available at www.sciencedirect.com
journal homepage: www.europeanurology.com

European Association of Urology

Kidney Cancer

Metabolic Modulation of Clear-cell Renal Cell Carcinoma with Dichloroacetate, an Inhibitor of Pyruvate Dehydrogenase Kinase

Adam Kinnaird[a,b], *Peter Dromparis*[b], *Bruno Saleme*[b], *Vikram Gurtu*[b], *Kristalee Watson*[c], *Roxane Paulin*[b], *Sotirios Zervopoulos*[b], *Trevor Stenson*[b], *Gopinath Sutendra*[b], *Desmond B. Pink*[d], *Katia Carmine-Simmen*[d], *Ronald Moore*[a], *John D Lewis*[d], *Evangelos D. Michelakis*[b,*]

[a] *Division of Urology, University of Alberta, Edmonton, Alberta, Canada;* [b] *Department of Medicine, University of Alberta, Edmonton, Alberta, Canada;* [c] *Department of Laboratory Medicine and Pathology, University of Alberta, Edmonton, Alberta, Canada;* [d] *Department of Oncology, University of Alberta, Edmonton, Alberta, Canada*

Article info

Article history:
Accepted September 10, 2015

Associate Editor:
James Catto

Keywords:
Metabolic modulation
Pyruvate dehydrogenase kinase
Warburg effect
Dichloroacetate

Abstract

Background: Clear-cell renal cell carcinoma (ccRCC) exhibits suppressed mitochondrial function and preferential use of glycolysis even in normoxia, promoting proliferation and suppressing apoptosis. ccRCC resistance to therapy is driven by constitutive hypoxia-inducible factor (HIF) expression due to genetic loss of von Hippel-Lindau factor. In addition to promoting angiogenesis, HIF suppresses mitochondrial function by inducing pyruvate dehydrogenase kinase (PDK), a gatekeeping enzyme for mitochondrial glucose oxidation.
Objective: To reverse mitochondrial suppression of ccRCC using the PDK inhibitor dichloroacetate (DCA).
Design, setting, and participants: Radical nephrectomy specimens from patients with ccRCC were assessed for PDK expression. The 786-O ccRCC line and two animal models (chicken in ovo and murine xenografts) were used for mechanistic studies.
Outcome measurements and statistical analysis: Mitochondrial function, proliferation, apoptosis, HIF transcriptional activity, angiogenesis, and tumor size were measured in vitro and in vivo. Independent-sample *t*-tests and analysis of variance were used for statistical analyses.
Results: PDK was elevated in 786-O cells and in ccRCC compared to normal kidney tissue from the same patient. DCA reactivated mitochondrial function (increased respiration, Krebs cycle metabolites such as α-ketoglutarate [cofactor of factor inhibiting HIF], and mitochondrial reactive oxygen species), increased p53 activity and apoptosis, and decreased proliferation in 786-O cells. DCA reduced HIF transcriptional activity in an FIH-dependent manner, inhibiting angiogenesis in vitro. DCA reduced tumor size and angiogenesis in vivo in both animal models.
Conclusions: DCA can reverse the mitochondrial suppression of ccRCC and decrease HIF transcriptional activity, bypassing its constitutive expression. Its previous clinical use in humans makes it an attractive candidate for translation to ccRCC patients.
Patient summary: We show that an energy-boosting drug decreases tumor growth and tumor blood vessels in animals carrying human kidney cancer cells. This generic drug has been used in patients for other conditions and thus could be tested in kidney cancer that remains incurable.

* Corresponding author. Department of Medicine, University of Alberta, Edmonton T6G 2B7, Canada. Tel. +1 780 4071576.
E-mail address: em2@ualberta.ca (E. Michelakis).

http://dx.doi.org/10.1016/j.eururo.2015.09.014
0302-2838/

1. Introduction

Renal cell carcinoma (RCC) affects more than 270 000 patients annually worldwide but remains deadly and resistant to radiation therapy and chemotherapy [1]. Current treatments with vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and mammalian target of rapamycin (mTOR) antibodies and inhibitors, despite improving progression free survival, rarely provide a durable response [2]. The most common RCC subtype, clear-cell RCC (ccRCC), exhibits loss-of-function mutations or gene silencing of the von Hippel-Lindau (VHL) factor in 70–90% of patients, which leads to elevated levels of hypoxia-inducible factor (HIF), even in normoxia, because of inhibition of the prolyl-hydroxylase–dependent proteasome machinery that normally destabilizes HIF [3–5]. In addition to directly promoting angiogenesis via induction of several proangiogenic genes, HIF upregulates many enzymes of cytoplasmic glycolysis and suppresses mitochondrial glucose oxidation, in part via upregulation of its gatekeeping enzyme pyruvate dehydrogenase kinase (PDK) [6–8]. This normoxic suppression of mitochondrial function, known as the Warburg effect, promotes suppression of mitochondrial-dependent apoptosis and shifts carbohydrates towards biomass biosynthesis, in part via the pentose phosphate pathway (PPP; since the carbohydrates are no longer oxidized in mitochondria) [9–11]. This suppression also leads to a decrease in the production of Krebs cycle metabolites such as α-ketoglutarate (αKG) and the generation of mitochondrial reactive oxygen species (mROS) from the electron transport chain. Both are diffusible and their decrease can have secondary tumor-promoting biologic effects, including inhibition of the redox-sensitive transcription factor p53, further promoting proliferation and resistance to apoptosis [10,12].

Thus, HIF activation in ccRCC underlies the strong proangiogenic and—largely through mitochondrial suppression—antiapoptotic and proproliferative environment. However, the presence of a genetic trigger for HIF stability (ie, VHL loss) limits pharmacologic therapy options, although there are early efforts to develop small-molecule HIF inhibitors [13,14]. The essentially constitutive activation of HIF in normoxia is a constant driver of the metabolic downstream effects and angiogenic products, perhaps explaining the long-term failure of essentially all ccRCC therapies.

While HIF is not ubiquitinated in the absence of VHL, its transcriptional activity can still be inhibited by other pathways. For example, factor inhibiting HIF (FIH) hydroxylates HIF at a site different to the prolyl-hydroxylases that promote its ubiquitination, preventing its binding to DNA hypoxia response elements. FIH is linked to mitochondrial function since it requires αKG as a cofactor. Thus, decreases in αKG levels inhibit the function of FIH, removing this potential defense to HIF activation. Another way to inhibit HIF transcriptional activity may be activation of p53 (which is inhibited by suppressed mitochondria) [15], since they both compete for p300, a cofactor for the transcriptional machinery of both p53 and HIF [16]. A promising ccRCC therapy approach might be a mitochondrial activator that could inhibit HIF function, in addition to promoting apoptosis and suppressing non–HIF-driven pathways via an increase in αKG or activation of p53. Such therapy would be more attractive if it involved a small molecule with an acceptable toxicity profile in humans. The aim of this study was to reverse the mitochondrial suppression of ccRCC using dichloroacetate (DCA), a small molecule that has several of these properties. Our group and others have previously shown that because of its inhibition of PDK, DCA is a potent mitochondrial activator with antitumor activity in several solid tumors [10,17–20]. We report that by inhibiting PDK, DCA activates pyruvate dehydrogenase (PDH) and thus increases the flux of pyruvate into mitochondria in ccRCC. This leads to an increase in the production of Krebs cycle metabolites such as αKG and mROS, activating p53 and inhibiting tumor growth in vitro and in vivo. We also show that DCA inhibits HIF transcriptional activity and decreases angiogenesis in ccRCC, and we propose an αKG- and FIH-dependent mechanism.

2. Materials and methods

2.1. Patient samples

We received institutional Health Research Ethics Board approval to obtain specimens from patients undergoing radical nephrectomy for RCC. All specimens (tumor and surrounding normal tissue from each patient) were fixed with formalin and embedded in paraffin and diagnosed by a pathologist as ccRCC versus normal.

2.2. Cell culture and reagents

The 786-O human ccRCC line was purchased from ATCC (Manassas, VA, USA) and maintained in RPMI 1640 medium (Gibco, Gaithersburg, MD, USA; 10% fetal bovine serum, 1% PSF). 786-O cell lines stably infected with an empty vector (–VHL) or functional hemagglutinin-tagged VHL (+VHL) were a gift from Dr. Kaelin (Harvard Medical School, Boston, MA, USA). Human renal proximal tubular cells were purchased from ScienCell (Carlsbad, CA, USA) and maintained in EpiCM medium. DCA powder (TCI America, Portland, OR, USA) was diluted in sterile water and the solution was adjusted to pH 7.4.

2.3. Nude mouse xenografts

Male *nu/nu* mice (6 wk old; Charles River, Wilmington, MA, USA) were injected with ~3 million 786-O cells (in 250 μl of phosphate-buffered saline) in a 1:1 ratio with Matrigel after institutional animal ethics committee approval [21]. DCA was administered in drinking water as previously described and the dose delivered was determined by daily measurement of the water consumption by each mouse [10].

2.4. In ovo xenografts and angiogenesis

Chick chorioallantoic membrane (CAM) in ovo and angiogenesis assays with 786-O cells were used to study the response to DCA compared to vehicle (water) as previously described [22–24]. Angiogenesis was also measured with a standard Matrigel assay in vitro and with stereoscopic quantification of lectin signaling in tumors as previously described [10].

2.5. Other assays

Metabolites were measured by mass spectroscopy and standard biochemical assays [10,25] and mitochondrial respiration was measured using a Seahorse analyzer (Seahorse Bioscience, Billerica, MA, USA).