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Research Article

Pyruvate dehydrogenase kinase expression and metabolic changes following dichloroacetate exposure in anoxic human colorectal cancer cells

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ABSTRACT

Dichloroacetate (DCA) is a small molecule that inhibits pyruvate dehydrogenase kinase (PDK) to constrain the aerobic glycolytic pathway observed in many cancer cells and effectively kill them with limited cytotoxicity on normal cells. We previously showed that DCA induced a cytoprotective effect in different human colorectal cancer (CRC) cell lines under anoxic conditions. In this study, we investigated the molecular and metabolic changes that may be providing this cytoprotection. The expression profiles of PDK isoforms in SW480 and LS174T cells along with subsequent changes in pyruvate dehydrogenase (PDH) phosphorylation were assessed following DCA exposure. Changes in mitochondrial activity and subsequent glucose consumption and lactate production were then examined. We show evidence of differential regulation in PDH phosphorylation between different human CRC cells leading to differences in mitochondrial activity following DCA exposure. However, these effects did not lead to significant changes in cellular metabolism nor growth. In conclusion, DCA may only be beneficial in treating a subset of tumor types based on their molecular profiles of different PDK isoforms.

Introduction

Cancer cells undergo a metabolic transition into a state of aerobic glycolysis known as the "Warburg Effect", characterized by a paradoxical reliance on glycolytic pathways for pyruvate reduction under environments of high oxygen tension [1]. This unique metabolic profile provides cancer cells with numerous selective advantages, including adaptation to hypoxia, resistance to mitochondria-mediated apoptosis, and acidification of the tumor microenvironment leading to increased tumor invasion and metastasis [2,3]. Studies have correlated the glycolytic state of tumor cells with cancer aggressiveness, further establishing a critical role for the Warburg Effect [4].

Due to the highly disorganized and chaotic nature of the tumor vasculature, regions of solid tumors are transiently and/or chronically exposed to ischemia and reperfusion, leading to hypoxic/anoxic conditions, which are also known to be mutagenic [5]. The impact of the various microenvironments found in solid tumors

Abbreviations: CRC, colorectal cancer; DCA, dichloroacetate; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; MA, mitochondrial activity.

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on the effectiveness of anti-cancer therapies is not well understood. The Warburg Effect enables cancer cells to use glycolytic pathways regardless of the microenvironment, preventing metabolic dictation and possibly less efficient switching between different strategies.

A key branching point in the glycolytic pathway for this metabolism is the production of pyruvate, which in anaerobic conditions is converted to lactate. Under normoxic conditions, the pyruvate dehydrogenase (PDH) complex (PDC) catalyzes the oxidative decarboxylation of pyruvate to acetyl-CoA for use in the tricarboxylic acid cycle for cellular respiration. PDC is composed of three catalytic components: pyruvate dehydrogenase (E1), dihydrolipoamid acetyl-transferase (E2), and dihydrolipoamide dehydrogenase (E3). PDC activity is inhibited in response to site-specific phosphorylation at three residues on the PDH (E1) subunit: Ser232, Ser293, and Ser300 [6]. Phosphorylation is catalyzed by one of four pyruvate dehydrogenase kinases (PDK) that exhibit tissue-specific expression [7]. PDK2 is reported to be ubiquitously expressed, while PDK1 is expressed in the heart, pancreatic islets, liver and skeletal muscle [8]. PDK3 expression is primarily limited to the testis, but is also expressed at low levels in the lung, kidney, spleen, heart and brain [8]. PDK4 is highly expressed in skeletal muscle and heart while weakly expressed in the kidney, liver and lung [8]. PDK1, 3 and 4 are reported to be hypoxia-responsive proteins under the control of transcription factor HIF-α and are often upregulated during tumorigenesis, playing a crucial role in the Warburg Effect [9–11]. Furthermore, PDK1 has been proposed as a key regulator of the Warburg Effect [10], possibly useful as a biomarker of poor prognosis in patients with gastric cancer and head and neck squamous cell cancer [12,13].

Dichloroacetate (DCA) is an inexpensive small molecule that is currently being evaluated in phase I/II clinical trials for solid tumors, including glioblastoma and glioma [14]. DCA inhibits PDK activity, thereby indirectly activating PDH and shifting metabolism to mitochondrial glucose oxidation [15]. Bonnet et al. initially showed that DCA exposure led to a significant regression of tumors derived from A549 lung carcinoma cells xenografted to rats [16]. Other studies have also demonstrated DCA-induced apoptosis in different cancer models, including ovarian [17], endometrial [18], neuroblastoma [19] and colorectal cancer (CRC) [20]. However, our laboratory previously determined that some human CRC cells are protected from apoptosis upon exposure to DCA under anoxic conditions in vitro and in vivo [21]. Colorectal cancer is the third most common cancer worldwide, and is the second leading cause of cancer-related deaths in the United States [22]. Due to its high prevalence, a better understanding of the responses of CRC to DCA is needed. In this study, we further elucidate the mechanisms by which DCA may be acting on human CRC cells under different oxygen tensions with particular emphasis on PDK expression and PDH regulation.

Materials and methods

Cell culture

Human colorectal cancer cell lines SW480 and LS174T and rat intestinal epithelial cell line IEC-18 were obtained from ATCC (Manassas, VA, USA). Cells were maintained in Dulbecco's modified Eagle medium (DMEM; Sigma-Aldrich, Oakville, ON, Canada) supplemented with 10% fetal bovine serum (FBS; Life Technologies, Grand Island, NY, USA), 1 mM sodium pyruvate (Sigma-Aldrich), and 50 μg/ml gentamicin (Sigma-Aldrich). Normal human colon mucosal epithelial cell line NCM460 was obtained from INCELL (San Antonio, TX, USA) and maintained in M3:base culture media (INCELL) supplemented with 10% FBS (Life Technologies). All cell lines were incubated at 37 °C in a humidified atmosphere containing 5% CO_2 in room air ("normoxia").

In vitro exposure to anoxia and/or DCA

Cells were seeded into plates and incubated under standard cell culture conditions overnight. Thereafter, plates were assigned to control and anoxia treatment, with or without 10 mM DCA (Sigma-Aldrich) for the indicated time periods. Anoxic conditions were achieved using a Modular Incubator Chamber (Billups-Rothenberg Inc., Del Mar, CA, USA) modified to allow continuous flushing of the chamber with a humidified mixture of 95% N_2 and 5% CO_2; the oxygen content in the chamber was less than 0.1% in all anoxic experiments.

Measurement of cell growth

Cell number was approximated through crystal violet staining. Briefly, 5×10^3 cells were seeded into a 96-well plate and cultured in the presence or absence of DCA in normoxia or anoxia for 72 h. Post-treatment media were aspirated and crystal violet solution (1% crystal violet, 20% methanol) was added to each well and incubated for 10 min at room temperature. Plates were then aspirated, rinsed and left to dry overnight. 10% acetic acid was used to dissolve the crystals and the absorbance at 570 nm was recorded. Cell numbers were extrapolated through standard curves previously generated for each cell line. For NCM460 cells, 1×10^4 cells were seeded for the experiments described above.

qRT-PCR for PDK mRNA expression

Following 24 h treatment, RiboZol (AMRESCO, OH, USA) was used to homogenize cells and isolate total RNA according to the manufacturer's protocol. Samples were further purified using the Aurum Total RNA Fatty and Fibrous Kit (Bio-Rad). iScript Reverse Transcription Supermix reagent (Bio-Rad) was used for cDNA synthesis under the following thermal cycle conditions: 25 °C for 5 min, 42 °C for 30 min, 85 °C for 5 min. qRT-PCR was performed using the SsoFast EvaGreen Supermix reagent (Bio-Rad) and reaction conditions were optimized according to the MIQE guidelines [23] to achieve an assay performance efficiency of 90–110%. The following primers for qRT-PCR analysis were obtained using Primer3 software [24]:

PDK1: forward 5′-CTATGAAAATGCTAGGCGTCT-3′, reverse 5′-AACCACTTGTATTGGCTGTCC-3′; PDK2: forward 5′-AGGACACCTACGGCGATGA-3′, reverse 5′-TGCCGATGTGTTTGGGATGG-3′; PDK3: forward 5′-GCCAAAGCGCCAGACAAAC-3′, reverse 5′-CAACTGTCGCTCTCATTGAGT-3′: PDK4: forward 5′-TTATCATACTCCACTGCACCA-3′, reverse 5′-ATAGACTCAGAAGACAAAGCCT-3′; β-actin: forward 5′-AAGATCAAGATCATTGCTCCTC-3′; reverse 5′-CAACTAAGTCATAGTCCGCC-3′. qRT-PCR was conducted in a CFX-96 Real-Time System (BioRad) under the following conditions: 95 °C for 30 s followed by 40 cycles of 95 °C for 10 s and 59 °C for 10 s. Melt curve analysis was performed