898

Abstract. A number of mammalian target of rapamycin (mTOR) inhibitors have been approved for the treatment of certain types of cancer or are currently undergoing clinical trials. However, mTOR targeted therapy exerts selective pressure on tumour cells, which leads to the preferential growth of resistant subpopulations. There are two classes of mTOR inhibitors: i) The rapalogs, such as rapamycin, which bind to the 12-kDa FK506-binding protein/rapamycin-binding domain of mTOR; and ii) the ATP-competitive inhibitors, such as AZD8055, which block the mTOR kinase domain. Cardamonin inhibits mTOR by decreasing the expression of regulatory-associated protein of mTOR (Raptor), a mechanism of action which differs from the currently available mTOR inhibitors. The present study investigated the inhibitory effects of cardamonin on mTOR inhibitor-resistant cancer cells. HeLa cervical cancer cells and MCF-7 breast cancer cells were exposed to high concentrations of mTOR inhibitors, until resistant clones emerged. Cytotoxicity was measured using the MTT and colony forming assays. The inhibitory effect of cardamonin on mTOR signalling was assessed by western blotting. The resistant cells were less sensitive to mTOR inhibitors compared with the parental cells. Consistent with the anti-proliferation effect, rapamycin and AZD8055 had no effect on the phosphorylation of rapamycin-sensitive sites on ribosomal protein S6 kinase B1 (S6K1) and AZD8055-sensitive sites on protein kinase B and eukaryotic translation initiation factor 4E binding protein 1 (Thr 37/46), respectively, in rapamycin- and AZD8055-resistant cells. Cardamonin inhibited cell proliferation and decreased the phosphorylation of mTOR and S6K1, as well as the protein level of raptor, in the mTOR inhibitor-resistant cells. Therefore, cardamonin may serve as a therapeutic agent for patients with cervical and breast cancer resistant to mTOR inhibitors.

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Key words: cardamonin, mTOR inhibitor, drug resistance, Raptor, breast cancer, cervical cancer

Introduction

The mammalian target of rapamycin (mTOR) plays a central role in cell physiology and controls several cellular functions, including proliferation, growth, survival, autophagy and metabolism (1). mTOR has emerged as a critical effector of cell-signalling pathways commonly upregulated in several types of human cancer and is a major target for cancer therapy (2).

mTOR exists in two functionally and structurally distinct multiprotein complexes termed mTOR complex (mTORC) 1 and mTORC2 (Fig. 1). mTORC1 contains the regulatory-associated protein of mTOR (Raptor), proline-rich protein kinase B (Akt) substrate 40 kDa, G-protein β-subunit-like protein/LST8 (GβL) and DEP domain containing mTOR-interacting protein (DEPTOR). Raptor is an essential component of mTORC1 and recruits ribosomal protein S6 kinase B1 (S6K1) to mTOR for phosphorylation (3). mTORC2 is mainly comprised of a rapamycin-insensitive companion of mTOR, GβL and DEPTOR. mTORC2 phosphorylates Akt on Ser 473 and eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1) on Thr 37/46 (4).

Two classes of mTOR inhibitors are currently in clinical use or undergoing clinical trials for cancer treatment (5). Rapamycin was the first mTOR inhibitor to be identified. The rapalogs (rapamycin and its analogues) form a gain-of-function complex with 12-kDa FK506-binding protein (FKBP12), which binds to the FKBP12/rapamycin-binding (FRB) domain of mTOR (6). The rapamycin/FKBP12 complex allosterically inhibits kinase activity of mTOR and disrupts the association of Raptor with mTORC1 (7). Additionally, it inhibits the phosphorylation of S6K1, but has a lesser impact on the phosphorylation of EIF4EBP1 (Thr 37/46) (8). Rapalogs may be used to treat a wide range of malignancies and numerous clinical trials have been performed in cancer patients (9,10). However, the efficacy of rapalogs as monotherapy for patients with breast cancer, kidney cancer and pancreatic neuroendocrine tumours is not as promising as initially expected, as only a subset of patients exhibit objective responses to rapalogs and the responses are frequently short-lived (10). Acquired resistance has emerged as a barrier to the antineoplastic activity of

this class of mTOR inhibitors (11,12). The second generation of mTOR inhibitors includes mTORC1/mTORC2 dual inhibitors, such as AZD8055, torin 1 and PP242. These inhibitors target the ATP-binding site of the mTOR kinase domain and are collectively called ATP-competitive mTOR inhibitors (13). AZD8055 exhibited more powerful antiproliferative and proapoptotic effects, as well as more complete inhibition of mTORC1, compared with rapalogs in preclinical studies, which likely results from its additional inhibitory effect on Akt and EIF4EBP1 phosphorylation (14). Several ATP-competitive mTOR inhibitors have been or are currently being investigated in clinical trials for a wide variety of malignancies (11,15). Unfortunately, primary or acquired resistance has begun to emerge (16). A previous study investigating a xenografted model of human breast cancer revealed that AZD8055 treatment completely inhibited tumour growth. However, after 11 days of treatment, tumour regrowth was observed (17).

Targeted cancer therapy exerts selective pressure on tumour cells, which leads to the preferential growth of resistant subpopulations and necessitates the development of next generation therapies to treat the resistant cancer. Cardamonin is as an mTOR inhibitor that has been shown to decrease the proliferation of various cancer cells (18-20). Recent studies have demonstrated that cardamonin and its analogues decrease the proliferation of non-small-cell lung cancer cells and prevent metastasis by inhibiting the mTOR signalling pathway (21,22). Furthermore, Jin *et al* (23) revealed that cardamonin modulates cell metabolism by repressing the activities of mTOR and S6K1 in breast cancer cells. Cardamonin, unlike rapamycin, inhibits mTOR without the involvement of FKBP12 (20). Additionally, cardamonin has no effect on the phosphorylation of Akt, which is decreased by AZD8055 (24). Cardamonin has previously been shown to inhibit the mTORC1 signalling pathway by decreasing the protein level of Raptor (24,25). You *et al* (26) demonstrated that cardamonin exerts cardioprotective effects in left ventricular remodelling by disrupting the mTOR-Raptor association, suggesting that cardamonin is a specific mTORC1 inhibitor.

In the present study, resistant MCF-7 breast cancer cells and HeLa cervical cancer cells were generated by exposing the parental cells to gradually increasing concentrations of rapamycin or AZD8055. The inhibitory effect of cardamonin on the proliferation and the mTOR signaling pathway in the rapamycin- and AZD8055-resistant cells was subsequently investigated.

Materials and methods

Chemical reagents. Cell culture supplies were purchased from Gibco; Thermo Fisher Scientific, Inc. AZD8055 was obtained from AstraZeneca Pharmaceuticals. Cardamonin, rapamycin and MTT were purchased from Sigma-Aldrich; Merck KGaA. Antibodies against mTOR (rabbit mAb; cat. no. 2972), phosphorylated (p)-mTOR (Ser 2448; rabbit mAb; cat. no. 2971), S6K1 (rabbit mAb; cat. no. 9202), p-S6K1 (Thr 389; rabbit mAb; cat. no. 9205), Akt (rabbit mAb; cat. no. 9272), p-Akt (Ser 473; rabbit mAb; cat. no. 4060), EIF4EBP1 (rabbit mAb; cat. no. 9452), p-EIF4EBP1 (T37/46; rabbit mAb; cat. no. 9459), Raptor (rabbit mAb; cat. no. 2280), β-actin (rabbit mAb; cat. no. 4970) and the secondary antibody (anti-rabbit IgG, mouse horseradish peroxidase-linked; cat. no. 7074) were purchased from Cell Signalling Technologies, Inc.

Cell culture. MCF-7 and HeLa cells were obtained from The Cell Bank of the Type Culture Collection of the Chinese Academy of Sciences. MCF-7 cells were cultured in a 1:1 mixture of DMEM:F12 medium (HyClone; GE Healthcare Life Sciences) supplemented with 4 mM glutamine. HeLa cells were cultured in RPMI 1640 media (HyClone; GE Healthcare Life Sciences). Both media were supplemented with 10% FBS (HyClone; GE Healthcare Life Sciences), penicillin (100 U/ml) and streptomycin (100 μg/ml). The cells were incubated at 37˚C with 5% CO_2.

Selection of drug resistant clones. Resistant MCF-7 breast cancer and HeLa cervical cancer cells were generated by exposing the parental cells to a gradually increasing concentration (5-500 nM) of either rapamycin or AZD8055 over 8 months. The media was replaced weekly. The resistant cells were subsequently generated through a single cell clone selection and tested for sensitivity to rapamycin or AZD8055. The resistant cells were passaged in drug-free media over 12 months. The sensitivity of the two clones with the greatest resistance (i.e., the highest IC_{50} to rapamycin and AZD8055, respectively) to rapamycin or AZD8055 was assessed.

Cell viability assay. The MTT assay was used to analyse the effect of cardamonin on cell viability as previously described (20). Parental MCF-7, HeLa and mTOR inhibitor resistant MCF-7, HeLa cells ($5x10^3$ cells per well) were seeded in 96-well plates and cultured overnight. A total of 20 μl rapamycin or AZD8055 at the indicated concentrations (0.1, 0.3, 1, 3, 10, 30, 100, 300, 1,000, 3,000 and 10,000 nM) or cardamonin at the indicated concentrations (1, 1.8, 3.2, 5.6, 10, 18, 32, 56, 100, 180 and 320 μM) was added to each well and the cells were incubated for 48 h. A total of 10 μl MTT (5 mg/ml) solution was added to each well and the cells were incubated for an additional 4 h. The purple formazan crystals were dissolved using DMSO and the number of surviving cells was assessed by determining the optical density at a wavelength of 570 nm using a microplate reader. The IC_{50} was determined by fitting to a standard 4-parameter logistic using GraphPad Prism software (version 5; GraphPad Software, Inc.).

Clonogenic survival assay. The clonogenic survival assay was performed as previously described (20). Parental MCF-7, HeLa and mTOR inhibitor resistant MCF-7, HeLa cells ($1x10^3$/well) were seeded in a 6-well plate and incubated overnight. The cells were treated with rapamycin or AZD8055 for 48 h, followed by two washes with their respective media. The cells were subsequently cultured for 7 days. Cells were fixed with ethanol (75%) at room temperature for 15 min and stained with 1% crystal violet at room temperature for 60 min. Colonies (>30 cells/colony) were counted using a Leica DMIL LED microscope (magnification, x200; Leica Microsystems GmbH) in triplicate wells. Five independent experiments were performed.

Western blotting. The parental MCF-7, HeLa and mTOR inhibitor resistant MCF-7, HeLa cells were treated with rapamycin or AZD8055 for 48 h. The cells were subsequently

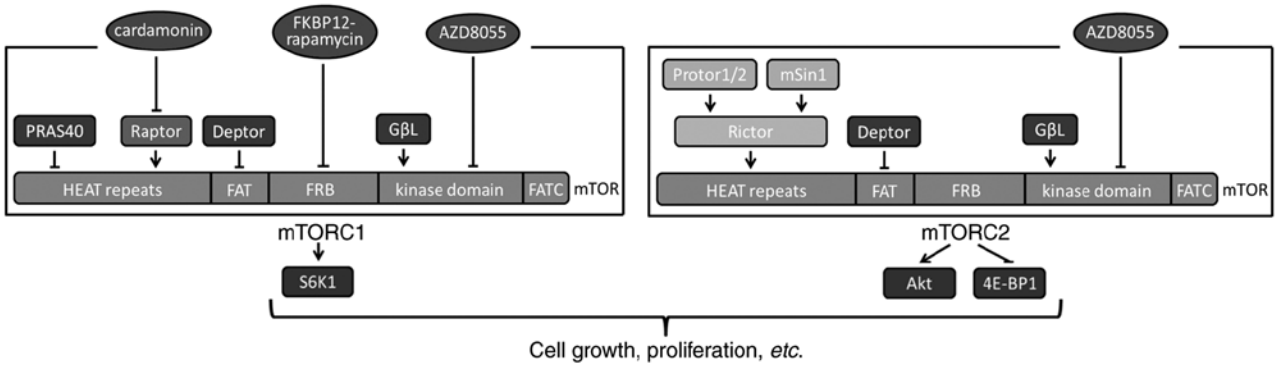

Figure 1. Established components and inhibitors of mTORC1 and mTORC2. mTORC, mammalian target of Rapamycin complex; Akt, protein kinase B; S6K1, ribosomal protein S6 kinase B1; Raptor, regulatory-associated protein of mTOR; Deptor, DEP domain containing mTOR-interacting protein.

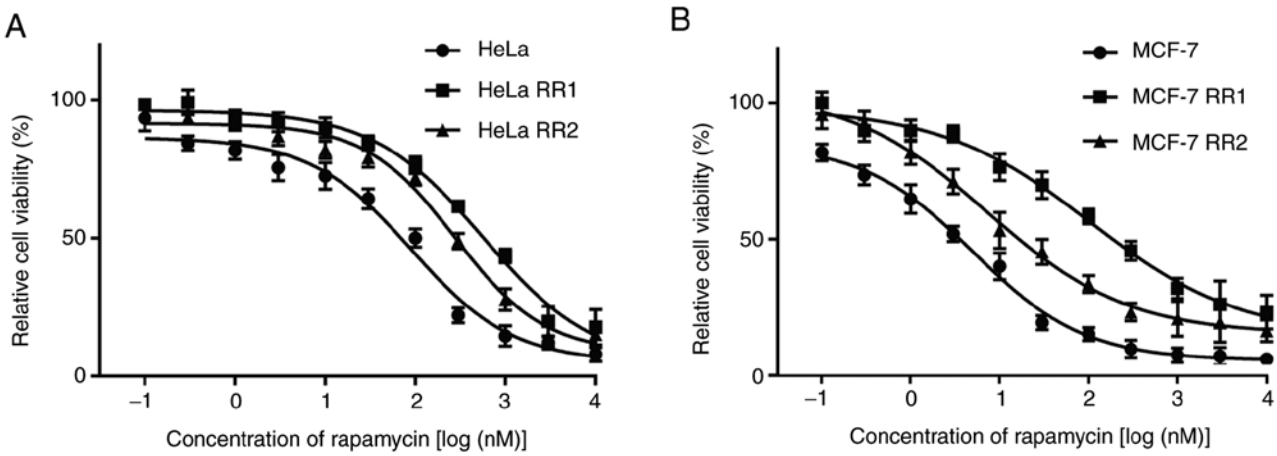

Figure 2. Dose-dependent cell growth inhibition curves of parental and RR cells. (A) HeLa RR1 and HeLa RR2 and (B) MCF-7, MCF-7 RR1 and MCF-7 RR2 cells were treated with rapamycin for 48 h. Cell viability was detected using the MTT assay as the absorbance ratio between drug-exposed cells and untreated control cells. Data are presented as the mean ± standard deviation. n=5. All experiments were repeated at least three times. RR, rapamycin-resistant.

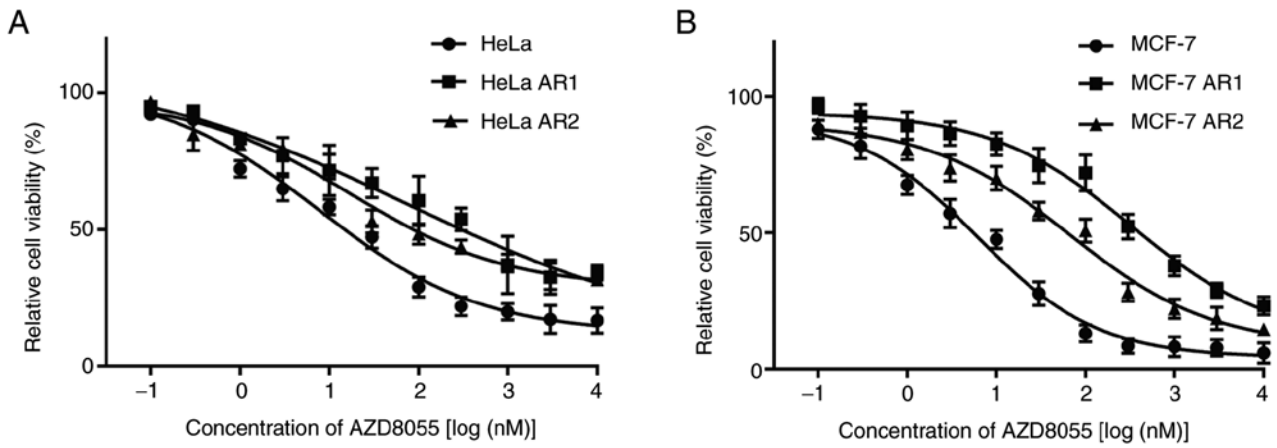

Figure 3. Dose-dependent cell growth inhibition curves of parental and AR cells. (A) HeLa, HeLa AR1 and HeLa AR2 and (B) HeLa, HeLa AR1 and HeLa AR2 cells were treated with AZD8055 for 48 h. Cell viability was detected using the MTT assay as the absorbance ratio between drug-exposed cells and untreated control cells. Data are presented as the mean ± standard deviation. n=5. All experiments were repeated at least three times. AR, AZD8055-resistant.

washed twice with ice-cold PBS and lysed using radioimmunoprecipitation assay lysis buffer [20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% NP-40, 1% sodium deoxycholate, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4 and 1 μg/ml leupeptin] and 1 mM phenylmethylsulfonyl fluoride for 20 min at 4˚C. The lysates were centrifuged at 15,000 x g at 4˚C for 20 min. The total protein concentration in the supernatant was determined using a bicinchoninic acid assay and 40 μg protein/lane was separated via 6-12% SDS-PAGE. The separated proteins

were subsequently transferred onto a polyvinylidene difluoride membrane and blocked for 1 h at room temperature with 5% bovine serum albumin (Cell Signalling Technologies, Inc.) in 1X TBST (0.1% Tween 20). The membrane was incubated overnight at 4˚C with the primary antibodies (all used at a 1:1,000). The membrane was subsequently incubated the horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin (1:2,000) at room temperature for 1 h. Protein bands were visualized using enhanced chemiluminescence (SignalFire ECL reagent; cat. no. 6883; Cell Signalling Technologies, Inc) and an X-ray film.

Statistical analysis. All data are presented as the mean ± standard deviation. Statistical analyses were performed using SPSS software (version 19; IMB Corp.). The one-way analysis of variance followed by the Tukey-Kramer multiple comparison test was used to compare the different groups. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Generation of HeLa and MCF-7 clones with acquired resistance to rapamycin or AZD8055. Rapamycin-resistant (RR) and AZD8055-resistant (AR) HeLa and MCF-7 cells were generated by culturing parental HeLa and MCF-7 cells with a gradually increasing concentration of rapamycin or AZD8055 for 8 months. Parental HeLa and MCF-7 cells were sensitive to rapamycin and AZD8055. The resistance of the selected cell clones was determined using the MTT assay. The sensitivity of the two clones with the greatest resistance (i.e., the highest IC_{50} to rapamycin and AZD8055, respectively) to rapamycin or AZD8055 was assessed. RR and AR clones were significantly less sensitive to their respective drugs than the parental cells at 48 h (Figs. 2 and 3; Tables I and II). The clone with the highest resistance was selected for subsequent experiments. These clones retained resistance to rapamycin or AZD8055 when passaged in drug-free media over 12 months, demonstrating that the acquired resistance was not transient.

Cardamonin inhibits proliferation of the mTOR inhibitor-resistant cells. The inhibitory activity of cardamonin in the parental and resistant cells was investigated. Cardamonin inhibited the proliferation of HeLa and MCF-7 cells. However, unlike rapamycin and AZD8055, cardamonin also had an inhibitory effect on the growth of RR and AR HeLa and MCF-7 cells (Fig. 4). The inhibitory efficacy of cardamonin on the resistant cells was similar to the parental cells (Table III).

Cardamonin inhibits clone formation of the mTOR inhibitor-resistant cells. Consistent with the results of the proliferation assay, the RR and AR MCF-7 and HeLa cells were less sensitive to rapamycin and AZD8055 in the clone formation assay when compared with the parental cells. The resistant cells maintained full sensitivity to cardamonin (Figs. 5 and 6).

Cardamonin inhibits the activity of mTORC1 in the mTOR inhibitor-resistant cells. In the rapamycin-resistant cells, phosphorylation of the rapamycin-sensitive site on S6K1 (T389) was unaffected even at higher concentrations of rapamycin (300 nM). Phosphorylation of the key mTORC2 effectors, Akt and EIF4EBP1, was unaffected by rapamycin

Table I. IC_{50} of rapamycin in the cells.

Cells	IC_{50} (μM)
HeLa	91.22±13.21
HeLa RR1	569.8±32.25[a]
HeLa RR2	279.5±20.54[a]
MCF-7	5.01±0.61
MCF-7 RR1	204.4±28.43[b]
MCF-7 RR2	27.32±5.13[b]

Varying concentrations of rapamycin were tested *in vitro* on HeLa and HeLa RR as well as MCF-7 cells and MCF-7 RR. The IC_{50} was determined by fitting to a standard 4-parameter logistic using GraphPad Prism software (version 5). [a]$P<0.01$ vs. HeLa, [b]$P<0.01$ vs. MCF-7. Data are presented as the mean ± standard deviation. n=5. RR, rapamycin-resistant; AR, AZD8055-resistant.

Table II. IC_{50} of AZD8055 in the cells.

Cells	IC_{50} (μM)
HeLa	9.75±1.29
HeLa AR1	247.4±21.25[a]
HeLa AR2	76.8±9.05[a]
MCF-7	6.28±1.64
MCF-7 AR1	325.6±22.65[b]
MCF-7 AR2	66.78±7.43[b]

Varying concentrations of AZD8055 were tested *in vitro* on HeLa and HeLa AR as well as MCF-7 and MCF-7 AR. The IC_{50} was determined by fitting to a standard 4-parameter logistic using GraphPad Prism software (version 5). [a]$P<0.01$ vs. HeLa, [b]$P<0.01$ vs. MCF-7. Data are presented as the mean ± standard deviation. n=5. RR, rapamycin-resistant; AR, AZD8055-resistant.

Table III. IC_{50} of cardamonin in the cells.

Cells	IC_{50} (μM)
HeLa	23.71±3.96
HeLa RR	28.56±2.43
HeLa AR	30.40±3.08
MCF-7	35.52±3.87
MCF-7 RR	37.35±3.92
MCF-7 AR	38.14±2.04

Varying concentrations of cardamonin were tested *in vitro* on HeLa and HeLa RR and HeLa AR as well as MCF-7, MCF-7 RR and MCF-7 AR. The IC_{50} was determined by fitting to a standard 4-parameter logistic using GraphPad Prism software (version 5). Data are presented as the mean ± standard deviation. n=5. RR, rapamycin-resistant; AR, AZD8055-resistant.

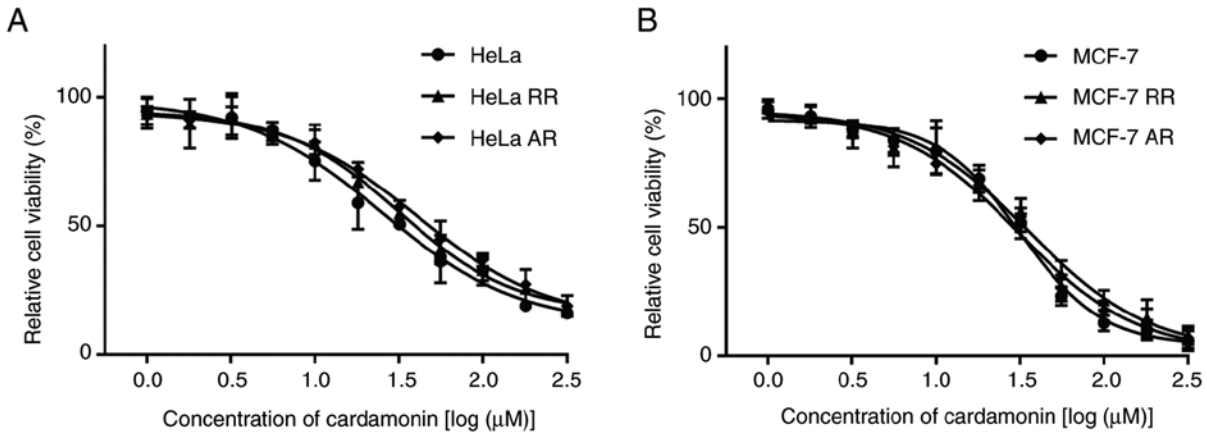

Figure 4. Dose-dependent cell growth inhibition curves of cardamonin on parental and resistant cells. (A) Hela, HeLa RR, HeLa AR and (B) MCF-7, MCF-7 RR, MCF-7 AR cells were treated with cardamonin for 48 h. Cell viability was detected using the MTT assay as the absorbance ratio between drug-exposed cells and untreated control cells. Data are presented as the mean ± standard deviation. n=5. All experiments were repeated at least three times. RR, rapamycin-resistant; AR, AZD8055-resistant.

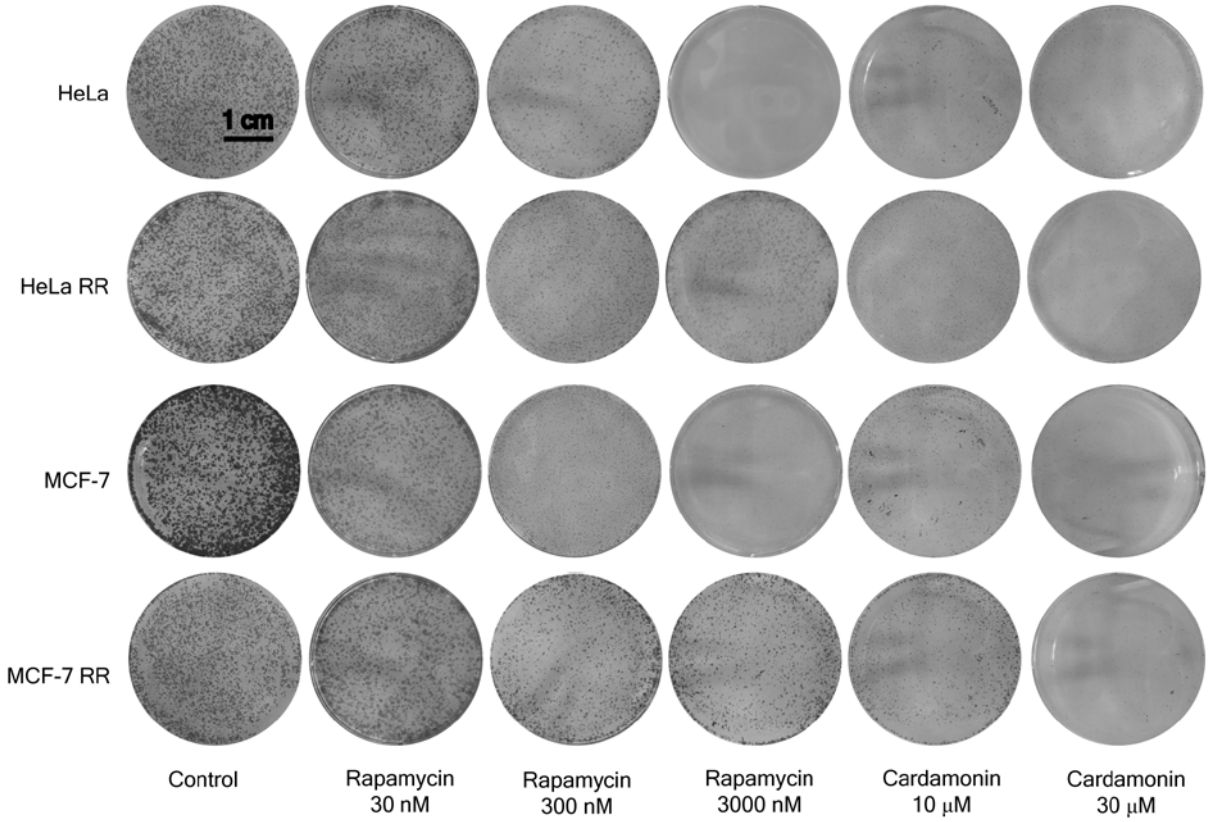

Figure 5. Effect of cardamonin on the clone formation of RR cells. HeLa, HeLa RR, MCF-7, MCF-7 RR cells were trypsinized, counted and plated at a density of $1x10^3$ cells/well in 6-well plates. Cells were treated with rapamycin for 48 h. Cells were then cultured in medium containing 10% foetal bovine serum and allowed to proliferate for 7 days. Colonies were then stained with crystal violet. HeLa RR, rapamycin-resistant HeLa cells; MCF-7 RR, rapamycin-resistant MCF-7 cells.

but strongly reduced by AZD8055. In the AR cells, phosphorylation of Akt and EIF4EBP1 was less sensitive to AZD8055. As expected, in the parental and resistant cells, the phosphorylation of mTOR and its substrate S6K1 was inhibited by cardamonin while that of Akt and EIF4EBP1 was not affected. Interestingly, compared with AZD8055 and rapamycin, cardamonin decreased the protein level of Raptor in RR and AR cells (Figs. 7 and 8).

Discussion

The PI3K/Akt/mTOR signalling pathway is commonly upregulated in cancer and increasing evidence has demonstrated that mTOR is a key signalling transduction node in this pathway. Increased mTOR activity is a prominent feature of cancer cells (5,27). Therefore, mTOR has emerged as an important molecular target for the treatment of cancer (28).

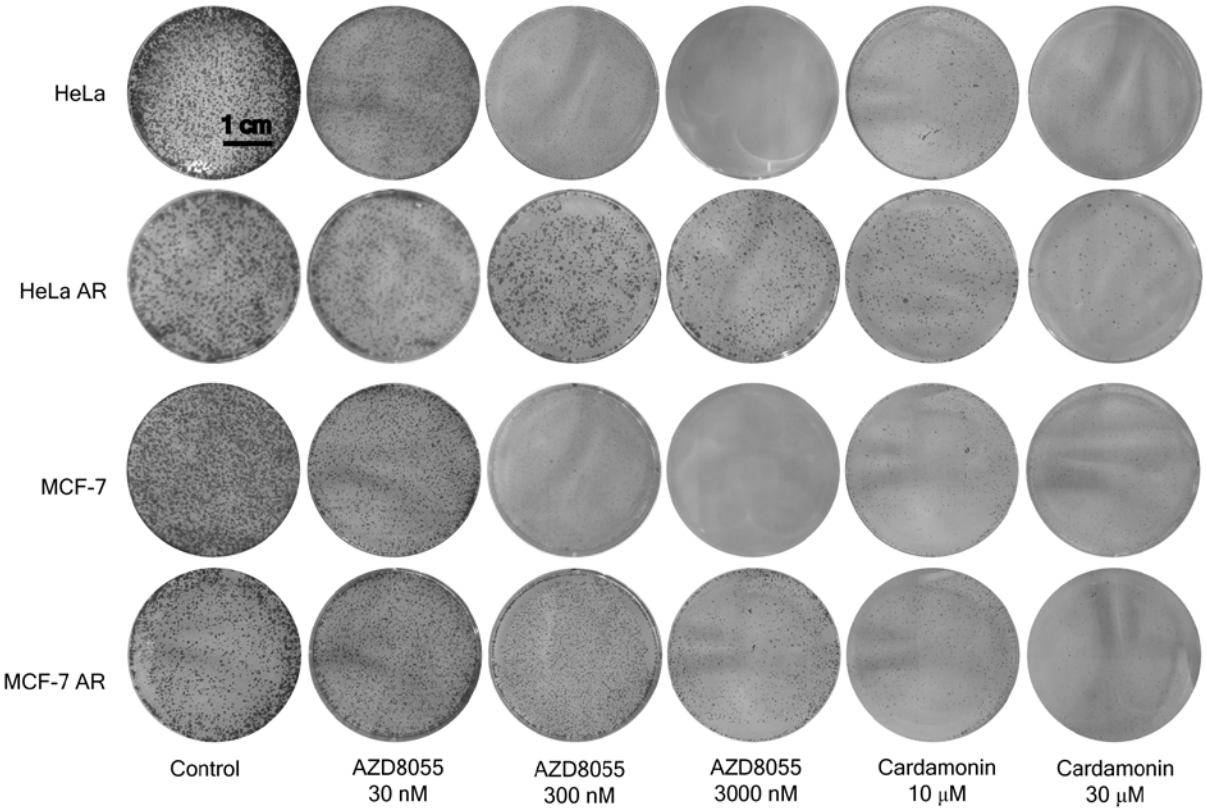

Figure 6. Effect of cardamonin on the clone formation of AR cells. HeLa, HeLa AR, MCF-7, MCF-7 AR cells were trypsinized, counted and plated at a density of $1x10^3$ cells/well in 6-well plates. Cells were treated with AZD8055 for 48 h. Cells were then cultured in medium containing 10% foetal bovine serum and allowed to proliferate for 7 days. Colonies were then stained with crystal violet. HeLa AR, AZD8055-resistant HeLa cells; MCF-7 AR, AZD8055-resistant MCF-7 cells.

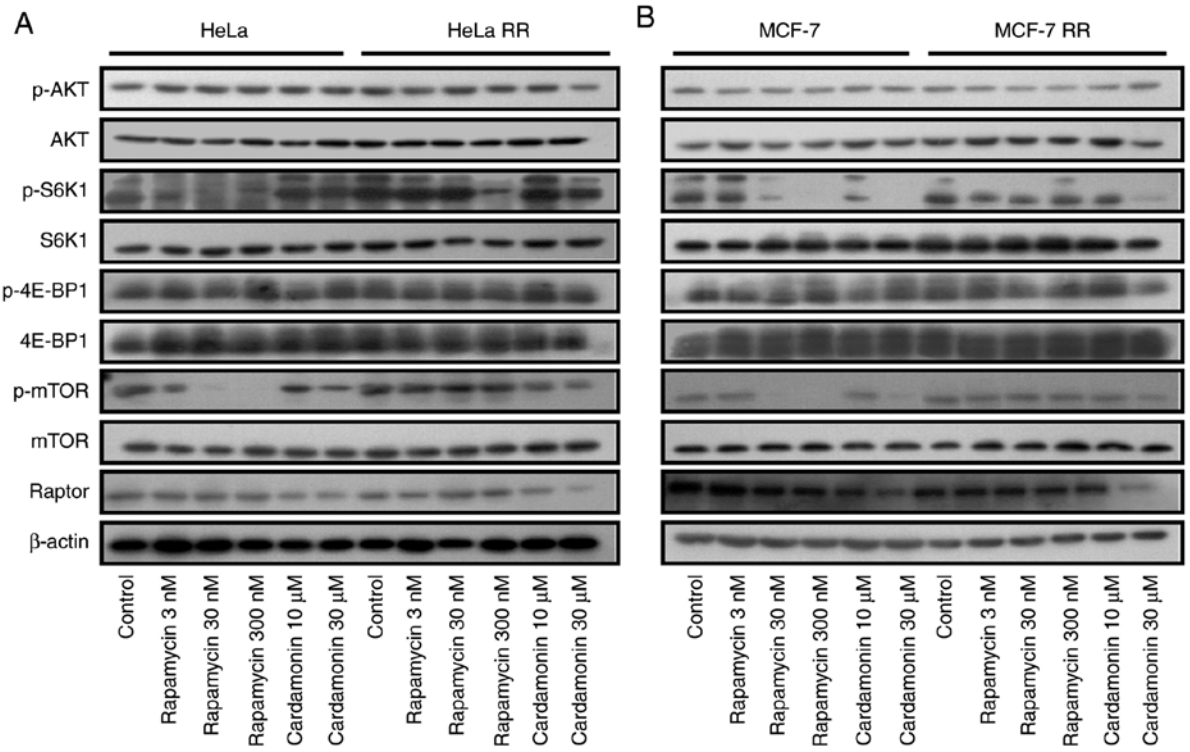

Figure 7. Cardamonin inhibits the mTORC1 signalling pathway in rapamycin-resistant cells. (A) HeLa, HeLa RR and (B) MCF-7, MCF-7 RR cells were treated with rapamycin for 48 h. The protein levels of Akt, p-Akt, S6K1, p-S6K1, 4E-BP1, p-4E-BP1, mTOR, p-mTOR and Raptor were determined by western blotting. β-actin was used as the loading control. n=3. HeLa RR, rapamycin-resistant HeLa cells; MCF-7 RR, rapamycin-resistant MCF-7cells. mTORC1, mammalian target of rapamycin complex 1; p, phosphorylated; S6K1, ribosomal protein S6 kinase B1; 4EBP1, eukaryotic translation initiation factor 4E binding protein 1; Akt, protein kinase B.

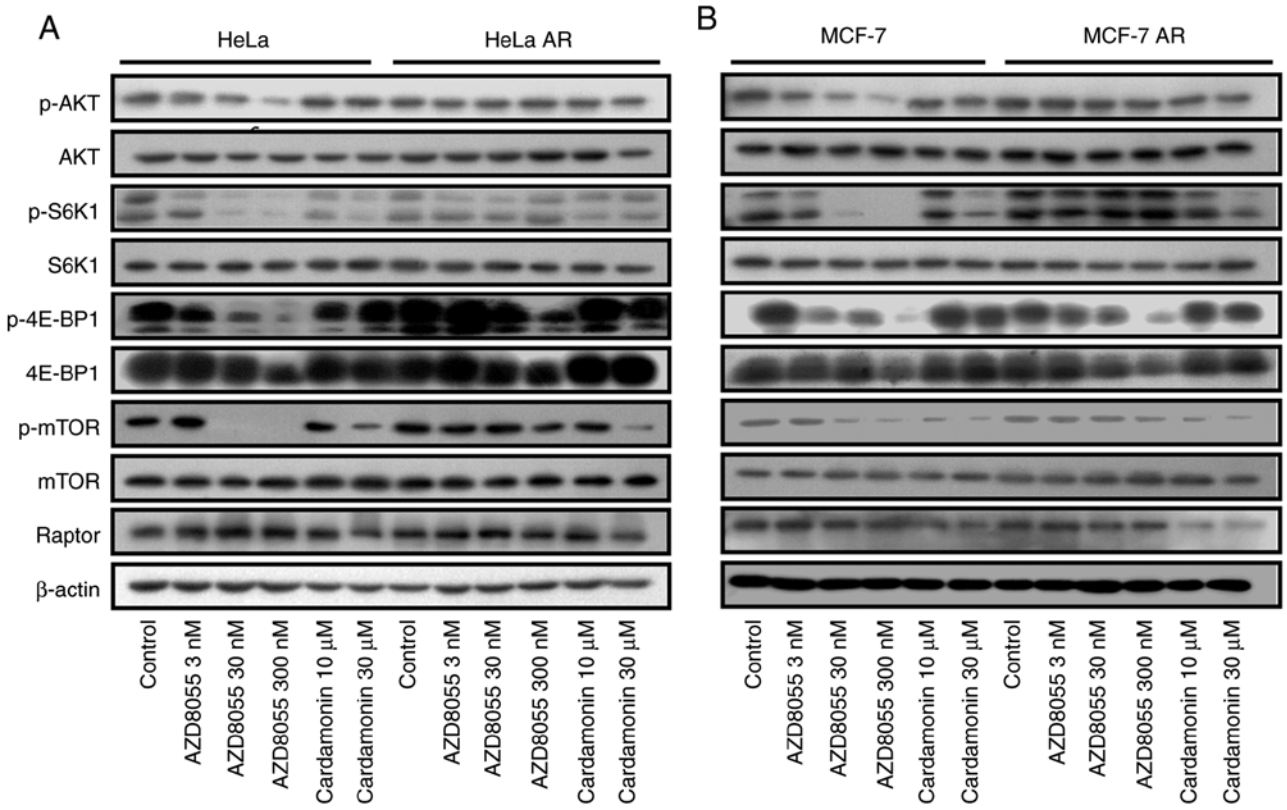

Figure 8. Cardamonin inhibits the mTORC1 signalling pathway in AR cells. (A) HeLa, HeLa AR and (B) MCF-7, MCF-7 AR cells were treated with AZD8055 for 48 h. The protein levels of Akt, p-Akt, S6K1, p-S6K1, 4E-BP1, p-4E-BP1, mTOR, p-mTOR and Raptor were determined by western blotting. β-actin was used as the loading control. n=3. HeLa AR, AZD8055-resistant HeLa cells; MCF-7 AR, AZD8055-resistant MCF-7 cells; mTORC1, mammalian target of rapamycin complex 1; p, phosphorylated; S6K1, ribosomal protein S6 kinase B1; 4EBP1, eukaryotic translation initiation factor 4E binding protein 1; Akt, protein kinase B.

Several mTOR inhibitors have already undergone clinical trials for various types of cancer; however, the results of these trials are not satisfactory (29,30).

Rapalogs, including temsirolimus and everolimus, have been approved by the FDA for the treatment of certain advanced-stage tumours (31,32). However, the efficacy of these agents is limited by the emergence of resistance (11). Exposure of MCF-7 breast cancer cells or HeLa cervical cancer cells to high doses of either rapamycin or AZD8055 for 8 months results in the emergence of resistant cells (33,34). A previous study reported that the mTOR sequence of AZD8055-resistant cells harboured mutations in the mTOR kinase domain while that of rapamycin-resistant cells contained mutations in the FRB domain (35). The present study did not perform mTOR sequencing, however, the resistant MCF-7 and HeLa cells generated were shown to be insensitive to rapamycin or AZD8055. Furthermore, the proliferation and clone formation of MCF-7 and HeLa cells were sensitive to rapamycin and AZD8055 at clinically relevant levels, while the RR and AR clones were significantly less so. In addition, in the RR cells, phosphorylation of the rapamycin-sensitive sites on S6K1 (T389) was unaffected even at high concentrations of rapamycin. In the AR cells, phosphorylation of Akt and EIF4EBP1 was less sensitive to AZD8055. The present study revealed that cardamonin significantly inhibited the viability and clone formation of parental and resistant cells. The emergence of acquired resistance is the main reason for the lack of efficacy of mTOR inhibitors in clinical practice (35). However, the role of mTOR inhibitors in cancer treatment continues to evolve as novel compounds are continuously being developed.

The mechanisms of acquired resistance to mTOR inhibitors have not been fully elucidated but may include metabolic alterations, S6K1-dependent feedback reactivation of the PI3K/Akt signalling pathway and mTOR mutations (36). mTOR mutations have attracted special interest and random mutagenesis screens in yeast demonstrated that single amino acid changes in the FRB domain conferred rapamycin resistance (37). The mutation in the FRB domain disrupted the interaction of mTOR with the FKBP12-rapalogs complex (35,38,39). As mutations occur in the FRB domain rather than the kinase domain, the mutant protein remains sensitive to inhibition by direct ATP-competitive mTOR kinase inhibitors (35). mTOR mutations in the kinase domain of AR clones may increase the understanding of the structure of the mTOR kinase domain-kinase inhibitor complex. AZD8055 binds to mTOR with similar affinities in both parental and mutant cancer cells; however, mutations in the kinase domain increase the activity of mTOR and cells with these mutations are still sensitive to rapalogs (35). An increased understanding of the mechanisms of acquired mTOR inhibitor resistance may lead to the development of novel therapeutic strategies.

Cardamonin is a specific mTORC1 inhibitor, which decreases the protein level of Raptor, disrupts the interaction

of mTOR and Raptor and interrupts the mTORC1 signalling cascade (24-26). Therefore, cardamonin inhibits mTOR independent of the FRB domain and the kinase domain. Recent studies suggested that Raptor should be included in the pharmacodynamic evaluation of mTOR inhibitor trials (40,41). Everolimus-resistant breast cancer cells exhibited recovery of mTORC1 signalling and Raptor upregulation (41). Earwaker *et al* (40) revealed that Raptor upregulation was implicated in resistance to mTOR kinase inhibitors in renal cancer cells. Therefore, the present study investigated whether cardamonin could overcome mTOR resistance by decreasing Raptor in breast and cervical cancer cells. As expected, the results demonstrated that the phosphorylation of mTORC1 and S6K1, as well as the protein level of Raptor, in RR and AR cells were decreased following treatment with cardamonin. The potential application of cardamonin in other types of cancer requires further investigation.

In conclusion, the results obtained in the present study suggested that cardamonin may serve as a novel therapeutic agent for the treatment of patients with cervical and breast cancer that have acquired resistance to either rapalogs or ATP-competitive inhibitors.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Natural Science Foundation of Fujian Province (grant. no. 2019J01515), the Innovative Medical Foundation of Fujian Provincial Health and Family Planning Commission (grant. no. 2016-CX-13) and Foundations of Fujian Provincial Maternity and Children's Hospital (grant. nos. YCXM 18-05 and YCXQ 18-18).

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Authors' contributions

PN and DS conceived and designed the experiments. JL performed the experiments. HC and JZ analysed the data. YZ prepared the material and performed the experiments. PN and DS wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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