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ScienceDirect
Biochimie 91 (2009) 408–415
BIOCHIMIE
www.elsevier.com/locate/biochi

Research paper

α-Ketoglutarate abrogates the nuclear localization of HIF-1α in aluminum-exposed hepatocytes

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Received 27 May 2008; accepted 28 October 2008
Available online 7 November 2008

Abstract

Aluminum (Al), a known environmental pollutant, has been linked to numerous pathologies such as Alzheimer's disease and anaemia. In this study, we show that α-ketoglutarate (KG) mitigates the Al-mediated nuclear accumulation of hypoxia inducible factor-1α (HIF-1α) in cultured human hepatocytes (HepG2). The nuclear localization of HIF-1α appeared to be triggered by the Al-induced perturbation of prolyl hydroxylase 2 (PHD2). This enzyme was markedly diminished in the Al-challenged hepatocytes. The fate of PHD2 and HIF-1α was intricately linked to the mitochondrial dysfunction observed during Al stress. BN-PAGE, immunoblot, and HPLC revealed that the loss of α-ketoglutarate dehydrogenase (KGDH) and succinate dehydrogenase (SDH) activities were coupled to the accumulation of succinate. However, the treatment of the Al-stressed cells with KG recovered the activity and expression of KGDH, SDH, and PHD2 with a concomitant decrease in the levels of HIF-1α in the nucleus. Taken together, these data indicate that the homeostasis of KG plays a pivotal role in aerobic and anaerobic respiration.

Keywords: Aluminum; Succinate; α-Ketoglutarate; Hypoxia inducible factor-1α; Prolyl hydroxylase 2

1. Introduction

Mammalian cells are known to invoke a hypoxic response in order to produce ATP via substrate level phosphorylation when oxygen is limiting [1]. This adaptive response is initiated by HIF-1, a heterodimeric transcription factor comprised of HIF-1α, HIF-2α, and HIF-1β. In contrast to HIF-1β which is constitutively expressed, HIF-1α is extremely sensitive to oxygen tension [2]. Under normoxic conditions HIF-1α is promptly degraded by prolyl hydroxylases (PHDs) and the ubiquitin-proteosomal degradation pathway [3]. PHD demarcates HIF-1α for degradation by the proteosome. PHD induces HIF-1α degradation by the hydroxylation of key proline residues in the oxygen-dependent degradation domain, a catalytic process which requires KG, Fe, and O_2. Three separate PHD isozymes (PHD 1–3) have been implicated in this process however, PHD2 is the most characterized. Hypoxia, Fe limitation, and oxidative stress appear to impede PHD activity thus stabilizing HIF-1α [3–5]. These events promote the activation of the hypoxic gene programs which encourage cell survival, glucose utilization, substrate level phosphorylation, erythropoiesis, and glycolysis. The activation of glycolysis during hypoxia is crucial to cell survival since it permits energy production without the participation of the mitochondria [6].

The mitochondria are the main site of cellular energy production generating 90% of the cell's ATP in an oxygen-dependent manner [7]. Aside from ATP production, the mitochondria have been shown to play a role in oxygen sensing and the activation of hypoxia signaling pathways [8]. Lack of oxygen, the accumulation of succinate, and abnormal ROS production have all been implicated in the mitochondrial-mediated activation of hypoxic response pathways [3,9,10]. Use of mitochondria-deficient cells, respiratory inhibitors, and ROS scavengers has helped delineate the role of ROS in the

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doi:10.1016/j.biochi.2008.10.014

oxygen sensing capabilities of this organelle. Due to inefficient electron transfer in cells treated with respiratory inhibitors, the resulting increase in mitochondrial ROS promotes HIF-1α stabilization [3,5]. The accumulation of some tricarboxylic acid (TCA) cycle intermediates such as succinate also participates in the stabilization of HIF-1α. Succinate accumulation as a result of lowered SDH expression and the concomitant stabilization of HIF-1α is observed in several types of cancers [6]. Furthermore, Fe limitation and toxic divalent metals such as Ni^{2+}, Co^{2+}, and Cu^{2+} have been shown to activate the hypoxic response [11–13].

Although Al is known as an environmental toxin, the precise molecular details that enable this trivalent metal to be toxic are still not well understood. Its ability to disturb Fe homeostasis and trigger the formation of an oxidative environment is postulated to be the source of Al toxic influence [14]. This trivalent metal has also been shown to interfere with ion channels, perturb Ca^{2+} homeostasis, and disrupt biological membranes [15]. Even though the impact of Al on some cellular processes has begun to emerge, the influence of this toxic metal on energy metabolism remains poorly understood. We have recently shown that the Al-mediated disruption of aerobic metabolism in HepG2 cells leads to the stabilization of HIF-1α and the enhanced anaerobic metabolism of glucose [16]. The disruption of aerobic metabolism by Al was characterized by the dedication of KG to ROS detoxification and the subsequent accumulation of succinate, a potent stabilizer of HIF-1α [17–19]. Here, the mechanistic details underlying the Al-mediated nuclear localization of HIF-1α have been probed. The ineffective PHD2 induced by the altered homeostasis of KG appeared to be responsible for the stabilization of HIF-1α. Treatment with KG restored PHD2 to control levels and abolished the nuclear accumulation of HIF-1α. The significance of KG in mitochondrial function and anaerobiosis is also discussed.

2. Materials and methods

2.1. Cell culture and subcellular fractionation

HepG2 cells were a gift from Dr. D. Templeton (University of Toronto) and were maintained in α-MEM supplemented with 5% FBS and 1% antibiotics and cultured as described previously [16]. The HepG2 cells were utilized since their metabolic profile does not deviate significantly from normal hepatocytes and are thus an excellent model to study hepatic metabolism [20]. Upon reaching 70% confluency, the cell cultures were re-supplemented with serum-free media containing citrate (2.5 mM; control) or Al chelated to citrate (0.5 mM Al:2.5 mM citrate; Al stress) and exposed for 4–24 h. The amount of Al used in these experiments was 13.4 μg/ml compared to 750 μg/ml in the serum of Alzheimer's patients [21]. Regulation experiments were performed on Al-stressed cells transferred to α-MEM containing 0.5–5 mM KG or 0.5 mM *N*-acetyl cysteine (NAC) for 24 h (*Note*: hepatocytes are known to have dicarboxylic acid transporters thus the use of derivatized substrates was not necessary [22]). Similar experiments were performed with control cells exposed to Al-citrate for 24 h. Cell viability was assessed using the Trypan Blue Exclusion Assay. At the desired time intervals cells were isolated and the cellular pellet was suspended in a cell storage buffer (CSB) (50 mM Tris–HCl, 1 mM phenylmethylsulfonylfluoride, 1 mM dithiothreitol, 250 mM sucrose, 2 mM citrate, 1 mg/ml pepstatin, 0.1 mg/ml pepstatin) and fractionated as described in Refs. [17,18,23]. The purity of the cellular fractions was confirmed using immunoblot and antibodies directed against F-actin, voltage dependent anion channel (VDAC), and histone 2A (H2A) for the cytosol, the mitochondria, and the nuclear extract respectively. The protein concentration of each fraction was assessed using the Bradford assay and BSA was used as the standard protein.

2.2. Measurement of H_2O_2

H_2O_2 levels were ascertained as described previously [18]. 2 mg/ml equivalent of CFE was incubated in a reaction buffer (25 mM Tris, 5 mM $MgCl_2$, pH 7.0) containing peroxidase, *p*-anisidine, 5 mM citrate, and 1 mM NAD. The reaction was stopped after 60 min and the absorbance was recorded at 458 nm.

2.3. In-gel activity assays

BN-PAGE was performed as described in Refs. [24–26]. Following the completion of electrophoresis, the gel was placed in reaction buffer (25 mM Tris, 5 mM $MgCl_2$ (pH 7.4)) for 15 min. Enzymatic activity was visualized using phenazine methosulfate (PMS) and iodonitrotetrazolium chloride (INT) [26]. KGDH activity staining was performed using reaction buffer, NAD^+ (0.5 mM), CoA (0.5 mM), KG (10 mM), PMS, and INT. Similarly SDH activity was ascertained using reaction buffer, 50 mM succinate, INT, and PMS. Band specificity was ensured using reactions devoid of substrate. Coomassie staining of the respective gels assured proper loading. The apparent molecular mass of each enzyme was confirmed using known standards and enzymes. Activity bands were quantified using SCION Imaging for Windows (SCION Corporation, Frederick, MD). Activity analyses were performed in triplicate.

2.4. Immunoblot analysis

Sodium dodecyl sulfate-PAGE (SDS-PAGE) was performed according to a modified protocol by Laemmli [27]. Briefly, samples were prepared in sample buffer (62.5 mM Tris–HCl (pH 6.8), 7% glycerol (w/v), 2% SDS (w/v), and 2% β-mercaptoethanol (v/v)) at a final concentration of 1 mg/ml and stored at −20 °C until needed. 30 μg protein was loaded into each well. Following electrophoresis, the proteins were transferred to a Hybond™-P polyvinylidene difluoride membrane for immunoblotting. Nonspecific binding sites were blocked using Blotto solution as described in Ref. [19]. Polyclonal antibodies directed against HIF-1α, (Santa Cruz, CA)

was used to determine the expression levels of their cognate epitopes. Antibodies directed specifically against PHD2 (Abcam, Cambridge, MA) were employed to assess the availability of this hydroxylase in the cytosol [28]. The antibodies raised against KGDH (E_2 subunit) and SDH (SDHB subunit) were generously supplied by Dr. G. Lindsay, University of Glasgow and Dr. Lemire, University of Alberta. F-actin, H2A (Santa Cruz, CA), and VDAC (Abcam, Cambridge, MA) were used as the loading controls. The secondary antibody consisted of horseradish peroxidase-conjugated mouse anti-rabbit, goat anti-mouse, or mouse anti-goat (Santa Cruz, CA). Following a 5 min incubation in Chemiglow (Alpha Innotech), the immunoblots were subsequently documented using the ChemiDoc XRS system (Biorad Imaging Systems). Band intensity was quantified using Alpha Innotech Software (Alpha Innotech Corporation). Experiments were performed in triplicate to ensure reproducibility.

2.5. Analysis of metabolites

To analyze the disparate metabolism in Al-stressed cells, metabolite levels were assessed using an Alliance HPLC (Waters). A 2 mg protein equivalent of mitochondria from control and Al-treated cells were incubated for 1 h at 37 °C in a phosphate reaction buffer (10 mM Na_2HPO_4, pH 7.4) containing 1 mM citrate and 0.1 mM NAD^+. Reactions were quenched on ice using a 0.5% (v/v) solution of perchloric acid diluted in mobile phase [29]. To determine the metabolite concentration in the cytosol and mitochondria in HepG2 cells exposed to control and Al-stressed conditions, 2 mg/ml of protein equivalent of the cytosol and mitochondria were diluted and treated with perchloric acid as described previously. Metabolites were separated using a C_{18} column with a polar cap (3.5 µm, amide cap, 4.6 mm × 150 mm inside diameter, Symmetry Column, Phenomenex®, Torrance, CA, USA). The mobile phase consisted of 20 mM KH_2PO_4 (pH 2.9 with 6 N HCl) and the column was eluted at a flow rate of 0.7 ml/min at 25 °C ambient temperature. Organic acids were detected at 210 nm. Peaks were identified by injecting known standards and by performing spiking experiments. The peaks corresponding to KG and succinate were quantified using EMPOWER software (Waters). The retention times of KG and succinate were further confirmed by collecting fractions and performing enzymatic assays [23].

2.6. Fluorescence microscopy

HepG2 cells were seeded at 50 000 cells/ml, exposed to citrate or Al-citrate for 24 h, and then prepared for microscopic analysis as described in Ref. [18]. Primary antibodies directed against HIF-1α (anti-HIF-1α 1/100 dilution) and secondary antibodies (anti-mouse FITC conjugate 1/1000) were utilized. The cells were then stained with propidium iodide (PI) (50 nM in 10 mM Na-citrate) and visualized with an inverted deconvoluting microscope (Zeiss). Merged images were generated by taking synchronous pictures using Axiovision software (Zeiss) for FITC and PI. This allowed the determination of the intracellular location of HIF-1α. The combination of the green fluorescence from FITC and the red fluorescence from PI provides a yellow which can be easily distinguished.

2.7. Statistical analysis

Data were expressed as mean ± SD. Statistical correlations of data were checked for significance using the Student *t* test. All experiments were performed twice and in triplicate.

3. Results

3.1. Aluminum toxicity alters the intracellular levels of KG and succinate

Al-mediated disruption of selected TCA cycle enzymes such as aconitase (ACN) and NAD^+-dependent isocitrate dehydrogenase (NAD^+-ICDH) is a well-documented phenomenon [30]. In addition, other TCA cycle enzymes such as KGDH and SDH display sensitivity to Al, Zn, and ROS [19,31]. The ability of the mitochondria from control and Al-treated cells to metabolize citrate was assessed by incubating 2 mg of protein equivalent to mitochondria in 1 mM citrate for 1 h. The Al-treated mitochondria accumulated KG, succinate, and citrate as opposed to the control mitochondria which metabolized the citrate much more readily (Fig 1, Panel 1). The perturbation of KG and succinate metabolism in the mitochondria from the Al-treated HepG2 cells prompted us to examine the activity and expression of KGDH and SDH in the mitochondria. In-gel activity analyses showed that Al treatment of the HepG2 cells diminished the activity of KGDH in the mitochondria. Band quantification revealed that KGDH activity was decreased sharply in the Al-stressed mitochondria in comparison to the control (Fig 1, Panel 2). However, over a period of 24 h the levels of the E_2 subunit were observed to be similar in the Al-treated and control mitochondria (Fig 1, Panel 3). SDH also displayed diminished activity in the Al-treated cells (Fig 1, Panel 4). Unlike KGDH however, the diminished activity of SDH in the Al-stressed HepG2 cells was due to a decrease in protein expression (Fig 1, Panel 5).

3.2. KG supplementation mitigates Al toxicity in HepG2 cells

KG is a known antioxidant and has been shown to produce succinate via the nonenzymatic sequestration of ROS molecules [18]. HepG2 cells exposed to Al contained higher amounts of ROS, a situation abrogated by KG (Table 1). Furthermore, the incubation of the Al-treated HepG2 cells in 5 mM KG improved cell viability (Table 1). As KG appeared to mitigate the toxicity of Al, the utilization of this keto acid was monitored by HPLC. Exposure of Al-treated HepG2 cells to KG for 24 h resulted in a 4-fold increase in KG in both the cytosol and the mitochondria in comparison to control. In addition a 2-fold increase and a 4-fold increase in succinate were also observed in the cytosol and mitochondria of Al-stressed HepG2 cells exposed to KG for 24 h (Fig. 2, Panel 1).

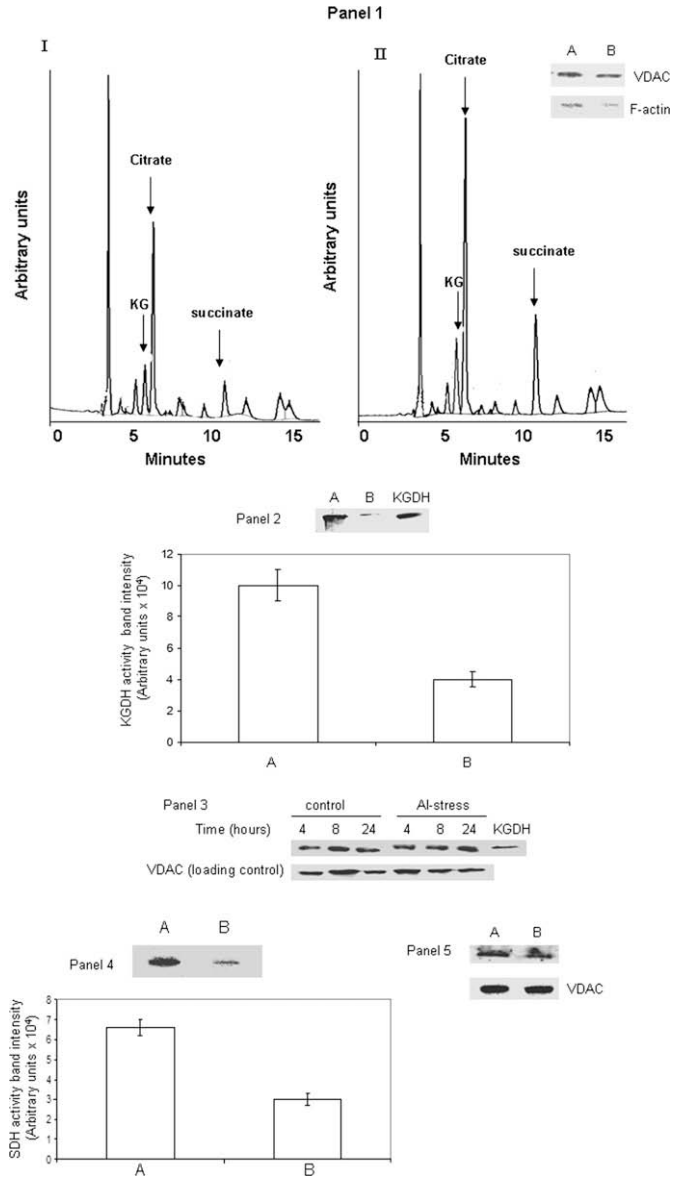

Fig. 1. Panel 1: Representative chromatographs showing the accumulation of succinate and KG. A 2 mg equivalent of mitochondrial protein from HepG2 cells exposed to I) citrate (control) and II) Al-citrate (Al stress) was incubated for 1 h at 37 °C in the presence of 1 mM citrate and 0.1 mM NAD^+ and analyzed by HPLC. Injections were performed in triplicate. Inset: The purity of the mitochondria and the cytosol was tested by immunoblotting for VDAC (mitochondria) and F-actin (cytosol) (A = control and B = Al stress). Panel 2: In-gel detection of KGDH in mitochondria from cells exposed to A) control and B) Al-stressed conditions for 24 h. The activity bands were quantified using SCION imaging for Windows. $n = 3$, S.D. ± mean, $p = 0.05$. Panel 3: Time profile for KGDH expression in HepG2 cells exposed to citrate and Al-citrate for 24 h. Cells were isolated at desired time intervals and KGDH was detected by immunoblot. KGDH = α-ketoglutarate dehydrogenase standard from Porcine heart (SIGMA). Panel 4: In-gel detection of SDH activity in mitochondria from cells exposed to A) control and B) Al-stressed conditions for 24 h. The activity bands were quantified using SCION imaging for Windows. $n = 3$, S.D. ± mean, $p = 0.05$. Panel 5: Immunodetection of SDH expression in the mitochondria of HepG2 exposed to A) control and B) Al-stressed conditions for 24 h.

These data suggest that KG sequesters the ROS generated by Al. KG supplementation also recovered the activity of KGDH and the expression of SDH (Fig 2, Panels 2 and 3). The recovery of SDH would ensure the metabolism of succinate in the TCA cycle. Experiments with control cells supplemented with KG indicated that this keto acid was metabolized immediately by the mitochondria (data not shown).

3.3. KG abrogates the Al-mediated nuclear accumulation of HIF-1α

The accumulation of succinate in the cytosol in the Al-stressed cells prompted us to discern if Al toxicity perturbs PHD2 and HIF-1α. Immunoblot revealed no band corresponding to PHD2 in the Al-exposed cells. However, the incubation of Al-treated HepG2 cells in KG rescued the level of PHD2. This amount appeared to be relatively similar to that of control cells (Fig. 3, Panel 1). This is not surprising since PHD2 depends on KG for its proper folding and activity. To further elucidate the importance of KG in this recovery process, the Al-stressed cells were exposed to 0.5 mM NAC, a known antioxidant. In this instance the recovery was lower than that observed for KG (Fig. 3, Panel 2).

Al exposure resulted in increased HIF-1α levels in the nucleus as opposed to control nuclei (Fig. 4, Panel 1). To test whether KG can prevent HIF-1α stabilization, we analyzed the nuclei from Al-treated HepG2 cells supplemented with KG. Indeed, exposure of Al-stressed HepG2 cells to 5 mM KG ablated the expression of HIF-1α in the nucleus (Fig. 4, Panel 1). In addition, NAC was not as efficient at reducing HIF-1α levels in the nucleus as indicated by immunoblot (Fig. 4, Panel 2). Indeed, a low intensity band was observed in the nucleus whereas Al-treated cells exposed to 0.5 mM KG contained no detectable HIF-1α in their nuclei. Immunofluorescence was used to confirm the Al-mediated localization of HIF-1α. FITC staining of HIF-1α (green) and PI (red) staining of the nucleus provided a yellow fluorescence in the merged image consistent with the migration of HIF-1α into the nuclear compartment (Fig. 4, Panel 3).

4. Discussion

Although Al has been shown to disrupt Fe homeostasis and macromolecules, the molecular details underlying its toxicity

Table 1
Assessment of the cell viability and H_2O_2 levels in Al-exposed cells treated with KG.

	Number of viable cells ($\times 10^5$ cells/ml)	Amount of H_2O_2 (nmol/mg of protein)
Control	7.12 ± 0.42	10.43 ± 2.11
Al stress	2.23 ± 0.19	35.78 ± 3.56
Al-stress treated with 5 mM KG	5.78 ± 0.91	15.32 ± 2.99
Control treated with Al	4.27 ± 0.65	20.87 ± 0.55

$n = 3$, mean ± S.D. $p \leq 0.05$. H_2O_2 was measured using *p*-anisidine following a 1 h reaction of a 2 mg/ml equivalent of CFE with 5 mM citrate, reaction buffer, NAD, and peroxidase. Cell viability was ascertained using the Trypan Blue Exclusion Assay.

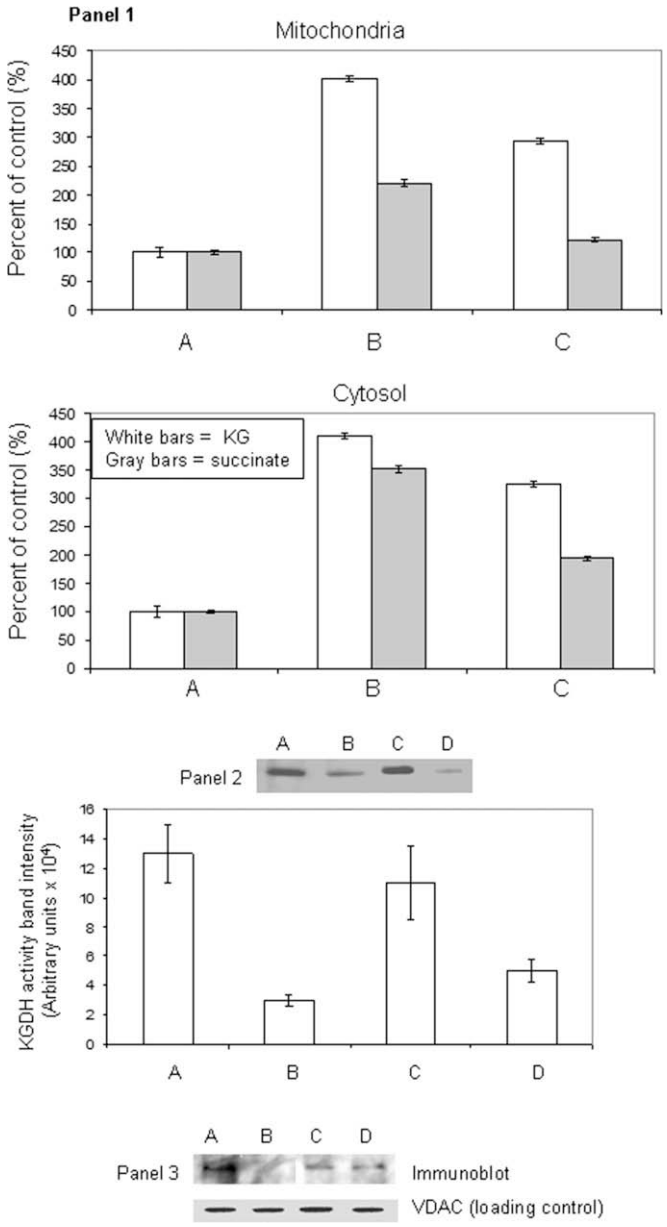

Fig. 2. Supplementation of HepG2 cells with KG protects them from Al toxicity. Cells were exposed to control or Al-stressed conditions for a 24 h period. For regulation assays, following a 24 h exposure to control and Al-stressed conditions, cultures were re-supplemented with α-MEM containing KG or Al-citrate. Panel 1: Analysis of α-ketoglutarate (white bars) and succinate (grey bars) levels in the cytosol and mitochondria of Al-treated HepG2 cells exposed to 5 mM KG and in control cells exposed to Al for 24 h. A 2 mg protein equivalent to cytosol and mitochondria was treated with a 0.5% solution of perchloric acid and injected into the HPLC. % values correspond to the amount of KG and succinate associated with each fraction (A = control, B = Al-stressed cells exposed to 5 mM KG, and C = control cells exposed to Al-citrate). The peaks were manually quantified using EMPOWER software (cytosol: 100% for KG and succinate is equivalent to an absorbance value of 0.0019 and 0.0014 μmol. Mitochondria: 100% for KG and succinate is equivalent to an absorbance value of 0.0026 and 0.0031 μmol). $n = 3$, mean ± S.D., $p \leq 0.05$. White bars = KG, Grey bars = succinate. Panel 2: In-gel detection of KGDH activity in HepG2 cells exposed to A) control, B) Al stress, C) Al-stressed cultures exposed to 5 mM KG, and D) control cells exposed to Al-citrate for 24 h. The activity bands were quantified using SCION imaging for Windows. $n = 3$, S.D. ± mean, $p = 0.05$. Panel 3: Immunodetection of SDH expression in HepG2 cells exposed to A) control, B) Al stress, C) Al-stressed cells exposed to 5 mM KG, and D) control cells exposed to Al-citrate for 24 h. VDAC was utilized as a loading standard.

remain poorly understood. We have recently shown that Al interferes with mitochondrial metabolism and activates the hypoxic response pathway [16,18,19]. In the present study, we demonstrate that the Al-mediated nuclear accumulation of HIF-1α is modulated by TCA cycle metabolism. The inability of the TCA cycle to function properly was characterized by the altered homeostasis of KG and succinate. The loss of KGDH and SDH function confers new cellular roles upon KG and succinate since energy metabolism is perturbed. While KG sequesters ROS, succinate activates anaerobic respiration. This survival mechanism would allow the mitochondria to signal the status of aerobic respiration to the nucleus when the mitochondria are dysfunctional. KGDH, a key mitochondrial enzyme that produces NADH and CO_2 via the decarboxylation of KG, displayed a sharp decrease in activity in the Al-treated cells. This is consistent with previous observations that Al, Zn, and H_2O_2 affect the activity and not the expression of KGDH [18,19,32]. The E_2 subunit of KGDH harbours a redox sensitive dihydrolipoic acid residue and an oxidative environment would deactivate the dihydrolipoic acid residue rendering the enzyme ineffective. The sensitivity of dihydrolipoic acid to oxidation has been suggested to serve as a defense mechanism aimed at promoting the accumulation of KG, a potent antioxidant [18,33,34]. The data in this study clearly point to the recovery of KGDH and SDH in Al-stressed cells treated with KG. Hence, this antioxidant may be promoting aerobic respiration, a phenomenon perturbed during Al toxicity [19]. We have recently shown that the nuclear migration of HIF-1α in the Al-exposed hepatocytes leads to anaerobiosis. In this instance, glycolytic enzymes such as hexokinase and lactate dehydrogenase were markedly increased [16].

Al is well known for its pro-oxidant effects on cells. Indeed, this metal is not only known for its ability to produce ROS but

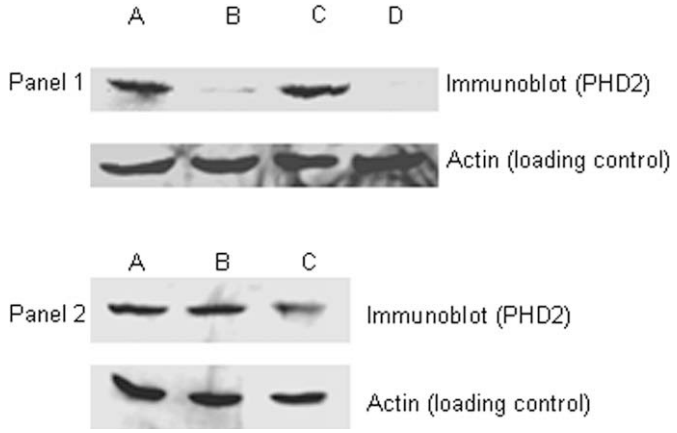

Fig. 3. Determination of PHD2 expression by immunoblot. Cells were exposed to control or Al-stressed conditions for a 24 h period. For regulation assays, following a 24 h exposure to control and Al-stressed conditions, cultures were re-supplemented with α-MEM containing KG, NAC, or Al-citrate. Panel 1: The expression of PHD2 in HepG2 cells exposed to A) control, B) Al stress, C) Al-stressed cells exposed to 5 mM KG, and D) control cells exposed to Al-citrate for 24 h. Panel 2: PHD2 expression in HepG2 cells exposed to A) control, B) Al-stressed cells exposed to 0.5 mM KG, and C) Al-stressed cells exposed to 0.5 mM NAC.

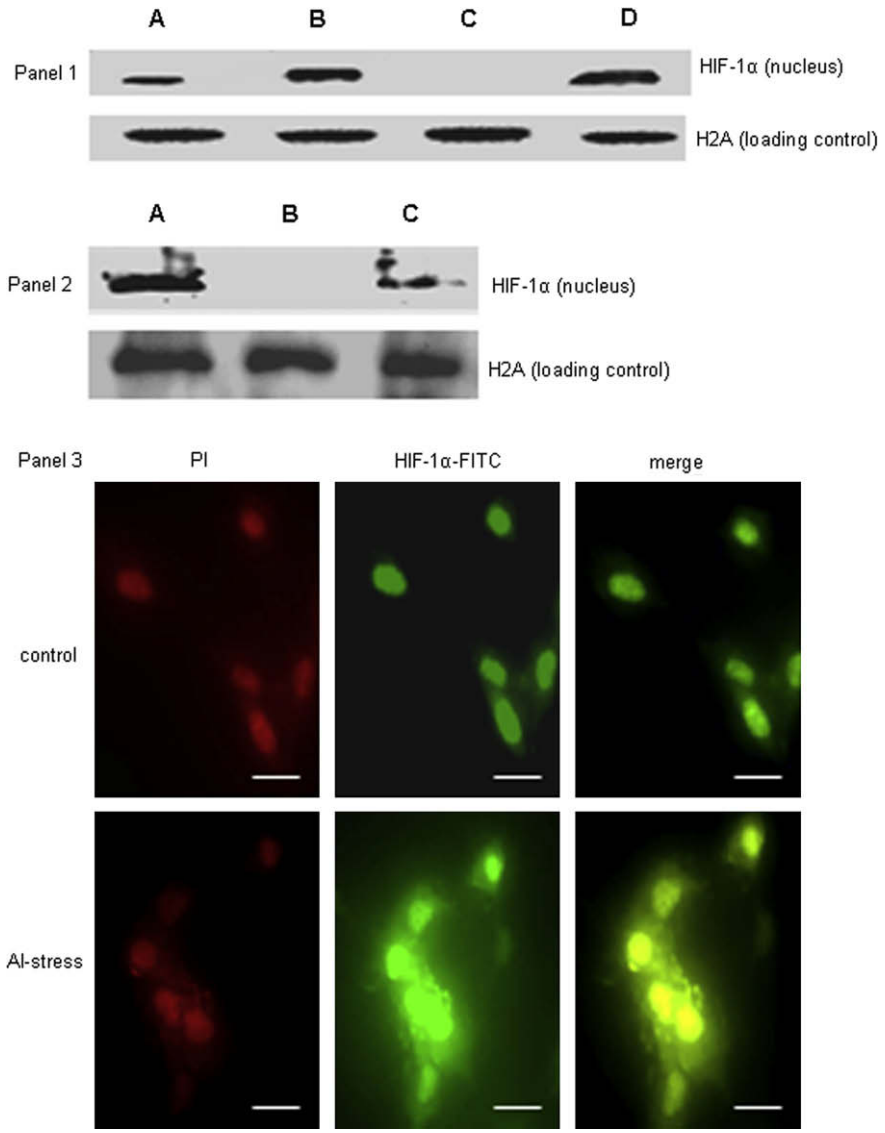

Fig. 4. Detection of HIF-1α in the nucleus of HepG2 cells. Cells were exposed to control or Al-stressed conditions for a 24 h period. For regulation assays, following a 24 h exposure to control and Al-stressed conditions, cultures were re-supplemented with α-MEM containing KG, NAC, or Al-citrate. Panel 1: Immunodetection of HIF-1α in the nuclear fraction of HepG2 cells exposed to A) control, B) Al stress, C) Al-citrate cells re-supplemented with 5 mM KG, and D) citrate cells re-supplemented with Al-citrate containing media. Panel 2: HIF-1α expression in HepG2 cells exposed to A) Al-stressed B) Al-stressed cells exposed to 0.5 mM KG for 24 h, and C) Al-stressed cells exposed to 0.5 mM NAC for 24 h. Panel 3: Immunofluorescence detection of HIF-1α. The localization of the nucleus was ascertained using propidium iodide (PI). Scale bar: 10 μm.

it also interferes with antioxidant enzymes such as SOD and catalase [35,36]. Thus, the accumulation of KG would be crucial in sustaining antioxidant defense. The presence of Al also severely diminished the activity and expression of SDH, a heme and Fe–S dependent enzyme. Fe-dependent TCA cycle enzymes are known targets of Al toxicity [37]. We have recently demonstrated that Fe-dependent enzymes such as aconitase, cytochrome *c* oxidase, and fumarase exhibit lowered activity and expression in Al, Zn, and H_2O_2-treated HepG2 cells [19,31]. The ability of Al to interfere with KGDH and SDH resulted in the accumulation of KG and succinate, two TCA cycle intermediates which participate in numerous processes within the cell. The former can sequester ROS generating succinate while the latter may signal a hypoxic situation. KG and succinate may signal the respiratory status of the mitochondria to the nucleus in order to maintain cellular ATP levels, an over-riding function for any cell.

Recent work has indicated that succinate promotes the stabilization of HIF-1α by interfering with PHD, a dioxygenase which initiates the degradation of HIF-1α [38]. This dicarboxylic acid is known to perturb substrate binding sites in the enzyme [10,39]. In the HepG2 cells exposed to Al, PHD2 was completely abolished. In sharp contrast, PHD2 expression was fully recovered in Al-treated cells exposed to 5 mM KG. The proper functioning of PHD2 is dependent upon KG, Fe, and ascorbic acid three cofactors that may be limiting during Al toxicity. Indeed, the absence of any of these cofactors significantly reduces the stability of PHD2 thus prompting its degradation [4]. The redirection of KG towards ROS detoxification may significantly limit the availability of this moiety

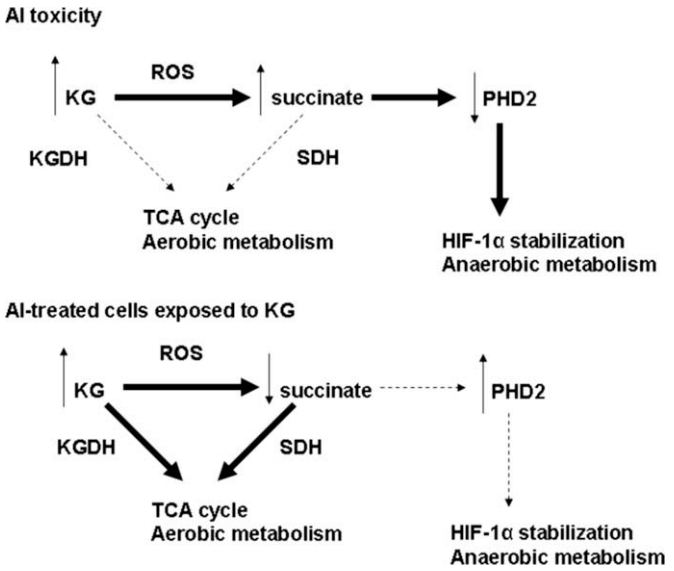

Fig. 5. The homeostasis of KG modulates aerobic and anaerobic metabolism in Al-treated cells. Bold arrows represent an increase. Broken arrows represent a decrease.

for PHD2 stabilization following translation. Furthermore, the KG-mediated detoxification of ROS produces succinate, a potent PHD inhibitor. NAC, a known antioxidant, did to some extent recover PHD2 and lower HIF-1α in the nucleus. However, KG was much more effective indicating that the homeostasis of this TCA cycle intermediate may be central to regulating aerobic and anaerobic respiration. In addition, Al may also compete for the Fe binding site in PHD2 destabilizing this dioxygenase. Al is known to perturb Fe-dependent enzymes [19,37]. Thus, numerous factors may act in parallel to disturb PHD2 levels in the Al-treated cells.

In conclusion, this study indicates that the Al-mediated disruption of key TCA cycle enzymes results in the localization of HIF-1α to the nucleus. These events are set into motion by the deregulation of KGDH and SDH and the concomitant intracellular increase in succinate, a signal for mitochondrial dysfunction. The resulting diminution in PHD2 promotes the nuclear accumulation of HIF-1α in the nuclei. Furthermore, the Al-mediated activation of hypoxic programs is completely abrogated by exposing the cells to KG, a multi-functional metabolite. To our knowledge this is the first demonstration of the ability of Al to activate a mitochondrial-to-nuclear signaling pathway which ensures the survival of the hepatocytes. Although these intriguing results were obtained in a model cell line system, it is tempting to propose that KG may serve as an important tool in treating metal-provoked hepatic disorders. Fig. 5 depicts a mechanism of Al-induced HIF-1α localization to the nucleus and the pivotal role of KG homeostasis in this process. Thus, the Al-induced localization of HIF-1α to the nucleus is an important adaptive response that promotes cell survival during Al toxicity and mitochondrial dysfunction.

Acknowledgements

This research was funded by Industry Canada.

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