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

Sung-Eun Hong [a, 1], Hyeon-Ok Jin [b, 1], Hyun-Ah Kim [c], Min-Ki Seong [c], Eun-Kyu Kim [d], Sang-Kyu Ye [e], Tae-Boo Choe [f], Jin Kyung Lee [b], Jong-Il Kim [g], In-Chul Park [a, *], Woo Chul Noh [c, **]

[a] Division of Radiation Cancer Research, Korea Institute of Radiological & Medical Sciences, 75 Nowon-ro, Nowon-gu, Seoul 01812, Republic of Korea
[b] KIRAMS Radiation Biobank, Korea Institute of Radiological & Medical Sciences, 75 Nowon-ro, Nowon-gu, Seoul 01812, Republic of Korea
[c] Department of Surgery, Korea Cancer Center Hospital, Korea Institute of Radiological & Medical Sciences, 75 Nowon-ro, Nowon-gu, Seoul 01812, Republic of Korea
[d] Department of Surgery, Breast Cancer Center, Seoul National University Bundang Hospital, Seoul National University College of Medicine, 82, Gumi-ro 173 Beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do 13620, Republic of Korea
[e] Department of Pharmacology, Seoul National University College of Medicine, 103 Daehangno, Jongno-gu, Seoul 03080, Republic of Korea
[f] Department of Microbiological Engineering, Kon-Kuk University, 120 Neungdong-ro, Gwangjin-gu, Seoul 05029, Republic of Korea
[g] Department of Food Science & Technology, Seoul Women's University, 621 Hwarangro, Nowon-gu, Seoul 01797, Republic of Korea

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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.

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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

* Corresponding author.
** Corresponding author.
E-mail addresses: parkic@kcch.re.kr (I.-C. Park), nohwoo@kcch.re.kr (W.C. Noh).
[1] Sung-Eun Hong and Hyeon-Ok Jin contributed equally to this study.

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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

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