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Research Article

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Dichloroacetic acid and rapamycin synergistically inhibit tumor progression

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Abstract: Mammalian target of rapamycin (mTOR) controls cellular anabolism, and mTOR signaling is hyperactive in most cancer cells. As a result, inhibition of mTOR signaling benefits cancer patients. Rapamycin is a US Food and Drug Administration (FDA)-approved drug, a specific mTOR complex 1 (mTORC1) inhibitor, for the treatment of several different types of cancer. However, rapamycin is reported to inhibit cancer growth rather than induce apoptosis. Pyruvate dehydrogenase complex (PDHc) is the gatekeeper for mitochondrial pyruvate oxidation. PDHc inactivation has been observed in a number of cancer cells, and this alteration protects cancer cells from senescence and nicotinamide adenine dinucleotide (NAD^+) exhaustion. In this paper, we describe our finding that rapamycin treatment promotes pyruvate dehydrogenase E1 subunit alpha 1 (PDHA1) phosphorylation and leads to PDHc inactivation dependent on mTOR signaling inhibition in cells. This inactivation reduces the sensitivity of cancer cells' response to rapamycin. As a result, rebooting PDHc activity with dichloroacetic acid (DCA), a pyruvate dehydrogenase kinase (PDK) inhibitor, promotes cancer cells' susceptibility to rapamycin treatment in vitro and in vivo.

Key words: Dichloroacetic acid (DCA); Rapamycin; Pyruvate dehydrogenase E1 subunit alpha 1 (PDHA1); Mammalian target of rapamycin (mTOR)

1 Introduction

Mammalian target of rapamycin (mTOR) controls cell anabolic metabolism, including protein translation and DNA and RNA synthesis (Beretta et al., 1996; Robitaille et al., 2013; Valvezan et al., 2017). This signaling is controlled by two individual TOR complexes: mTORC1 and mTORC2 (Kim and Guan, 2019). In most cancer cells, mTOR signaling is hyperactive as a result of demand for building blocks (Menon and Manning, 2008). mTORC2 promotes metastasis by controlling the actin cytoskeleton (Jacinto et al., 2004; Zhang et al., 2019). Meanwhile, TOR1 complex is inhibited by rapamycin-12-kDa FK506-binding protein (FKBP12) (Sabatini et al., 1994), which prevents substrates from entering the kinase active site (Yang et al., 2017). Although mTORC2 is insensitive to rapamycin, prolonged treatment with rapamycin can inhibit mTORC2 signaling by dissociating mTORC2 assembly (Sarbassov et al., 2006; Lamming et al., 2012). Moreover, rapamycin severely reduces tumor angiogenesis by inhibiting mTOR signaling (Phung et al., 2006). To date, mTOR inhibition has shown little effect in restricting the growth of cancer cells compared to chemotherapeutics. Inhibition of mTOR induces autophagy, which protects cancer cells from the stress of poor nutrition (Levy et al., 2017; Bai et al., 2022). In addition, mTOR signaling inhibition limits the cancer-cell growth rate rather than induces apoptosis (Kim and Guan, 2019). As a result, combining mTOR inhibition with other therapies produces significantly better therapeutic effects (Lorusso, 2016; Zhuang et al., 2016).

Pyruvate dehydrogenase complex (PDHc) catalyzes pyruvate converting to acetyl-coenzyme A (AcCoA), which is further synthesized to citrate (Patel et al., 2014). PDHc enzymatic activity is tightly controlled

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by reversible phosphorylation of pyruvate dehydrogenase E1 subunit alpha 1 (PDHA1), which is mediated by pyruvate dehydrogenase kinase (PDK) and pyruvate dehydrogenase phosphatase 1/2 (PDP1/2) (Holness and Sugden, 2003). In several cancer cells, PDHc activity is downregulated, and this downregulation promotes nicotinamide adenine dinucleotide (NAD^+) regeneration (Luengo et al., 2021) and resistance to senescence (Kaplon et al., 2013).

Recent studies have demonstrated that mitochondrial Ras homolog enriched in brain (Rheb) regulates PDHc activity via binding to PDP1/2, independent of mTOR signaling (Yang et al., 2021). However, we observed that not only rapamycin treatment but also amino acid starvation strongly induces PDHA1 phosphorylation by inhibiting mTOR signaling. Therefore, this study aims to investigate the interplay between mTOR signaling and PDHA1 phosphorylation in cancer cells, and the potential therapeutic implications of dichloroacetic acid (DCA) and rapamycin.

2 Results

2.1 PDHA1 phosphorylation induced by rapamycin treatment

A previous study demonstrated that Rheb regulates PDHc activity via PDHA1 phosphorylation, independent of mTOR signaling (Yang et al., 2021). However, rapamycin (a well-known mTORC1 inhibitor) and its clinical derivative compound everolimus significantly promote the phosphorylation of PDHA1 in MDA-MB-231 cells (Fig. 1a). In line with this, we found that rapamycin treatment significantly increased phosphorylation of PDHA1: P-s232 and P-s300 in 4T1 cells; P-s300 in CT26 cells; P-s232 in B16F10 cells; and P-s232 and P-s300 in Hepa1-6 cells. However, it almost completely inhibited phosphorylation of ribosomal protein S6 in several different cell lines (Figs. 1b–1e). Moreover, even a much lower concentration of rapamycin (2.5 nmol/L) completely inhibited S6 phosphorylation and activated PDHA1 phosphorylation (Fig. 1f). These results indicated that rapamycin treatment induced PDHA1 phosphorylation, and this was a widespread phenomenon in different cell lines.

2.2 mTORC1 involved in PDHA1 regulation

Since rapamycin is a specific mTORC1 inhibitor, we speculated that it induces PDHA1 phosphorylation dependent on mTOR signaling. Next, to demonstrate this hypothesis, we starved cells with different amino acids for 6 h to activate mTOR signaling. We observed that arginine and glutamine starvation significantly inhibited S6 phosphorylation; however, leucine starvation did not affect S6 phosphorylation within 6 h (Fig. 2a), so it may require prolonged starvation. Notably, phosphorylation of s293-PDHA1 was upregulated by arginine and glutamine starvation (Fig. 2a), indicating that inhibition of mTOR signaling induces PDHA1 phosphorylation. In addition, S6 phosphorylation was almost completely inhibited by rapamycin within 1 h (Fig. 2b), and three phosphorylation sites of PDHA1 increased in a time-dependent manner. Especially, the s293-PDHA1 responded to rapamycin treatment at an early time point—30 min (Fig. 2b). This phenomenon was also confirmed with time-dependent glutamine starvation and re-addition (Figs. 2c and 2d). Finally, we found that fetal bovine serum (FBS) and glutamine stimulated S6 phosphorylation and inhibited PDHA1 phosphorylation (Fig. 2e); however this effect was inversed by rapamycin treatment (Fig. 2e). Taken together, rapamycin-inhibited mTOR signaling increases PDHA1 phosphorylation.

2.3 Role of DCA in rapamycin-induced PDHA1 phosphorylation

PDKs and PDP1/2 reversibly regulate the phosphorylation of PDHA1 (Holness and Sugden, 2003). Thus, we wondered whether rapamycin-promoted PDHA1 phosphorylation could be eliminated by PDK inhibition. We therefore treated MDA-MB-231 cells with DCA, a PDK inhibitor (Tso et al., 2014). We observed that three phosphorylation sites of PDHA1 were greatly decreased in control and rapamycin-treated cells (Fig. 3a). In addition, we observed that DCA treatment did not affect S6 phosphorylation (Fig. 3a), indicating that although mTOR signaling controls PDHc enzymatic activity, the converse is not valid. Similar results were also obtained for B16F10 cells (Fig. 3b). These findings demonstrate that PDK inhibition could overcome rapamycin-induced PDHA1 phosphorylation.

2.4 Synergistic effect of co-treatment with DCA and rapamycin on cancer cell growth in vitro

Since mTOR signaling is hyperactive in most human cancers (Menon and Manning, 2008), mTOR signaling inhibition has potential therapeutic benefits in

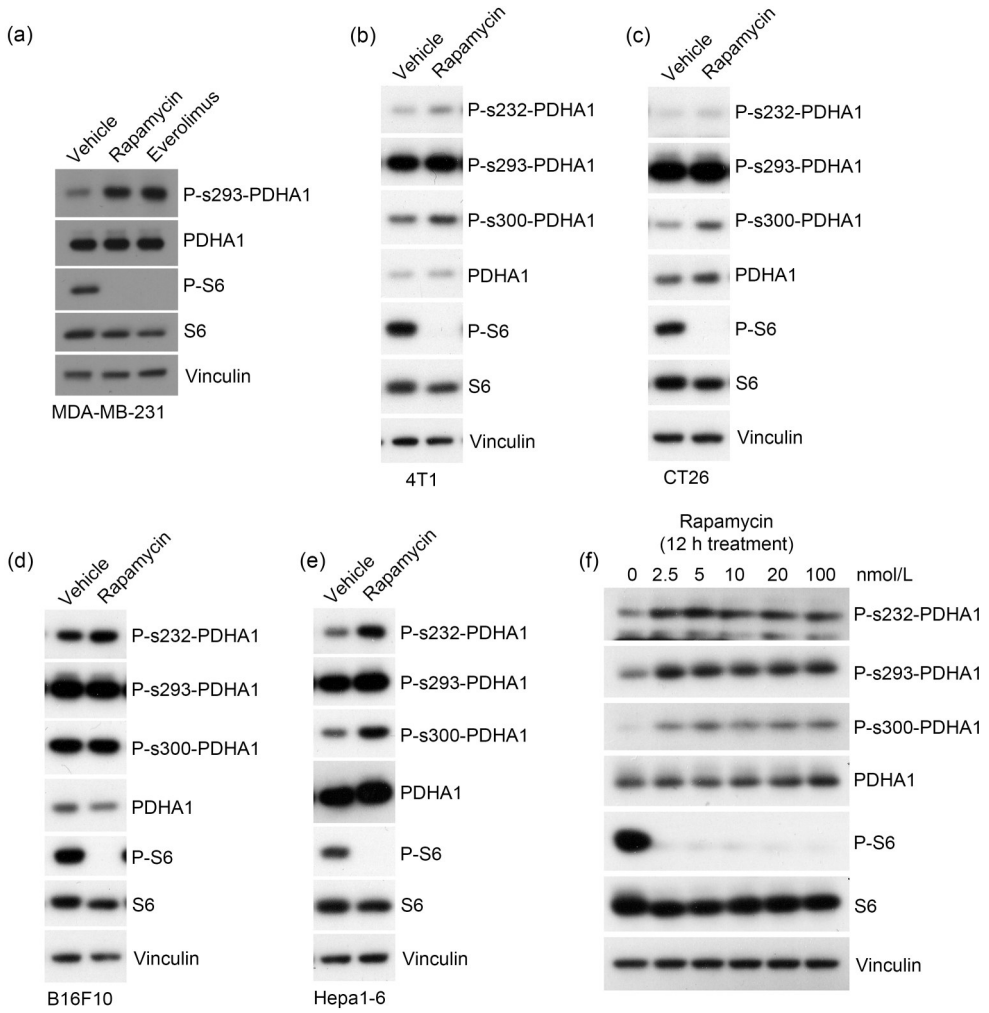

Fig. 1 PDHA1 phosphorylation induced by rapamycin treatment. (a–e) The indicated cells were treated with rapamycin (100 nmol/L, 12 h) or everolimus (1 μmol/L, 12 h) and subjected to immunoblotting analysis of the indicated proteins. (f) MDA-MB-231 cells were treated with the indicated concentrations of rapamycin for 12 h, and cell lysates were subjected to immunoblotting analysis of indicated proteins. PDHA1: pyruvate dehydrogenase E1 subunit alpha 1; P-: phosphorylated.

different types of cancer (Guba et al., 2002; Ghobrial et al., 2016; Rodrik-Outmezguine et al., 2016). DCA significantly decreased the growth of MDA-MB-231 cells under rapamycin or everolimus treatment (Fig. 4a). In addition, we observed that cell growth was significantly inhibited by 1 nmol/L of rapamycin, but this inhibition did not increase with higher concentrations of rapamycin in several cancer cell lines (Figs. 4b and 4c). Importantly, DCA treatment enhanced rapamycin-induced cell-growth inhibition (Figs. 4b and 4c), indicating that the activation of PDHc activity may weaken cancer cells which are resistant to mTOR inhibition. We found that rapamycin treatment reduced the cell colonization of MDA-MB-231 cells (Fig. 4d), although 2 mmol/L DCA treatment had no effect on cell growth in MDA-MB-231 cells. However, DCA combined with rapamycin had higher growth-inhibiting efficacy than rapamycin alone (Fig. 4d). In addition, we observed that rapamycin significantly inhibited cell colonization but did not affect colony numbers in 4T1 cells (Fig. 4e). However, DCA treatment significantly reduced colony numbers in rapamycin-treated 4T1 cells (Fig. 4e). Next, we found that DCA only, or DCA combined with rapamycin, activated the apoptosis protein marker cleaved poly(ADP-ribose) polymerase (PARP); but this phenomenon was not evident for treatment with rapamycin alone (Fig. 4f), indicating that PDHc activation may induce cancer cell apoptosis. It is evident from these results that DCA sensitizes cancer cells to rapamycin-induced growth inhibition.

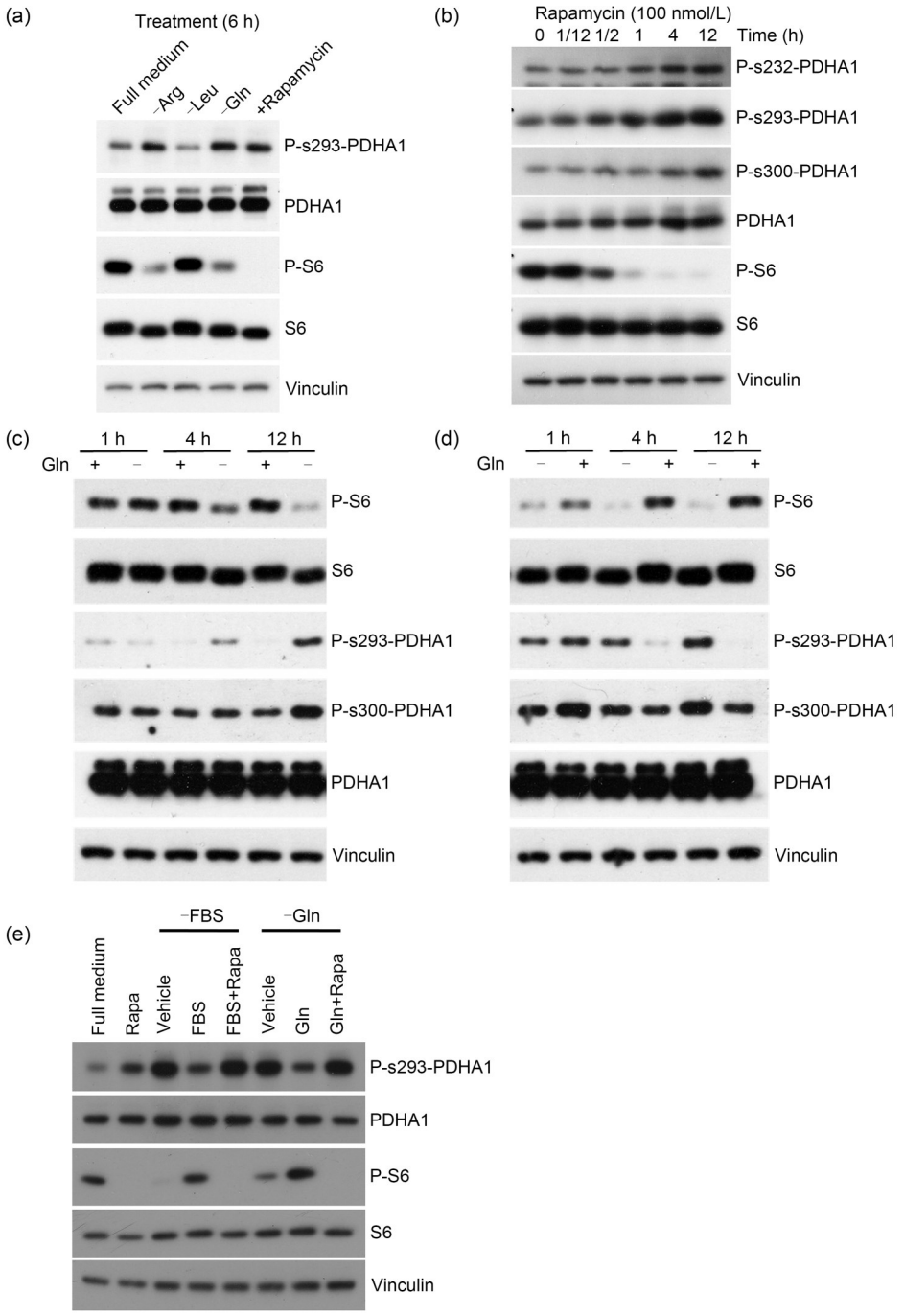

Fig. 2 mTORC1 involved in PDHA1 regulation, dependent on mTOR signaling. (a) MDA-MB-231 cells were starved with the indicated amino acids for 6 h, using rapamycin as a positive control, and cell lysates were used for immunoblotting analysis of the indicated proteins. (b) MDA-MB-231 cells were treated with rapamycin (100 nmol/L) for the indicated time periods and followed by immunoblotting analysis of the indicated proteins. (c) MDA-MB-231 cells were starved with glutamine for the indicated time periods, and cell lysates were subjected to immunoblotting analysis of the indicated proteins. (d) MDA-MB-231 cells were pre-starved with glutamine for 12 h, and then glutamine (4 mmol/L) was re-added to the culture medium for the indicated time periods. Cell lysates were used to analyze the indicated proteins. (e) MDA-MB-231 cells were starved with glutamine or FBS, together with rapamycin treatment. After 8 h of treatment, we re-added glutamine (4 mmol/L) or FBS to the culture medium for 4 h. Cell lysates were used to analyze the indicated proteins. PDHA1: pyruvate dehydrogenase E1 subunit alpha 1; mTOR: mammalian target of rapamycin; mTORC1: mTOR complex 1; FBS: fetal bovine serum; Arg: arginine; Leu: leucine; Gln: glutamine; Rapa: rapamycin; P-: phosphorylated.

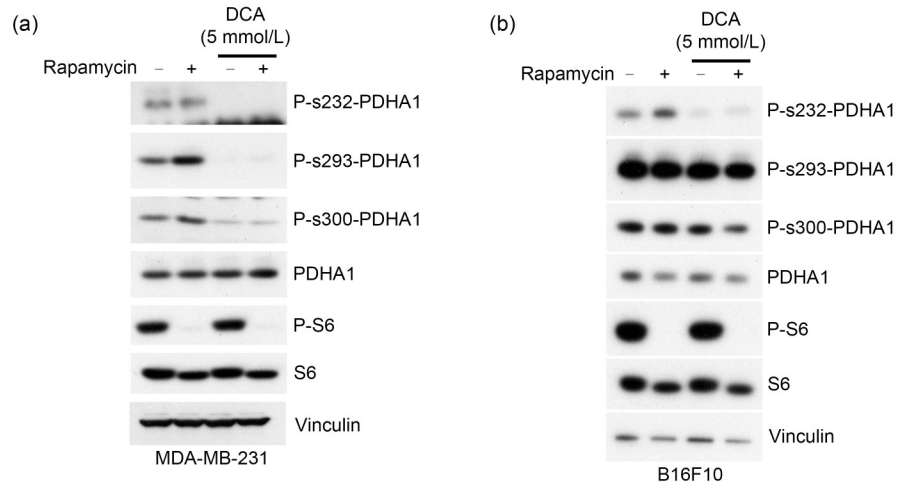

Fig. 3 Role of DCA in rapamycin-induced PDHA1 phosphorylation. MDA-MB-231 cells (a) and B16F10 cells (b) were treated with rapamycin (100 nmol/L), DCA (5 mmol/L), or both for 12 h. Cell lysates were also subjected to immunoblotting analysis of the indicated proteins. DCA: dichloroacetic acid; PDHA1: pyruvate dehydrogenase E1 subunit alpha 1; P-: phosphorylated.

2.5 Effects of the combined treatment of DCA and rapamycin on cancer cells

To further confirm the synergistic efficacy of DCA and rapamycin in vivo, we performed a drug-combination study in vivo (Fig. 5a). We found that rapamycin treatment inhibited tumor growth, and DCA treatment only showed slight effects (Figs. 5b and 5c). However, DCA combined with rapamycin significantly suppressed tumor volume and weight compared to rapamycin treatment alone (Figs. 5b–5d), indicating that combined treatment with DCA and rapamycin synergistically inhibited tumor growth in vivo. Previous research demonstrated that patients treated with rapamycin had a decreased spleen size due to its immunosuppressive role (Araújo et al., 2014). Consistently, we observed that the spleens of vehicle-treated mice were greatly enlarged compared to those treated with rapamycin (Figs. 5e and 5f). In addition, mice that underwent combined DCA and rapamycin treatment exhibited the smallest spleens compared to other groups (Figs. 5e and 5f), arguing that DCA may inhibit cancer metastasis in vivo (Jacobs et al., 2012). These results demonstrate that DCA cooperates with rapamycin to inhibit tumor progression in vivo.

3 Discussion and conclusions

mTOR signaling is hyperactive for the anabolic metabolism of rapidly proliferating cancer cells. Thus, mTOR inhibition is a potent therapeutic strategy for cancer patients. Many mTOR inhibitors have been used in clinical trials, including rapamycin and rapamycin derivatives known as rapalogs (Benjamin et al., 2011). However, rapamycin and rapalogs have proved to reduce tumor growth but not induce cell apoptosis, perhaps because the mTORC2-protein kinase B (AKT) complex, a pro-survival regulator, is insensitive to these inhibitors (Hsieh et al., 2012). The second-generation mTOR inhibitors (Torin1, WAY-600, and PP242) are designed to compete for adenosine-triphosphate (ATP) near the catalytic site of the kinase domain. These inhibitors bind directly to the kinase domain of both mTORC1 and mTORC2, resulting in considerable therapeutic effects (Ghobrial et al., 2016; Fan et al., 2017). Unfortunately, long-term treatment with these inhibitors can reboot the AKT pathway (Rodrik-Outmezguine et al., 2011), which is the primary limitation of the second-generation inhibitors (Ghobrial et al., 2016; Rodrik-Outmezguine et al., 2016).

In this study, we discovered that mTOR inhibition by rapamycin induces PDHA1 phosphorylation, which is inconsistent with the recently reported result in liver-cancer cells (Yang et al., 2021). In addition, we observed that amino acid starvation induced PDHA1 phosphorylation followed by mTOR inhibition. These findings strongly indicate that mTOR inhibition reduces PDHc activity. In addition, rapamycin-activated PDHA1 phosphorylation is suppressed by DCA. A previous report demonstrated that PDK4 inactivation switches

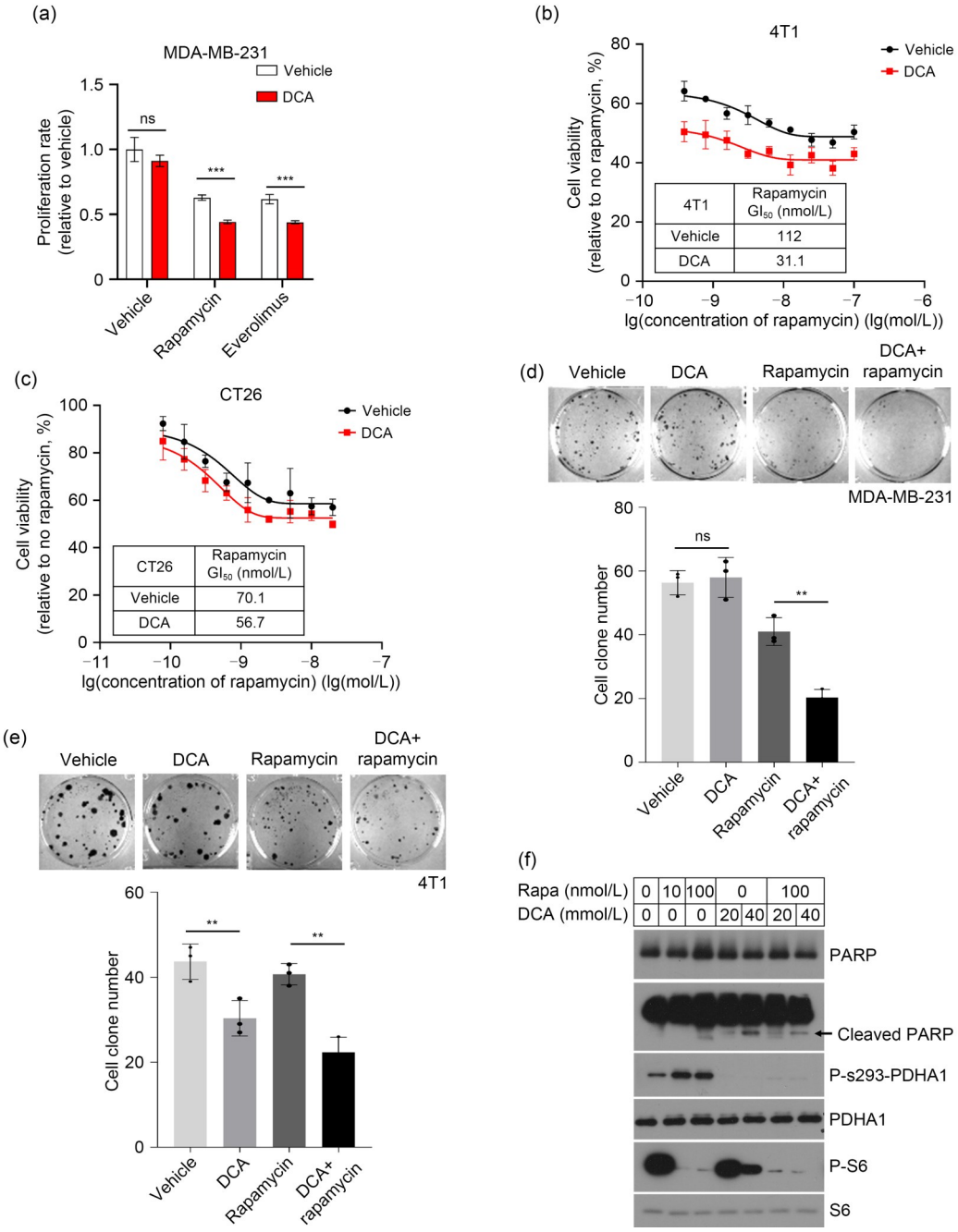

Fig. 4 Synergistic effect of co-treatment with DCA and rapamycin on cancer cell growth in vitro. (a) MDA-MB-231 cells were treated with the indicated compounds (5 mmol/L DCA, 100 nmol/L rapamycin, and 1 μmol/L everolimus), and proliferation rate was tested using SRB assay (n=4). (b) 4T1 cells were treated with different concentrations of rapamycin (0, 0.390 625, 0.781 25, 1.5625, 3.125, 6.25, 12.5, 25, 50, and 100 nmol/L) for 96 h, and cell viability was tested using SRB assay (n=3). (c) CT26 cells were treated with different concentrations of rapamycin (0, 0.078 125, 0.156 25, 0.3125, 0.625, 1.25, 2.5, 5, 10, and 20 nmol/L) for 72 h and cell viability was tested using SRB assay (n=3). (d, e) MDA-MB-231 and 4T1 cells were plated in 12-well plates and treated with the indicated drugs (2 mmol/L DCA and 10 nmol/L rapamycin) for 6 d. Cell clones were stained with SRB followed by imaging and quantification (n=3). (f) MDA-MB-231 cells were treated with the indicated drugs for 24 h, and then cell lysates were used for analysis of the indicated proteins. All values are expressed as mean±standard deviation (SD). The star (*) symbol indicates statistical significance as determined by a paired Student's t-test: $^{**}P$<0.01, $^{***}P$<0.001, and ns is not significant. DCA: dichloroacetic acid; SRB: sulforhodamine B; PARP: poly(ADP-ribose) polymerase; PDHA1: pyruvate dehydrogenase E1 subunit alpha 1; P-: phosphorylated; GI_{50}: concentration causing 50% cell growth inhibition.

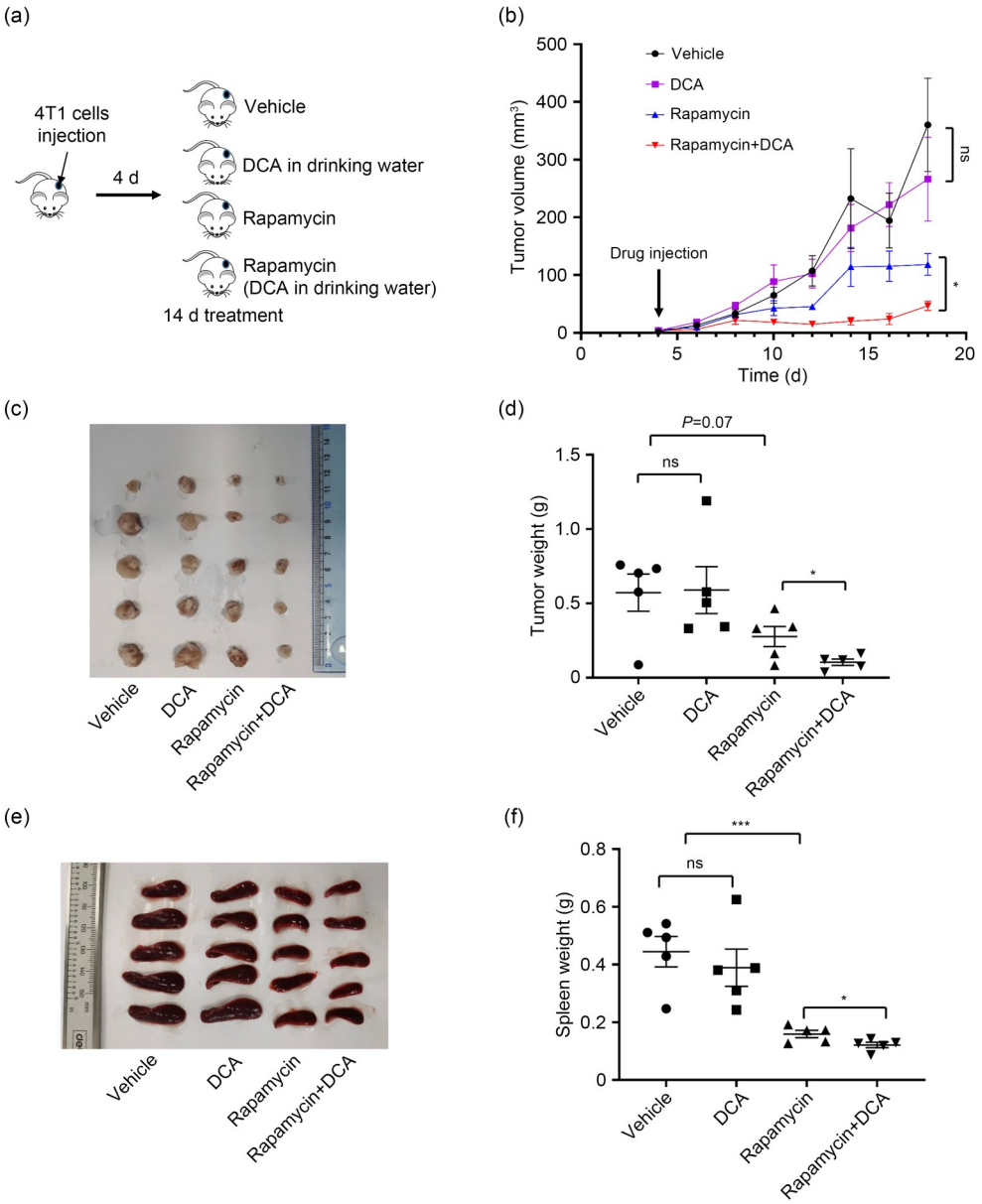

Fig. 5 Effects of the combined treatment of DCA and rapamycin on cancer cells. (a) Diagram of DCA and rapamycin treatment procedure in mice injected with 4T1 cells. DCA: 0.75 g/L in drinking water; Rapamycin: 2 mg/kg intraperitoneally injected daily. (b) Tumor volume was measured every two days using a caliper, and tumor volume was calculated using the standard formula $0.5\times L\times W^2$, where L is the longest diameter and W is the shortest diameter. (c, d) Tumors were removed and imaged after treatment with the indicated drugs for 14 d, and tumor weight was measured (n=5). (e, f) Spleens from mice were removed and imaged after treatment with the indicated drugs for 14 d, and spleen weight was measured (n=5). All values are expressed as mean±standard deviation (SD). The star (*) symbol denotes statistical significance as determined by a paired Student's t-test: $^{*}P<0.05$, $^{***}P<0.001$, and ns is not significant.

cells from pro-survival to pro-apoptosis mode under inflammatory stress (Wu et al., 2018). We also observed that DCA sensitized tumor cells to rapamycin treatment, so we argue that reduced PDHc activity can reduce cell proliferation from mTOR-signaling inhibition-induced growth arrest.

PDHc activity reduction promotes NAD^+ regeneration (Luengo et al., 2021) and increases cells' resistance to senescence (Kaplon et al., 2013). However, we observed that DCA treatment had limited efficacy in inhibiting growth of cancer cells in vitro and in vivo, consistent with previous studies (Wu et al., 2018;

Tataranni and Piccoli, 2019). DCA combined with rapamycin treatment exhibited much smaller tumor lumps compared to DCA or rapamycin alone in a homograft mouse model, confirming that DCA may have better therapeutic benefits in cooperation with other anticancer drugs (Cao et al., 2008; Lucido et al., 2018; Verma et al., 2019). Hypoxia inducible factor-1α (HIF-1α), a key regulator for metastasis, is downregulated when mTOR signaling is inhibited (Bernardi et al., 2006). We consistently observed that spleen volume was reduced in the mice treated by the combination of DCA and rapamycin. Since DCA also inhibits HIF-1α expression (Sutendra et al., 2013; Škorja Milić et al., 2021), we speculate that DCA and rapamycin may synergistically inhibit cancer metastasis by suppressing the HIF-1α signaling pathway.

Taken together, our observations support the conclusion that PDHc inactivation can promote cancer cell resistance to mTOR signaling inhibition, which provides a new, effective therapeutic strategy for clinics. One way to achieve this inactivation is combined treatment with DCA and rapamycin.

Materials and methods

Detailed methods are provided in the electronic supplementary materials of this paper.

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Author contributions

Huan CHEN and Hai-long PIAO conceived the project, designed and performed most of the experiments and the data analysis, and wrote the manuscript with input from all other authors. Hai-long PIAO supervised the project. Kunimg LIANG performed the most of experiments. Huan CHEN, Kunimg LIANG, and Cong HOU provided significant intellectual input. 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

Huan CHEN, Kunming LIANG, Cong HOU, and Hai-long PIAO declare that they have no conflict of interest.

All work performed with animals was approved by the Medical Animal Care and Use Committee of China Medical University, Shenyang, China (No. KT2020061).

References

Araújo NC, Sampaio Goncalves de Lucena SB, da Silveira Rioja S, 2014. Effect of rapamycin on spleen size in longstanding renal transplant recipients. *Transplant Proc*, 46(5):1319-1323. https://doi.org/10.1016/j.transproceed.2014.03.011

Bai ZS, Peng YL, Ye XY, et al., 2022. Autophagy and cancer treatment: four functional forms of autophagy and their therapeutic applications. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 23(2):89-101. https://doi.org/10.1631/jzus.B2100804

Benjamin D, Colombi M, Moroni C, et al., 2011. Rapamycin passes the torch: a new generation of mTOR inhibitors. *Nat Rev Drug Discov*, 10(11):868-880. https://doi.org/10.1038/nrd3531

Beretta L, Gingras AC, Svitkin YV, et al., 1996. Rapamycin blocks the phosphorylation of 4E-BP1 and inhibits cap-dependent initiation of translation. *EMBO J*, 15(3):658-664.

Bernardi R, Guernah I, Jin D, et al., 2006. PML inhibits HIF-1α translation and neoangiogenesis through repression of mTOR. *Nature*, 442(7104):779-785. https://doi.org/10.1038/nature05029

Cao WG, Yacoub S, Shiverick KT, et al., 2008. Dichloroacetate (DCA) sensitizes both wild-type and over expressing *Bcl-2* prostate cancer cells in vitro to radiation. *Prostate*, 68(11):1223-1231. https://doi.org/10.1002/pros.20788

Fan QW, Aksoy O, Wong RA, et al., 2017. A kinase inhibitor targeted to mTORC1 drives regression in glioblastoma. *Cancer Cell*, 31(3):424-435. https://doi.org/10.1016/j.ccell.2017.01.014

Ghobrial IM, Siegel DS, Vij R, et al., 2016. TAK-228 (formerly MLN0128), an investigational oral dual TORC1/2 inhibitor: a phase I dose escalation study in patients with relapsed or refractory multiple myeloma, non-Hodgkin lymphoma, or waldenström's macroglobulinemia. *Am J Hematol*, 91(4):400-405. https://doi.org/10.1002/ajh.24300

Guba M, von Breitenbuch P, Steinbauer M, et al., 2002. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. *Nat Med*, 8(2):128-135. https://doi.org/10.1038/nm0202-128

Holness MJ, Sugden MC, 2003. Regulation of pyruvate dehydrogenase complex activity by reversible phosphorylation. *Biochem Soc Trans*, 31(6):1143-1151. https://doi.org/10.1042/bst0311143

Hsieh AC, Liu Y, Edlind MP, et al., 2012. The translational landscape of mTOR signalling steers cancer initiation and metastasis. *Nature*, 485(7396):55-61. https://doi.org/10.1038/nature10912

Jacinto E, Loewith R, Schmidt A, et al., 2004. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. *Nat Cell Biol*, 6(11):1122-1128. https://doi.org/10.1038/ncb1183

Jacobs KE, Visser BC, Gayer G, 2012. Changes in spleen volume after resection of hepatic colorectal metastases. *Clin Radiol*, 67(10):982-987. https://doi.org/10.1016/j.crad.2012.03.013

Kaplon J, Zheng L, Meissl K, et al., 2013. A key role for mitochondrial gatekeeper pyruvate dehydrogenase in oncogene-induced senescence. *Nature*, 498(7452):109-112. https://doi.org/10.1038/nature12154

Kim J, Guan KL, 2019. mTOR as a central hub of nutrient signalling and cell growth. *Nat Cell Biol*, 21(1):63-71. https://doi.org/10.1038/s41556-018-0205-1

Lamming DW, Ye L, Katajisto P, et al., 2012. Rapamycin-induced insulin resistance is mediated by mTORC2 loss and uncoupled from longevity. *Science*, 335(6076):1638-1643. https://doi.org/10.1126/science.1215135

Levy JMM, Towers CG, Thorburn A, 2017. Targeting autophagy in cancer. *Nat Rev Cancer*, 17(9):528-542. https://doi.org/10.1038/nrc.2017.53

Lorusso PM, 2016. Inhibition of the PI3K/AKT/mTOR pathway in solid tumors. *J Clin Oncol*, 34(31):3803-3815. https://doi.org/10.1200/JCO.2014.59.0018

Lucido CT, Miskimins WK, Vermeer PD, 2018. Propranolol promotes glucose dependence and synergizes with dichloroacetate for anti-cancer activity in HNSCC. *Cancers (Basel)*, 10(12):476. https://doi.org/10.3390/cancers10120476

Luengo A, Li ZQ, Gui DY, et al., 2021. Increased demand for NAD^+ relative to ATP drives aerobic glycolysis. *Mol Cell*, 81(4):691-707.e6. https://doi.org/10.1016/j.molcel.2020.12.012

Menon S, Manning BD, 2008. Common corruption of the mTOR signaling network in human tumors. *Oncogene*, 27:S43-S51. https://doi.org/10.1038/onc.2009.352

Patel MS, Nemeria NS, Furey W, et al., 2014. The pyruvate dehydrogenase complexes: structure-based function and regulation. *J Biol Chem*, 289(24):16615-16623. https://doi.org/10.1074/jbc.R114.563148

Phung TL, Ziv K, Dabydeen D, et al., 2006. Pathological angiogenesis is induced by sustained Akt signaling and inhibited by rapamycin. *Cancer Cell*, 10(2):159-170. https://doi.org/10.1016/j.ccr.2006.07.003

Robitaille AM, Christen S, Shimobayashi M, et al., 2013. Quantitative phosphoproteomics reveal mTORC1 activates de novo pyrimidine synthesis. *Science*, 339(6125):1320-1323. https://doi.org/10.1126/science.1228771

Rodrik-Outmezguine VS, Chandarlapaty S, Pagano NC, et al., 2011. mTOR kinase inhibition causes feedback-dependent biphasic regulation of AKT signaling. *Cancer Discov*, 1(3):248-259. https://doi.org/10.1158/2159-8290.CD-11-0085

Rodrik-Outmezguine VS, Okaniwa M, Yao Z, et al., 2016. Overcoming mTOR resistance mutations with a new-generation mTOR inhibitor. *Nature*, 534(7606):272-276. https://doi.org/10.1038/nature17963

Sabatini DM, Erdjument-Bromage H, Lui M, et al., 1994. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. *Cell*, 78(1):35-43. https://doi.org/10.1016/0092-8674(94)90570-3

Sarbassov DD, Ali SM, Sengupta S, et al., 2006. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. *Mol Cell*, 22(2):159-168. https://doi.org/10.1016/j.molcel.2006.03.029

Škorja Milić N, Dolinar K, Miš K, et al., 2021. Suppression of pyruvate dehydrogenase kinase by dichloroacetate in cancer and skeletal muscle cells is isoform specific and partially independent of HIF-1α. *Int J Mol Sci*, 22(16):8610. https://doi.org/10.3390/ijms22168610

Sutendra G, Dromparis P, Kinnaird A, et al., 2013. Mitochondrial activation by inhibition of PDKII suppresses HIF1α signaling and angiogenesis in cancer. *Oncogene*, 32(13):1638-1650. https://doi.org/10.1038/onc.2012.198

Tataranni T, Piccoli C, 2019. Dichloroacetate (DCA) and cancer: an overview towards clinical applications. *Oxid Med Cell Longev*, 2019:8201079. https://doi.org/10.1155/2019/8201079

Tso SC, Qi XB, Gui WJ, et al., 2014. Structure-guided development of specific pyruvate dehydrogenase kinase inhibitors targeting the ATP-binding pocket. *J Biol Chem*, 289(7):4432-4443. https://doi.org/10.1074/jbc.M113.533885

Valvezan AJ, Turner M, Belaid A, et al., 2017. mTORC1 couples nucleotide synthesis to nucleotide demand resulting in a targetable metabolic vulnerability. *Cancer Cell*, 32(5):624-638.e5. https://doi.org/10.1016/j.ccell.2017.09.013

Verma A, Lam YM, Leung YC, et al., 2019. Combined use of arginase and dichloroacetate exhibits anti-proliferative effects in triple negative breast cancer cells. *J Pharm Pharmacol*, 71(3):306-315. https://doi.org/10.1111/jphp.13033

Wu JG, Zhao YL, Park YK, et al., 2018. Loss of PDK4 switches the hepatic NF-κB/TNF pathway from pro-survival to pro-apoptosis. *Hepatology*, 68(3):1111-1124. https://doi.org/10.1002/hep.29902

Yang HJ, Jiang XL, Li BR, et al., 2017. Mechanisms of mTORC1 activation by RHEB and inhibition by PRAS40. *Nature*, 552(7685):368-373. https://doi.org/10.1038/nature25023

Yang WC, Pang DJ, Chen MN, et al., 2021. Rheb mediates neuronal-activity-induced mitochondrial energetics through mTORC1-independent PDH activation. *Dev Cell*, 56(6):811-825.e6. https://doi.org/10.1016/j.devcel.2021.02.022

Zhang S, Qian GQ, Zhang QQ, et al., 2019. mTORC2 suppresses GSK3-dependent snail degradation to positively regulate cancer cell invasion and metastasis. *Cancer Res*, 79(14):3725-3736. https://doi.org/10.1158/0008-5472.CAN-19-0180

Zhuang HQ, Bai J, Chang JY, et al., 2016. MTOR inhibition reversed drug resistance after combination radiation with erlotinib in lung adenocarcinoma. *Oncotarget*, 7(51):84688-84694. https://doi.org/10.18632/oncotarget.12423

Supplementary information

Materials and methods