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Targeting HIF-1α is a prerequisite for cell sensitivity to dichloroacetate (DCA) and metformin

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ABSTRACT

Recently, targeting deregulated energy metabolism is an emerging strategy for cancer therapy. In the present study, combination of DCA and metformin markedly induced cell death, compared with each drug alone. Furthermore, the expression levels of glycolytic enzymes including HK2, LDHA and ENO1 were downregulated by two drugs. Interestingly, HIF-1α activation markedly suppressed DCA/metformin-induced cell death and recovered the expressions of glycolytic enzymes that were decreased by two drugs. Based on these findings, we propose that targeting HIF-1α is necessary for cancer metabolism targeted therapy.

1. Introduction

Cancer cells are known to have different metabolic properties than normal cells. Cancer cells are well documented to rewire cellular metabolism and energy production networks to demand rapid proliferation [1–3]. Cancer cells become heavily dependent on aerobic glycolysis, fatty acid synthesis and glutaminolysis [4]. Thus, targeting metabolic dependence might be an effective way of targeting cancers.

Metformin, an oral drug widely used in the treatment of type 2 diabetes, is associated with a decreased risk of cancer in diabetic patients using this drug. Metformin inhibits complex I of mitochondrial electron transport chain and cellular respiration [5–7]. In cancer cells, metformin inhibits cell proliferation in the presence of glucose but induces cell death upon glucose starvation [8]. Dichloroacetate (DCA) was shown to decrease glucose uptake and inhibit glycolysis, thus inducing cell injury in cancers of the breast, prostate, lung, medullary thyroid, endometrial cancers, myelomas and glioblastoma multiforme [9–16]. DCA, a synthetic PDK inhibitor, was shown to reverse glycolysis by oxidative phosphorylation through PDH activation [17]. However, not all studies found apoptosis induction with DCA alone at clinically relevant concentrations when tested in vitro [18]. Results of recent studies have indicated that DCA-mediated reprogramming of glucose metabolism enhances metformin-cytotoxicity in cancer cells [19].

In addition to metabolic alterations, tumor hypoxia and activation of hypoxia pathways appears to be strongly associated with aggressive malignancy [20]. Hypoxia inducible factor (HIF) is a transcription factor mediating responses to hypoxia, and HIF target genes overlap strongly with genes implicated in dysregulated tumor metabolism. Importantly, hypoxia has been associated with

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the resistance of cancer cells to radiation therapy and anticancer drugs [21]. Cancer cells can survive under hypoxic conditions by metabolic reprogramming to achieve a high level of glycolysis, which contributes to the development of chemoresistance [22]. Therefore, targeting hypoxic conditions in human tumors may be an effective cancer therapy.

In this study, we observed that co-treatment with DCA and metformin led to a dramatic induction of cell death in MCF-7 breast cancer cells and H1299 lung cancer cells. HIF-1α activation was able to recover cell death induced by DCA/metformin via an up-regulation of glycolytic enzymes. Our results strongly suggest that inhibiting hypoxia may be an effective strategy in metabolism-targeted cell death for cancer cells.

2. Materials and methods

2.1. Cell cultures and reagents

MCF-7, H1299, HDF and MCF-10A cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA). MCF-7 and HDF cells were maintained in DMEM and H1299 cells in RPMI1640 (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS. MCF-10A cells were cultured in DMEM/F12 Ham's Mixture supplemented with 5% horse serum, 20 ng/ml EGF, 10 μg/ml insulin, 0.5 mg/ml hydrocortisone, and 100 ng/ml cholera toxin. Cell lines were cultured in air with 5% CO_2 at 37 °C. For culture under hypoxic conditions, cells were incubated in a hypoxic chamber (Forma Anaerobic System; Thermo Scientific, MA) with 5% CO2/1.0% O2 and 94.0% N2 (all v/v), respectively. DCA and metformin, phenformin were purchased from Sigma–Aldrich (St Louis, MO, USA).

2.2. Measurement of cell viability

Cell viability was determined by measuring the mitochondrial conversion of 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) to a colored product. After treatment the medium was removed and serum-free medium containing the MTT reagent (0.5 mg/ml) was added. After 1 h at 37 °C, the medium was removed, the formazan crystals in the cells were dissolved in dimethyl sulfoxide (DMSO), and the absorbance of the formazan solution was measured by an ELISA reader at a wavelength of 595 nm.

2.3. Evaluation of cell death

Cells were stained with Annexin V-FITC and propidium iodide (PI) for the assessment of cell death, as described previously [23].

2.4. Synergy determination by isobologram analysis

The synergy between two agents was determined by their isobolograms [24]. Cells were treated with different concentrations of metformin (0–50 mM) and DCA (0–45 mM). Combinations yielding 50 ± 5% cell viability were plotted as the percentage of each single agent alone versus the equivalent level of cell viability (fractional inhibitory concentration (FIC): concentration of each agent in the combination/concentration of each agent alone). When the sum of the FIC was = 1, the combination was additive and the graph was expressed as a straight line. When the sum was < 1, the combination was synergistic and the graph had a concave shape. When the sum was > 1, the combination was antagonistic and the graph had a convex shape.

2.5. siRNA transfection

HIF-1α (sequence: GGGAUUAACUCAGUUUGAACUAACUdTdT) [25] and control (sequence: CCUACGCCACCAAUUUCGUdTdT) siRNAs were synthesized by Bioneer (Daejeon, Republic of Korea). Transfection experiments with siRNAs were transiently performed using Lipofectamine RNAi MAX™ according to the manufacturer's instructions (Invitrogen).

2.6. Western blot analysis

Cell lysates were separated via SDS–PAGE and transferred to a nitrocellulose membrane, followed by immunoblotting with the specified primary and horseradish peroxidase-conjugated secondary antibodies. Immunoreactive bands were visualized with SuperSignal West Pico chemiluminescent substrates (Thermo Scientific Pierce, Rockford, IL, USA). The following antibodies were used: Lactate dehydrogenase A (LDHA) (#3582), Enolase 1 (ENO1) (#3810), Hexokinase 2 (HK2) (#2867), and cleaved PARP (#9541) obtained from Cell Signaling Technology (Beverly, MA, USA), HIF-1α (610958) from BD Biosciences (San Diego, CA, USA) and β-actin (#A5361) from Sigma–Aldrich.

2.7. Statistical analysis

Data are presented as the means ± standard deviations. Comparisons between groups were made using Student's t-test. Asterisks (***P < 0.001, **P < 0.01, *P < 0.05) indicate statistical significance.

3. Results

3.1. DCA and metformin synergistically enhance cell death

We first investigated the effect of DCA or metformin on MCF-7 breast cancer cell death. MCF-7 cells were incubated with various concentrations of DCA or metformin for 48 h, and cell death was determined by Annexin V/PI-positive staining. Less than 20% cell death was observed despite the presence of high concentrations of 20 mM DCA [9] and 20 mM metformin (Fig. 1A and B) [26]. We examined the combined effects of 10 mM DCA and 10 mM metformin in subsequent experiments. As shown in Fig. 1C, treatment with DCA or metformin induced 7% or 6% cell death, respectively. However, co-treatment with DCA and metformin led to a dramatic induction of apoptosis (Fig. 1C and E). Combination of DCA and phenformin, a lipophilic analog of metformin also resulted in an increased apoptosis compared with drug alone (Fig. 1D–E). The combination treatment of DCA and metformin was synergistic according to isobolographic analysis (Fig. 1F). A synergistic induction of cell death by the two drugs was also observed in H1299 lung cancer cells (Fig. 1G). However, no significant cell death was observed in normal cells, such as MCF-10A (non-tumorigenic immortalized breast epithelial cells) and HDF (human dermal fibroblasts), suggesting that a combination of DCA and metformin induces greater cell death in cancer cells than in normal cells (Fig. 1H and I).

3.2. Hypoxia condition reduces cell death from DCA/metformin

Regions of tumor hypoxia are common in the microenvironment of many solid tumors [27,28]. Hypoxia is associated with increased tumor resistance to radiation treatment and chemotherapy [27]. Therefore, we investigated the effect of hypoxia in cells with DCA and metformin. As shown in Fig. 2A and B, hypoxia (1% O_2) increased the expression of HIF-1α and attenuated cell death

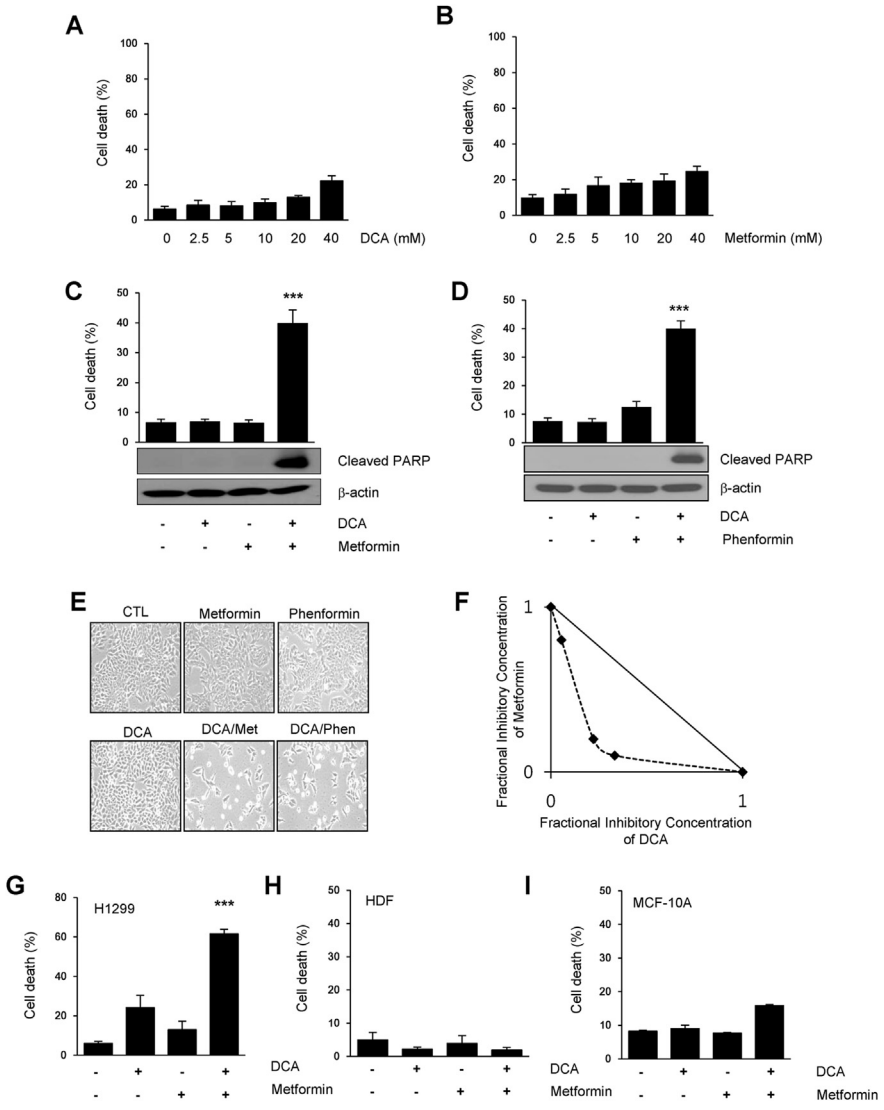

Fig. 1. DCA and metformin synergistically induce cell death in cancer cells. (a–b) MCF-7 cells were treated with the indicated concentrations of DCA or metformin for 48 h (c, e) MCF-7 cells were treated with or without 10 mM DCA or/and 10 mM metformin for 48 h (d-e) MCF-7 cells were treated with or without 10 mM DCA or/and 50 μM phenformin for 48 h (f) MCF-7 cells were treated with DCA (0–45 mM) in combination with metformin (0–50 mM) for 48 h and were examined for synergy by isobologram analysis as described in the Materials and Methods. (g–i) H1299, HDF and MCF-10A cells were treated with or without 10 mM DCA or/and 10 mM metformin for 48 h. Cell death was evaluated via flow cytometry after Annexin V and PI staining (a-d, g-i). Data are presented as the means of triplicate samples and error bars reflect standard deviation (SD). Levels of the indicated proteins were measured by Western blot analysis (c–d). The blot is representative of three independent experiments. Cell morphology images were obtained under a microscope (e). ***$p < 0.001$ vs. DCA or metformin-treated group (c, g), ***$p < 0.001$ vs. DCA or phenformin-treated group (d). Met; Metformin, Phen; Phenformin.

induced by DCA and metformin. We simulated hypoxic conditions with cobalt chloride ($CoCl_2$) to further investigate the effect of hypoxia in cells with DCA and metformin. $CoCl_2$ also increased HIF-1α protein expression in a dose-dependent manner and recovered the cell death phenotype induced by DCA/metformin in MCF-7 (Fig. 2C–E) and H1299 (Fig. 2F and G) cells. These data indicate that hypoxia condition induces resistance to DCA/metformin in cancer cells.

3.3. Resistance to DCA/metformin-induced cell death in hypoxia is HIF-1α dependent

Hypoxia-inducible factor-1-alpha (HIF-1α) is a subunit of a heterodimeric transcription factor of HIF-1 that is a critical regulator of cellular and systemic responses to hypoxia [20]. Thus, to assess the role of HIF-1α in hypoxia-induced resistance to DCA/metformin, HIF-1α expression was suppressed by siRNAs (Fig. 3C). As shown in Fig. 3A–C, HIF-1α knock-down further induced cell death by DCA and metformin. These data suggest that resistance to DCA/metformin-induced cell death in hypoxia is HIF-1α dependent.

3.4. HIF-1α is involved in hypoxia-induced resistance to DCA/metformin by regulating metabolic enzymes

Recent findings highlighted metabolic enzymes as direct modulators of cell death pathways [29–31]. Therefore, we investigated several key glycolytic enzymes that are involved in MCF-7 cells using DCA and metformin. As shown in Fig. 4A, expression levels of HK2, ENO1 and LDHA were markedly down-regulated upon treatment with both DCA and metformin, compared to DCA and metformin alone. We investigated whether hypoxia condition recovers the protein expression of glycolytic enzymes reduced by DCA/metformin. MCF-7 cells were pre-treated with hypoxia condition (1% O_2 or $CoCl_2$), followed by DCA/metformin. Hypoxic conditions

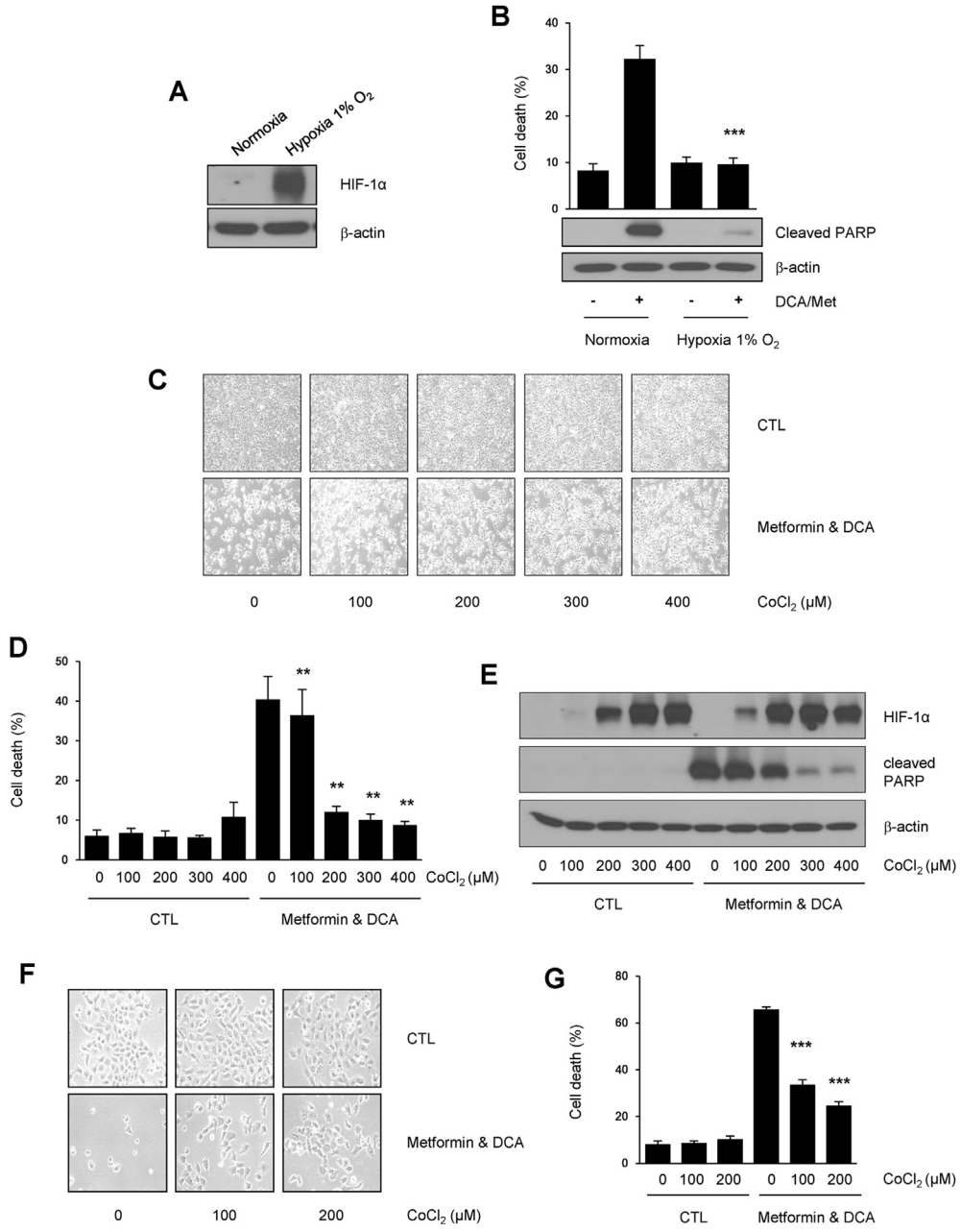

Fig. 2. Hypoxia condition reduces MCF-7 cell death induced by DCA and metformin. (a) MCF-7 cells were exposed to hypoxia (1% O_2) or kept in normoxia for 7 h (b) MCF-7 cells were treated with or without 10 mM DCA and 10 mM metformin followed by hypoxia (1% O_2) or normoxia for 48 h (c–e) MCF-7 cells were pretreated with or without the indicated concentrations of $CoCl_2$ for 30 min, followed by a 48 h treatment with or without 10 mM DCA and 10 mM metformin. (f–g) H1299 cells were pretreated with indicated concentrations of $CoCl_2$ for 30 min, followed by a 48 h treatment with or without 10 mM DCA and 10 mM metformin. Cell morphology images were obtained under a microscope (c, f). Cell death was evaluated via flow cytometry after Annexin V and PI staining (b, d, g). Data are presented as the means of triplicate samples and error bars reflect SD. Levels of the indicated proteins were measured by Western blot analysis (a, b, e). The blot is representative of three independent experiments. ***$p < 0.001$ vs. DCA and metformin-treated group under normoxia condition (b), ***$p < 0.001$ vs. DCA and metformin-treated group (d, g).

significantly recovered the protein expression of HK2, ENO1 and LDHA reduced by DCA/metformin (Fig. 4B and C). However, down-regulation of HIF-1α significantly decreased glycolytic enzyme protein expression after DCA/metformin treatment in the presence of $CoCl_2$ (Fig. 4D), suggesting that HIF-1α is involved in hypoxia-induced resistance to DCA/metformin by regulating glycolytic enzymes.

4. Discussion

Cancer cells reprogram their metabolic machinery to meet the bioenergetic and biosynthetic demands for growth and proliferation [32]. Metabolic pathways promoting cancer cell survival and growth have been linked to therapeutic resistance. Thus, targeting metabolic dependence might be an effective way of targeting cancers.

Metformin is widely used in the treatment of type 2 diabetes. Metformin has been shown to inhibit cell proliferation and induce

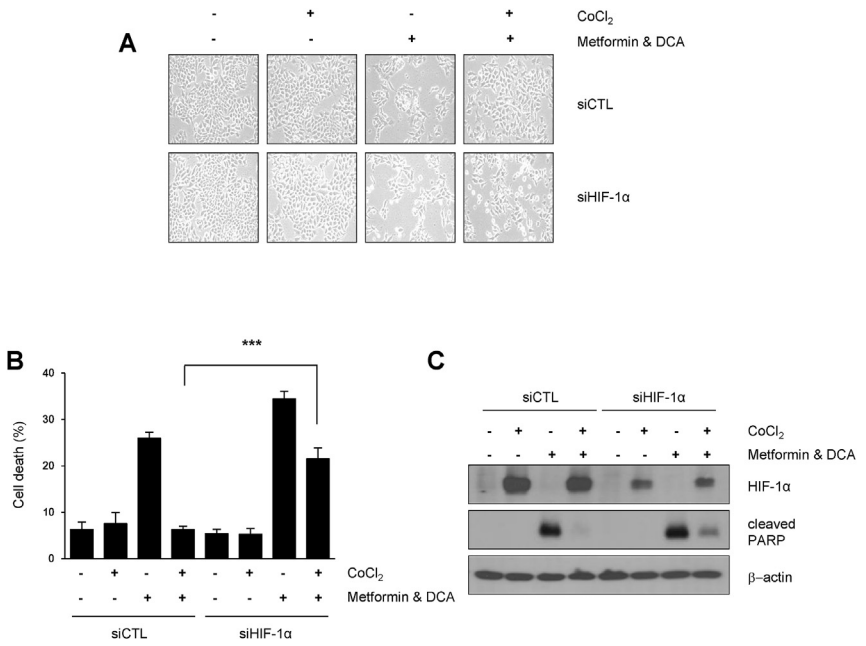

Fig. 3. HIF-1α is an essential factor in hypoxia-induced DCA/metformin resistance. (a–c) MCF-7 cells were transiently transfected with HIF-1α or control siRNA for 6 h and pre-treated with or without 300 μM $CoCl_2$ for 30 min, followed by a 48 h treatment with or without 10 mM DCA and 10 mM metformin. Morphology images were obtained under a microscope (a). Cell death was evaluated via flow cytometry after Annexin V and PI staining (b). Levels of the indicated proteins were measured by Western blot (c). The blot is representative of three independent experiments. Data are presented as the means of triplicate samples and error bars reflect SD. ***$p < 0.001$ vs. siCTL/$CoCl_2$/DCA/metformin-treated group (b).

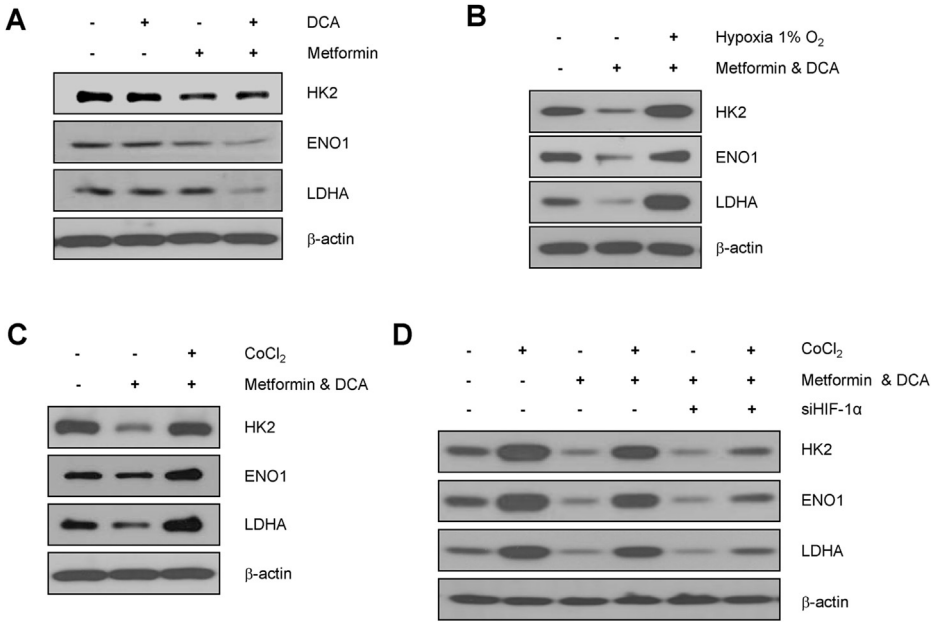

Fig. 4. HIF-1α alters the metabolic protein expression of DCA and metformin-treated MCF-7 cells.(a) MCF-7 cells were treated with or without 10 mM DCA or/and 10 mM metformin for 48 h (b) MCF-7 cells were treated with or without 10 mM DCA and 10 mM metformin followed by hypoxia (1% O_2) or normoxia for 48 h (c) MCF-7 cells were pretreated with or without 300 μM $CoCl_2$ for 30 min, followed by a 48 h treatment with or without 10 mM DCA and 10 mM metformin. (d) MCF-7 cells were transiently transfected with HIF-1α or control siRNA for 6 h and pretreated with or without 300 μM $CoCl_2$ for 30 min, followed by a 48 h treatment with or without 10 mM DCA and 10 mM metformin. Levels of the indicated proteins were measured by Western blot (a–d). The blot is representative of three independent experiments.

cell death in cancer [8]. However, we observed limited apoptotic cell death (Fig. 1B) in MCF-7 and H1299 cells, despite high concentrations of metformin (≥10 mM). In our experiments, MCF-7 or H1299 cells were incubated in *media containing* 25 or 11 mM glucose, respectively. When glucose was increased to 10 mM or greater, breast cancer cells became less responsive to metformin, suggesting that lowering the glucose potentiates metformin-induced cell death by reducing metformin-stimulated glycolysis [33]. DCA is a well-characterized inhibitor of PDK that can inactivate pyruvate dehydrogenase. PDK inhibition increases the flux of pyruvate into the mitochondria, promoting glucose oxidation over glycolysis. However, glycolysis inhibitors, such as 2-DG, 3-BrPA and

DCA, did not induce apoptosis when used as monotherapy because the high doses required to achieve therapeutic efficacy are often associated with toxicity [18]. According to the previous report, a combination of DCA and metformin synergistically induced cell death in breast cancer cells [34]. We also observed that DCA and metformin led to a dramatic induction of apoptosis in MCF-7 and H1299 cells compared to normal cells, such as MCF-10A and HDF cells (Fig. 1). Recent studies showed that glycolysis inhibition by lactate export inhibitors also enhances the cancer cells sensitivity to metformin or penformin [35–37].

Interestingly, several glycolytic enzymes including HK2, ENO1 and LDHA were markedly down-regulated by DCA and metformin, compared to DCA and metformin alone (Fig. 4A). Glycolytic enzymes have frequently been found to be overexpressed in malignant tumors and thus targeted for cancer treatment. Knock-down of HK2, ENO1 or LDHA using siRNA uncovered several interesting effects on transformed cells, including cell cycle delay and increased apoptosis (HK2) [29], decreased cell invasion and migration (ENO1) [30] and increased sensitivity to hypoxia (LDHA) [31].

Solid tumors frequently encounter hypoxic stress because of structurally disorganized blood vessels and rapidly proliferating cancer cells. Hypoxic conditions lead to anticancer chemotherapy and radiotherapy resistance, as well as a predisposition for increased tumor metastases [27]. The hypoxia condition (1% O_2 or $CoCl_2$) recovered DCA/metformin-induced cell death in MCF-7 (Fig. 2A–E) and H1299 cells (Fig. 2F and G). In addition, as shown in Fig. 4B and C, hypoxia condition (1% O_2 or $CoCl_2$) led to significantly increased protein expression of HK2, ENO1 and LDHA that was reduced by DCA/metformin. These data suggest that hypoxia can cause resistance to apoptosis by DCA/metformin in cancer cells.

The transcription factor HIF-1 are a major regulators of cellular adaptation to hypoxic stress. HIF-1 regulates a number of genes, including those involved in glucose metabolism, cell survival, erythropoiesis, stem cell maintenance, angiogenesis and resistance to chemotherapy and radiation therapy [38]. Specially, HIF-1α induces over-expression and increased activity of several glycolytic proteins, including transporters (GLUT1, GLUT3) and enzymes (HK1, HK2, PFKL, ALDA, ALDC, PGK1, ENOα, PYKM2, LDHA, PFKFB3) in cancer cells [39]. As shown in Fig. 3A–C and Fig. 4D, knock-down of HIF-1α further led to decreased protein expression of HK2, ENO1 and LDHA that and induced cell death after DCA/metformin treatment in the presence of $CoCl_2$. These data suggest that HIF-1α is involved in hypoxia-induced drug resistance to DCA/metformin by regulating glycolytic enzymes.

In summary, hypoxic conditions block MCF-7 cells from cell death induced by DCA and metformin. Treatment with DCA and metformin decreased HK2, ENO1 and LDHA protein expression. HIF-1α activation recovers the decreased metabolic protein expression induced by DCA and metformin. These results indicate that HIF-1α protein expression plays a role in protecting MCF-7 cells against DCA and metformin. Based on these results, we strongly suggest that targeting hypoxia may be an essential prerequisite for cell sensitivity to drug combinations targeting cancer cell metabolism.

Conflict of interest

The authors declare no conflicts of interest.

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References

[1] R.J. DeBerardinis, J.J. Lum, G. Hatzivassiliou, C.B. Thompson, The biology of cancer: metabolic reprogramming fuels cell growth and proliferation, Cell. Metab. 7 (2008) 11–20.
[2] C.V. Dang, G.L. Semenza, Oncogenic alterations of metabolism, Trends Biochem. Sci. 24 (1999) 68–72.
[3] P.S. Ward, C.B. Thompson, Metabolic reprogramming: a cancer hallmark even warburg did not anticipate, Cancer Cell. 21 (2012) 297–308.
[4] M.G. Vander Heiden, L.C. Cantley, C.B. Thompson, Understanding the Warburg effect: the metabolic requirements of cell proliferation, Science 324 (2009) 1029–1033.
[5] S. Ota, K. Horigome, T. Ishii, M. Nakai, K. Hayashi, T. Kawamura, A. Kishino, M. Taiji, T. Kimura, Metformin suppresses glucose-6-phosphatase expression by a complex I inhibition and AMPK activation-independent mechanism, Biochem. Biophys. Res. Commun. 388 (2009) 311–316.
[6] M.Y. El-Mir, V. Nogueira, E. Fontaine, N. Averet, M. Rigoulet, X. Leverve, Dimethylbiguanide inhibits cell respiration via an indirect effect targeted on the respiratory chain complex I, J. Biol. Chem. 275 (2000) 223–228.
[7] M.R. Owen, E. Doran, A.P. Halestrap, Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain, Biochem. J. 348 Pt 3 (2000) 607–614.
[8] W.W. Wheaton, S.E. Weinberg, R.B. Hamanaka, S. Soberanes, L.B. Sullivan, E. Anso, A. Glasauer, E. Dufour, G.M. Mutlu, G.S. Budigner, N.S. Chandel, Metformin inhibits mitochondrial complex I of cancer cells to reduce tumorigenesis, Elife 3 (2014) e02242.
[9] L.H. Stockwin, S.X. Yu, S. Borgel, C. Hancock, T.L. Wolfe, L.R. Phillips, M.G. Hollingshead, D.L. Newton, Sodium dichloroacetate selectively targets cells with defects in the mitochondrial ETC, Int. J. Cancer 127 (2010) 2510–2519.
[10] R.C. Sun, M. Fadia, J.E. Dahlstrom, C.R. Parish, P.G. Board, A.C. Blackburn, Reversal of the glycolytic phenotype by dichloroacetate inhibits metastatic breast cancer cell growth in vitro and in vivo, Breast Cancer Res. Treat. 120 (2010) 253–260.
[11] W. Cao, S. Yacoub, K.T. Shiverick, K. Namiki, Y. Sakai, S. Porvasnik, C. Urbanek, C.J. Rosser, Dichloroacetate (DCA) sensitizes both wild-type and over expressing Bcl-2 prostate cancer cells in vitro to radiation, Prostate 68 (2008) 1223–1231.
[12] K. Ayyanathan, S. Kesaraju, K. Dawson-Scully, H. Weissbach, Combination of sulindac and dichloroacetate kills cancer cells via oxidative damage, PLoS One 7 (2012) e39949.
[13] D. Flavin, Medullary thyroid carcinoma relapse reversed with dichloroacetate: A case report, Oncol. Lett. 1 (2010) 889–891.
[14] J.Y. Wong, G.S. Huggins, M. Debidda, N.C. Munshi, I. De Vivo, Dichloroacetate induces apoptosis in endometrial cancer cells, Gynecol. Oncol. 109 (2008) 394–402.
[15] W.Y. Sanchez, S.L. McGee, T. Connor, B. Mottram, A. Wilkinson, J.P. Whitehead, S. Vuckovic, L. Catley, Dichloroacetate inhibits aerobic glycolysis in multiple myeloma cells and increases sensitivity to bortezomib, Br. J. Cancer 108 (2013) 1624–1633.
[16] E.D. Michelakis, G. Sutendra, P. Dromparis, L. Webster, A. Haromy, E. Niven, C. Maguire, T.L. Gammer, J.R. Mackey, D. Fulton, B. Abdulkarim, M.S. McMurtry, K.C. Petruk, Metabolic modulation of glioblastoma with dichloroacetate, Sci. Transl. Med. 2 (2010), 31ra34.
[17] E.D. Michelakis, L. Webster, J.R. Mackey, Dichloroacetate (DCA) as a potential metabolic-targeting therapy for cancer, Br. J. Cancer 99 (2008) 989–994.
[18] H. Pelicano, D.S. Martin, R.H. Xu, P. Huang, Glycolysis inhibition for anticancer treatment, Oncogene 25 (2006) 4633–4646.
[19] Y.W. Choi, I.K. Lim, Sensitization of metformin-cytotoxicity by dichloroacetate via reprogramming glucose metabolism in cancer cells, Cancer Lett. 346 (2014) 300–308.
[20] N. Masson, P.J. Ratcliffe, Hypoxia signaling pathways in cancer metabolism: the importance of co-selecting interconnected physiological pathways, Cancer Metab. 2 (2014) 3.
[21] N. Rohwer, T. Cramer, Hypoxia-mediated drug resistance: novel insights on the functional interaction of HIFs and cell death pathways, Drug Resist Updat 14 (2011) 191–201.
[22] F. Chen, M. Zhuang, C. Zhong, J. Peng, X. Wang, J. Li, Z. Chen, Y. Huang, Baicalein reverses hypoxia-induced 5-FU resistance in gastric cancer AGS cells through suppression of glycolysis and the PTEN/Akt/HIF-1alpha signaling pathway, Oncol. Rep. 33 (2015) 457–463.
[23] H.N. Choi, H.O. Jin, J.H. Kim, S.E. Hong, H.A. Kim, E.K. Kim, J.K. Lee, I.C. Park, W.C. Noh, Inhibition of S6K1 enhances glucose deprivation-induced cell death via downregulation of anti-apoptotic proteins in MCF-7 breast cancer cells, Biochem. Biophys. Res. Commun. 432 (2013) 123–128.
[24] M.C. Berenbaum, Criteria for analyzing interactions between biologically active agents, Adv. Cancer Res. 35 (1981) 269–335.
[25] K. Momose, A. Minami, Y. Shimono, K. Mizutani, K. Nobutani, T. Azuma, Y. Takai, miR-214 and hypoxia down-regulate Necl-2/CADM1 and enhance ErbB2/ErbB3 signaling, Genes. Cells 18 (2013) 195–202.
[26] Q. Luo, D. Hu, S. Hu, M. Yan, Z. Sun, F. Chen, In vitro and in vivo anti-tumor effect of metformin as a novel therapeutic agent in human oral squamous

cell carcinoma, BMC Cancer 12 (2012) 517.
[27] J.A. Bertout, S.A. Patel, M.C. Simon, The impact of O2 availability on human cancer, Nat. Rev. Cancer 8 (2008) 967–975.
[28] I. Moen, L.E. Stuhr, Hyperbaric oxygen therapy and cancer—a review, Target Oncol. 7 (2012) 233–242.
[29] Q. Peng, Q. Zhou, J. Zhou, D. Zhong, F. Pan, H. Liang, Stable RNA interference of hexokinase II gene inhibits human colon cancer LoVo cell growth in vitro and in vivo, Cancer Biol. Ther. 7 (2008) 1128–1135.
[30] Y. Song, Q. Luo, H. Long, Z. Hu, T. Que, X. Zhang, Z. Li, G. Wang, L. Yi, Z. Liu, W. Fang, S. Qi, Alpha-enolase as a potential cancer prognostic marker promotes cell growth, migration, and invasion in glioma, Mol. Cancer 13 (2014) 65.
[31] V.R. Fantin, J. St-Pierre, P. Leder, Attenuation of LDH-A expression uncovers a link between glycolysis, mitochondrial physiology, and tumor maintenance, Cancer Cell. 9 (2006) 425–434.
[32] Y. Zhao, E.B. Butler, M. Tan, Targeting cellular metabolism to improve cancer therapeutics, Cell. Death Dis. 4 (2013) e532.
[33] Y. Zhuang, D.K. Chan, A.B. Haugrud, W.K. Miskimins, Mechanisms by which low glucose enhances the cytotoxicity of metformin to cancer cells both in vitro and in vivo, PLoS One 9 (2014) e108444.
[34] A.B. Haugrud, Y. Zhuang, J.D. Coppock, W.K. Miskimins, Dichloroacetate enhances apoptotic cell death via oxidative damage and attenuates lactate production in metformin-treated breast cancer cells, Breast Cancer Res. Treat. 147 (2014) 539–550.
[35] J.R. Doherty, C. Yang, K.E. Scott, M.D. Cameron, M. Fallahi, W. Li, M.A. Hall, A.L. Amelio, J.K. Mishra, F. Li, M. Tortosa, H.M. Genau, R.J. Rounbehler, Y. Lu, C.V. Dang, K.G. Kumar, A.A. Butler, T.D. Bannister, A.T. Hooper, K. Unsal-Kacmaz, W.R. Roush, J.L. Cleveland, Blocking lactate export by inhibiting the Myc target MCT1 disables glycolysis and glutathione synthesis, Cancer Res. 74 (2014) 908–920.
[36] I. Marchiq, R. Le Floch, D. Roux, M.P. Simon, J. Pouyssegur, Genetic disruption of lactate/H+ symporters (MCTs) and their subunit CD147/BASIGIN sensitizes glycolytic tumor cells to phenformin, Cancer Res. 75 (2015) 171–180.
[37] S. Granja, I. Marchiq, R. Le Floch, C.S. Moura, F. Baltazar, J. Pouyssegur, Disruption of BASIGIN decreases lactic acid export and sensitizes non-small cell lung cancer to biguanides independently of the LKB1 status, Oncotarget 6 (2015) 6708–6721.
[38] J.M. Heddleston, Z. Li, J.D. Lathia, S. Bao, A.B. Hjelmeland, J.N. Rich, Hypoxia inducible factors in cancer stem cells, Br. J. Cancer 102 (2010) 789–795.
[39] A. Marin-Hernandez, J.C. Gallardo-Perez, S.J. Ralph, S. Rodriguez-Enriquez, R. Moreno-Sanchez, HIF-1alpha modulates energy metabolism in cancer cells by inducing over-expression of specific glycolytic isoforms, Mini Rev. Med. Chem. 9 (2009) 1084–1101.