Scandinavian Journal of Gastroenterology. 2012; 47: 565–571

informa
healthcare

ORIGINAL ARTICLE

Alpha-ketoglutarate (AKG) inhibits proliferation of colon adenocarcinoma cells in normoxic conditions

WOJCIECH RZESKI[1,2], KATARZYNA WALCZAK[2], MAŁGORZATA JUSZCZAK[2], EWA LANGNER[2], PIOTR POŻAROWSKI[3], MARTYNA KANDEFER-SZERSZEN[1] & STEFAN G. PIERZYNOWSKI[2,4]

[1]*Department of Virology and Immunology, Institute of Microbiology and Biotechnology, Maria Curie-Skłodowska University, Akademicka, Lublin, Poland,* [2]*Department of Medical Biology, Institute of Agricultural Medicine, Jaczewskiego, Lublin, Poland,* [3]*Department of Clinical Immunology, Medical University of Lublin, Chodźki, Lublin, Poland, and* [4]*Department of Cell and Organism Biology, Lund University, Helgonavägen, Lund, Sweden*

Scand J Gastroenterol Downloaded from informahealthcare.com by 87.246.233.207 on 04/10/12
For personal use only.

Abstract

Background and objective. Alpha-ketoglutarate (AKG), a key intermediate in Krebs cycle, is an important biological compound involved in the formation of amino acids, nitrogen transport, and oxidation reactions. AKG is already commercially available as a dietary supplement and its supplementation with glutamine, arginine, or ornithine alpha-ketoglutarate has been recently considered to improve anticancer immune functions. It is well documented that AKG treatment of Hep3B hepatoma cells in hypoxia induced HIF-alpha (hypoxia-inducible factor) degradation and reduced vascular endothelial growth factor (VEGF) synthesis. Moreover, AKG showed potent antitumor effects in murine tumor xenograft model, inhibiting tumor growth, angiogenesis, and VEGF gene expression. However, the mechanisms of its anticancer activity in normoxia have not been examined so far. ***Results.*** Here, we report that in normoxia, AKG inhibited proliferation of colon adenocarcinoma cell lines: Caco-2, HT-29, and LS-180, representing different stages of colon carcinogenesis. Furthermore, AKG influenced the cell cycle, enhancing the expression of the inhibitors of cyclin-dependent kinases p21 Waf1/Cip1 and p27 Kip1. Moreover, expression of cyclin D1, required in G1/S transmission, was decreased, which accompanied with the significant increase in cell number in G1 phase. AKG affected also one the key cell cycle regulator, Rb, and reduced its activation status. ***Conclusion.*** In this study for the first time, the antiproliferative activity of AKG on colon adenocarcinoma Caco-2, HT-29, and LS-180 cells in normoxic conditions was revealed. Taking into consideration an anticancer activity both in hypoxic and normoxic conditions, AKG may be considered as a new potent chemopreventive agent.

Key Words: *α-ketoglutarate, cell cycle, normoxia, p21 Waf1/Cip1, proliferation, Rb*

Introduction

Alpha-ketoglutarate (AKG), a key intermediate in Krebs cycle, is an important biological compound involved in the formation of amino acids, nitrogen transport, and oxidation reactions. Although AKG is already commercially available as a dietary supplement, supplementation with glutamine (Glu), arginine (Arg), or ornithine alpha-ketoglutarate (OKG) has attracted recent attention for the potential to improve anticancer immune functions. Robinson et al. [1] reported that rats fed with OKG had increased macrophage cytostatic activity and NK cell cytotoxicity. Moreover, rats bearing tumors fed with OKG showed a better positive nitrogen balance, higher concentration of glutamine, and branched-chain amino acids in muscle [2]. Oxygen deprivation leading to hypoxia is a common feature of solid tumors, which often drives their progression and aggressiveness [3]. Under these conditions, a signaling pathway involving a key hypoxia-inducible factor (HIF)

Correspondence: Wojciech Rzeski, Department of Virology and Immunology, Institute of Microbiology and Biotechnology, Maria Curie-Skłodowska University, Akademicka 19, 20-030 Lublin, Poland. Tel: +48 81 5375947. Fax: +48 81 537 59 40. E-mail: rzeskiw@hektor.umcs.lublin.pl

(Received 6 September 2011; revised 7 December 2011; accepted 17 January 2012)
ISSN 0036-5521 print/ISSN 1502-7708 online © 2012 Informa Healthcare
DOI: 10.3109/00365521.2012.660539

RIGHTSLINK

Scand J Gastroenterol Downloaded from informahealthcare.com by 87.246.233.207 on 04/10/12
For personal use only.

is switched on. HIF is a transcription factor that, in hypoxia, drives expression of several genes involving angiogenesis, metastasis, and apoptosis. It has been reported that AKG treatment of Hep3B hepatoma cells induced alpha-HIF degradation *in vitro* and reduced VEGF protein synthesis [4]. Moreover, AKG showed potent antitumor effects in murine tumor xenograft model, inhibiting tumor growth, angiogenesis, and VEGF gene expression [5]. To date, most experiments concerned alpha-ketoglutarate activity in hypoxia. Therefore, the aim of the study was to examine whether alpha-ketoglutarate directly influences colon cancer cell proliferation and to figure out the related mechanism(s) in normoxic conditions.

Materials and methods

Cell lines

Human colon adenocarcinoma cell lines HT-29 and LS-180 were obtained from the ECACC (European Collection of Cell Cultures, Salisbury, UK). Human colon adenocarcinoma Caco-2 cells were obtained from ATCC (American Type Culture Collection, Manassas, VA, USA). HT-29 and LS-180 cells were grown in 1:1 mixture of DMEM and nutrient mixture Ham F-12 supplemented with 10% FBS. Caco-2 cells were grown in Eagle's Minimum Essential Medium (ATCC) supplemented with 20% FBS. The cells were maintained in a humidified atmosphere of 95% air and 5% CO_2 at 37°C. Culture media were supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml). All reagents were obtained from Sigma (Sigma Chemicals, St. Louis, MO, USA).

MTT assay

Tumor cells were plated on 96-well microplates (Nunc, Roskilde, Denmark) at a density 2×10^4 (Caco-2), 3×10^4 (HT-29, LS-180). Next day, the culture medium was removed and the cells were exposed to a fresh medium (control) or serial dilutions of AKG (5, 10, 25, 50 mM). Cell proliferation was assessed after 96 h by using the MTT method in which the yellow tetrazolium salt (MTT) is metabolized by viable cells to purple formazan crystals. Tumor cells were incubated for 3 h with MTT solution (5 mg/ml). Formazan crystals were solubilized overnight in SDS buffer (10% SDS in 0.01N HCl), and the product was quantified spectrophotometrically by measuring absorbance at 570 nm wavelength using ELx800 Microplate Reader (BioTek Instruments Inc., Highland Park, Winooski, Vermont, USA) ([6], modified).

BrdU assay

Cells were plated on 96-well microplates (Nunc) at a density of 4×10^4 (Caco-2) or 5×10^4 (HT-29, LS-180). Next day, the culture medium was removed and the cells were exposed to a fresh medium (control) or serial dilutions of AKG (5, 10, 25, 50 mM). Cell proliferation was quantified after 24 h by measurement of BrdU incorporation during DNA synthesis (Cell Proliferation ELISA BrdU, Roche Diagnostics GmbH, Penzberg, Germany). Tumor cells were incubated with 10 μM BrdU for 2 h. The cells were subsequently incubated with the Fix-Denat solution for 30 min and then exposed to monoclonal anti-BrdU antibodies conjugated to peroxidase. Color reaction was developed by the adding the TMB substrate solution and terminated by addition of 1 M H_2SO_4. The absorbance was measured at 450 nm wavelength using ELx800 Microplate Reader ([6], modified).

Assessment of cell death

Measurement of cell death was performed using Cell Death Detection ELISA^{PLUS} kit (Roche Diagnostics, Germany). The assay is based on a quantitative sandwich-enzyme-immunoassay principle using mouse monoclonal antibodies directed against DNA and histones, respectively. This allows the specific determination of mono- and oligonucleosomes in the cytoplasmic fraction of cell lysates. HT-29 cells growing on 96-well microplates were subjected to AKG (5, 10, 25, 50 mM) for 24 h, whereupon supernatants were removed and cells lysed with 200 μl of lysis buffer for 30 min. Subsequently, cell lysates were centrifuged at 200× g for 10 min. and 20 μl of the samples were carefully transferred into the streptavidin-coated 96-well microplate. The immunoreagent (80 μl) containing anti-histone-biotin and anti-DNA-POD mouse monoclonal antibody was added and incubated under gentle shaking (300 rpm) for 2 h at 20°C. The solution was removed by tapping, each plate well rinsed three times with 250 μl of incubation buffer, and finally 100 μl per well of substrate solution (ATBS) was applied and incubated at room temperature for 15 min on a plate shaker (250 rpm) until sufficient color developed. Absorbance was measured at 405 nm wavelength using E-max Microplate Reader.

Cell cycle analysis

HT-29 cells exposed to AKG (25 or 50 mM) or fresh medium (control) for 24 h were fixed with cold ethanol (75%) and cell cycle was analyzed in cytometer with the use of propidium iodide (PI)

RIGHTSLINK

method. After rehydration in PBS, the cells were stained by propidium iodide (PI; 10 μg/ml) in the presence of RNase A (100 μg/ml) for 30 min and analyzed by FACS Calibur flow cytometer (Becton Dickinson) [7].

Western blot analysis

After treatment, HT-29 cells were harvested, lysed in RIPA buffer (1% NP40, 0.5% sodium deoxycholate, 0.1% SDS, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3VO_4, 20 mM NaF, 0.5 mM DTT, 1 mM PMSF, and protease inhibitor cocktail in PBS (pH 7.4)) and then centrifuged 14,000× g for 10 min. For Western blot analysis, supernatants were solubilized in 6× Laemmli sample buffer (30% glycerol, 10% SDS, 0.5 M Tris-HCl, pH 6.8, 0.012% bromophenol blue, 5% β-mercaptoethanol) and boiled for 5 min at 100°C. Equal amounts of total cellular protein extract were electrophoresed on 10–12% SDS-PAGE under reducing conditions and transferred by electrobloting onto a PVDF membrane. After blocking for 1 h at RT with 5% nonfat milk in TBS-T (TBS + 0.1% Tween 20), membranes were probed overnight at 4°C with the primary antibody as follows: p21 Waf1/Cip1 (1:1000), p27 Kip1 (1:1000), cyclin D1 (1:2000), p-Rb (1:1000), β-actin (1:2000) (Cell Signaling Technology), then incubated with the secondary antibody coupled to horseradish peroxidase (1:2000 in 5% BSA in TBS-T) and visualized using enhanced chemiluminescence (Pierce, Rockford, IL) [8].

Immunofluorescence

HT-29 cells were grown on Lab-Tek Chamber slide (Nunc) and exposed to AKG 25 mM for 24 h. Then, cells were fixed with 3.7% paraformaldehyde in PBS for 10 min, permeabilized with 0.2% Triton X-100 in PBS for 7 min, and incubated for 30 min in 5% BSA in PBS. After that fixed cells were exposed to rabbit anti-phospho-Rb antibodies (1:100 in PBS; Cell Signaling) overnight at 4°C. Staining was visualized with FITC-conjugated goat anti-rabbit secondary antibodies (1:100, 2 h at room temperature; Sigma) by fluorescent microscopy (Olympus BX51 System Microscope, Olympus Optical CO., LTD, Tokyo, Japan) [8].

Data analysis

Data were presented as the mean value and standard error of the mean (SEM). Statistical analysis of proliferation and cell death assessment data were performed using one-way ANOVA with Tukey *post-hoc* test ($p < 0.05$ was considered statistically significant). Cell cycle analysis was statistically analyzed using paired t-test ($p < 0.05$ was considered statistically significant). The IC_{50} value (the concentration of drug necessary to induce 50% inhibition), together with confidence limits, was calculated using computerized linear regression analysis of quantal log dose-probit functions, according to the method of Litchfield and Wilcoxon [9].

Results

Antiproliferative activity of AKG (MTT assay and BrdU assay)

To determine antiproliferative effect of AKG in normoxia, colon adenocarcinoma cells were exposed to serial dilutions of AKG (5–50 mM) for 96 h. Proliferation of all tumor cell cultures was decreased in a concentration-dependent fashion, which was assessed by means of MTT assay. AKG significantly inhibited proliferation of Caco-2, HT-29, and LS-180 cells exposed to the tested compound with IC_{50} of 26.6 mM, 26.7 mM, and 35.3 mM, respectively (Figure 1A). The antiproliferative effect of AKG was confirmed in BrdU Assay. This effect was attributed to decreased cell division as determined by measurements of incorporation of BrdU during DNA synthesis. AKG inhibited DNA synthesis in all tested colon adenocarcinoma cell lines with IC_{50} of 55.3 mM (Caco-2), 64.3 mM (HT-29), and 67.8 mM (LS-180) (Figure 1B).

Colon adenocarcinoma HT-29 cell line was chosen as a model for further cell cycle and molecular analysis.

Cell death assessment

Cell death ELISA was applied to evaluate whether AKG induces apoptotic cell death. In our experiments, no increase in mono- and oligonucleosomes in cytoplasm of HT-29 cells treated with AKG was observed up to 50 mM (data not shown).

Cell cycle analysis

Cell cycle analysis of HT-29 cells exposed to AKG (25, 50 mM) for 24 h revealed a cell cycle arrest in G1 phase. A statistically significant increase in G1 cell population (56.45% in control cells vs. 80.88% in cells exposed to 50 mM AKG) associated with a decrease in the S (28.26% in control cells vs. 10.86% in cells exposed to 50 mM AKG) and G2/M cell population (15.29% in control cells vs. 8.27% in cells exposed to 50 mM AKG) was observed (Figure 2).

Scand J Gastroenterol Downloaded from informahealthcare.com by 87.246.233.207 on 04/10/12
For personal use only.

RIGHTSLINK

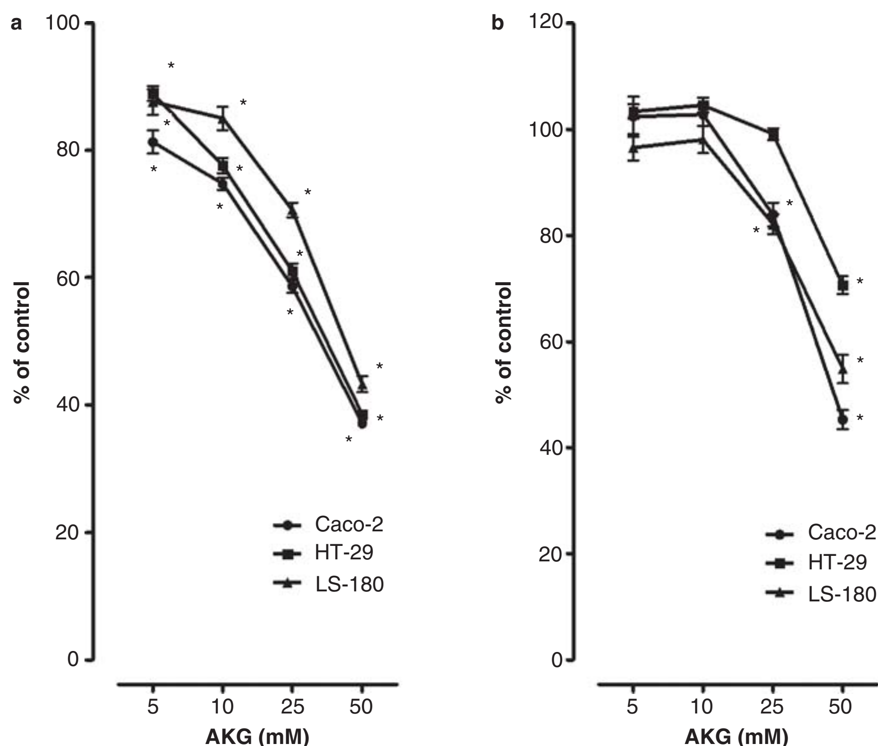


Figure 1. Antiproliferative effect of alpha-ketoglutarate (AKG). AKG exerted a concentration-dependent antiproliferative effect in colon adenocarcinoma cell lines – Caco-2, HT-29, LS-180. Cells were exposed to either culture medium alone (control) or AKG (5–50 mM) and proliferation was measured by means of MTT assay (A) and BrdU assay (B). Data represented the mean (% of control) ± SEM of six trials and were analyzed by means of linear regression (control = 100%). Values significant in comparison with control with **p* < at least 0.05 (one-way ANOVA with Tukey *post-hoc* test).

Scand J Gastroenterol Downloaded from informahealthcare.com by 87.246.233.207 on 04/10/12
For personal use only.

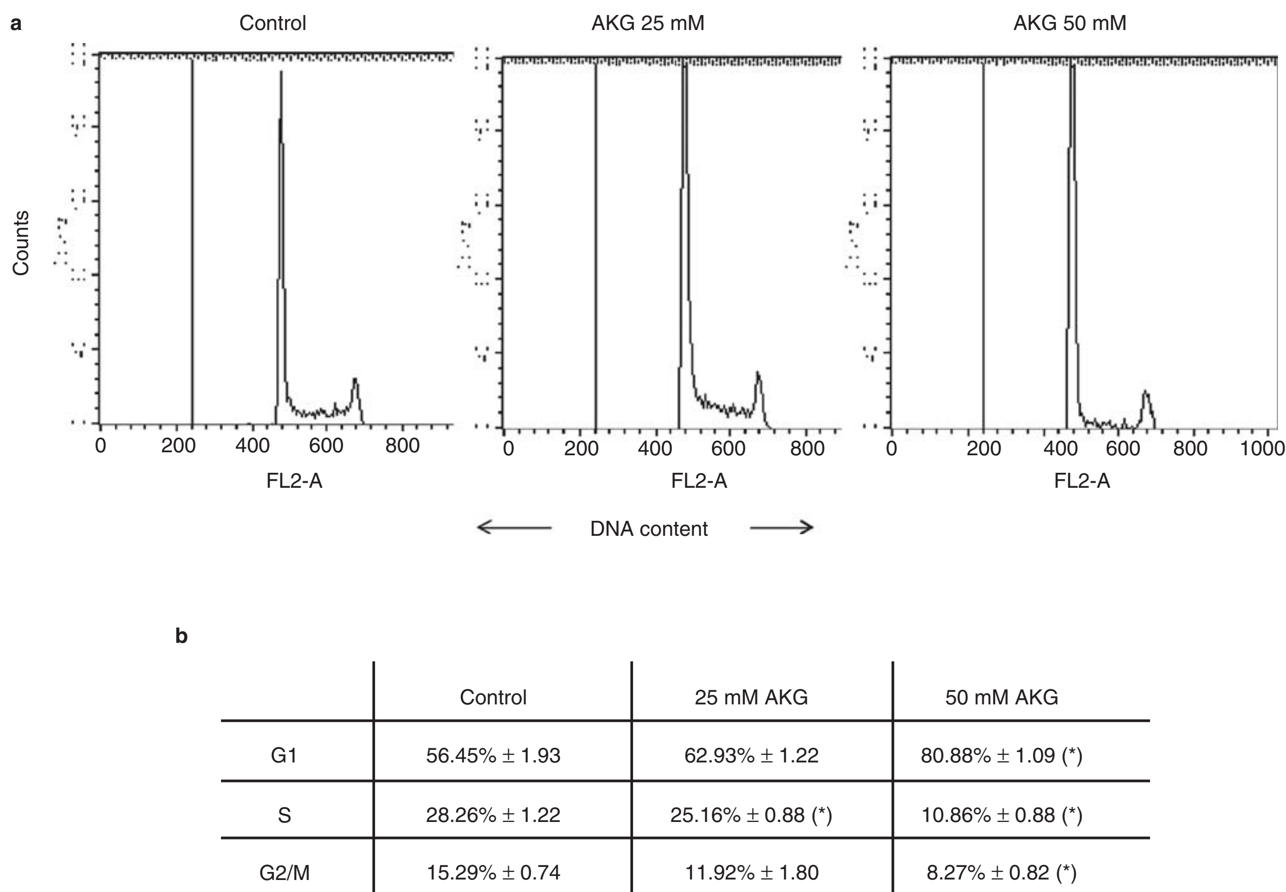
The influence of AKG on cell cycle regulation

To determine whether AKG influences expression and activation of cell cycle regulators, we analyzed their protein expression and phosphorylation in HT-29 cells exposed to AKG 25 mM by means of western blot. The expression of the inhibitors of cyclin-dependent kinases p21 Waf1/Cip1 and p27 Kip1 was significantly increased from 4 h and 24 h of AKG exposition, respectively. Moreover, expression of cyclin D1, required in G1/S transmission, was decreased. AKG affected also an activation of one of the key cell cycle regulator – retinoblastoma protein (Rb). One-hour incubation with AKG decreased Rb phosphorylation, but significant reduction was achieved after 24 h (Figure 3A). This result was confirmed by immunofluorescence, which revealed significant inhibition of Rb phosphorylation in HT-29 cells exposed to AKG for 24 h (Figure 3B,C).

Discussion

Although molecular mechanism of anticancer properties of AKG in hypoxia has been deeply investigated, in this study, for the first time we indicated its anticancer effect in normoxia. Hypoxia often drives the progression and aggressiveness of tumors and results in alternations in gene expression, which are involved in cellular metabolism, migration, survival, proliferation, and differentiation [10-12]. Matsumoto et al. [4] detected that AKG reduced alpha-HIF-1, erythropoietin, and VEGF expression under hypoxic conditions in the hepatoma cell line Hep3B. Moreover, to address clinical usefulness of AKG, they detected that intraperitoneal injection of AKG significantly inhibited tumor growth and angiogenesis in murine tumor xenograft model [5]. In the present study, the direct antiproliferative effect of AKG on colon adenocarcinoma cell lines representing different stages of colon carcinogenesis was found in normoxic conditions. Interestingly, AKG inhibited not only proliferation of Caco-2, HT-29, and LS-180 cells (IC_{50} of 26.6 mM, 26.7 mM, and 35.3 mM, respectively) but also DNA synthesis (IC_{50} of 55.3 mM, 64.3 mM, and 67.8 mM, respectively), which was measured by means of BrdU assay. Importantly, the tested concentrations of AKG were nontoxic for normal colon epithelial CCD 841 CoTr cells (data not shown). Tested compound did not induce apoptosis in HT-29 cells, which suggests that

RIGHTSLINK



	Control	25 mM AKG	50 mM AKG
G1	56.45% ± 1.93	62.93% ± 1.22	80.88% ± 1.09 (*)
S	28.26% ± 1.22	25.16% ± 0.88 (*)	10.86% ± 0.88 (*)
G2/M	15.29% ± 0.74	11.92% ± 1.80	8.27% ± 0.82 (*)

Figure 2. Representative flow cytometric analysis of HT-29 cells treated with AKG (25 or 50 mM) for 24 h. Data are presented as representative histograms (A) and percentage of the cell cycle phases (mean ± SEM) (B). Analysis revealed higher percentage of cells in G1 phase and lower in S and G2/M phase of the cell cycle following exposure to AKG compared with control. Values significant in comparison with control with $*p <$ at least 0.05 (paired t-test).

its antiproliferative activity is not even partially due to programmed cell death. HT-29 cells were chosen as a model of colon adenocarcinoma cell line to reveal molecular mechanism of antiproliferative action. Our studies demonstrated that AKG significantly interfered the cell cycle in HT-29 cells inducing a cell cycle arrest in G1 phase. Further molecular analysis indicated that AKG influenced several cell cycle regulators. In our study, the significant increase in CDK inhibitors p21 Waf1/Cip1 and p27 Kip1 protein expression was observed. Overexpression of p21 Waf1/Cip1 has been reported to inhibit all cyclin–CDK complexes in mammalian cells [13]. Its expression may be activated via p53-dependent and -independent pathway. Since mutation in p53 gene is well documented in HT-29 cell line, it is suggested that AKG induced overexpression of p21 Waf1/Cip1 through p53-independent way. Similarly, p27 Kip1 inhibits cyclin–CDK complexes involved in G0/G1 transition [14]. These CDK inhibitors subsequently inhibit DNA replication and are also partly responsible for the G1 growth arrest phenotype, which may explain the cell cycle arrest induced by AKG [15]. Active complexes of cyclin D1 with CDK4 and CDK6 promote cell cycle by phosphorylation and inactivation of the retinoblastoma protein Rb [16]. Importantly, AKG not only decreased protein level of cyclin D1, but also caused reduction of Rb phosphorylation maintaining it in its active form and, thus, regulated cell proliferation by controlling progression through the restriction point within the G1 phase of the cell cycle.

Taking into account the specificity of molecular mechanism of antiproliferative activity of millimolar concentrations of AKG and its physiological role as an intermediate metabolite of the Krebs cycle, the hypothesis that enhanced metabolic influx could influence cancer cell proliferation needs to be verified. Lemons et al. [17] reported that a high metabolic activity may be important for preservation of self-integrity of quiescent fibroblasts. Thus, overnutrition as an alternative mechanism of antiproliferative properties of AKG is worth further consideration.

Scand J Gastroenterol Downloaded from informahealthcare.com by 87.246.233.207 on 04/10/12
For personal use only.

RIGHTSLINK

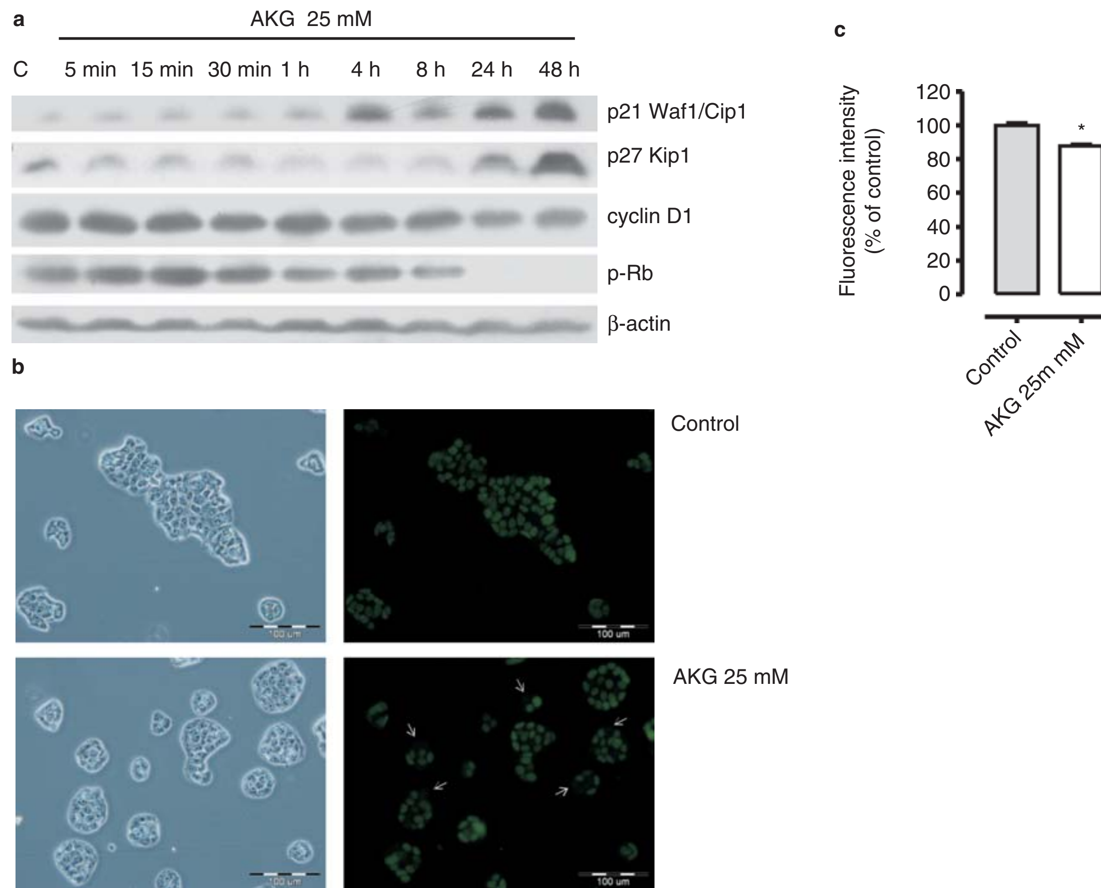


Figure 3. Modulation of protein expression and activation status of cell cycle regulators in AKG-treated HT-29 cells. (A) Western blot analysis of protein expression or phosphorylation status of cell cycle-related proteins in HT-29 cells after treatment with AKG 25 mM for 5 min – 48 h (C – control; not treated). (B) Immunofluorescent staining of phospho-Rb protein in HT-29 cells incubated with or without AKG 25 mM for 24 h. As a secondary antibody, anti-rabbit FITC-conjugated antibodies were used. Arrows show cells with decreased Rb phosphorylation. (C) Fluorescence intensity of corresponding images. Fluorescence intensity of 100 individual cells, randomly selected in two different fields of view. Data represented the mean (% of control) ± SEM (control = 100%). Values significant in comparison with control with $^{*}p <$ at least 0.05 (t-test).

Scand J Gastroenterol Downloaded from informahealthcare.com by 87.246.233.207 on 04/10/12
For personal use only.

It is worth to mention that dietary supplementation with AKG can ameliorate intestinal injury and increase the absorptive function of the small intestine in the endotoxin-challenged piglet [18]. Moreover, AKG increasing concentration of glutamine activated the mTOR signaling pathway in intestinal porcine epithelial cells [19]. mTOR signaling pathway plays a crucial role in the control of cell growth and proliferation as a major mechanism for the regulation of protein synthesis [20]. Despite proved beneficial effect of AKG supplementation on intestines, a possible interaction of AKG with mTOR signaling pathway in colon adenocarcinoma cells needs to be clarified.

Taking into consideration an anticancer activity of AKG both in hypoxia, mainly via restoration of prolyl hydroxylase activity and inhibition of angiogenesis, and in normoxia, via influence on cell cycle progression, its potential application in cancer chemoprevention is suggested. Chemoprevention refers to the use of specific natural or synthetic compounds to prevent, inhibit, or reverse process of carcinogenesis [21]. Several well-documented chemopreventive compounds such as curcumin, resveratrol, and catechins have been shown to interfere with cell cycle regulation [22,23]. Some of them have been introduced to clinic as a supplementation of anticancer chemotherapy.

Acknowledgements

This work was supported by the SGPlus, Sweden.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

[1] Robinson LE, Bussiere FI, Le Boucher J, Farges MC, Cynober LA, Field CJ, et al. Amino acid nutrition and

RIGHTSLINK

immune function in tumour-bearing rats: a comparison of glutamine-, arginine-, and ornithine 2-oxoglutarate-supplemented diets. Clin Sci (Lond) 1999;97:657–69.
[2] Le Bricon T, Cynober L, Field CJ, Baracos VE. Supplemental nutrition with ornithine alpha-ketoglutarate in rats with cancer-associated cachexia: surgical treatment of the tumor improves efficacy of nutritional support. J Nutr 1995;125: 2999–3010.
[3] Harris AL. Hypoxia—a key regulatory factor in tumour growth. Nat Rev 2002;2:38–47.
[4] Matsumoto K, Imagawa S, Obara N, Suzuki N, Takahashi S, Nagasawa T, et al. 2-Oxoglutarate downregulates expression of vascular endothelial growth factor and erythropoietin through decreasing hypoxia-inducible factor-1a and inhibits angiogenesis. J Cell Physiol 2006;209:333–40.
[5] Matsumoto K, Obara N, Ema M, Horie M, Naka A, Takahashi S, et al. Antitumor effects of 2-oxoglutarate through inhibition of angiogenesis in a murine tumor model. Cancer Sci 2009;100:1639–47.
[6] Rzeski W, Turski L, Ikonomidou C. Glutamate antagonists limit tumor growth. Proc Natl Acad Sci USA 2001;98:6372–7.
[7] Blagosklonny MV, Darzynkiewicz Z, Halicka HD, Pozarowski P, Demidenko ZN, Barry JJ, et al. Paclitaxel induces primary and postmitotic G1 arrest in human arterial smooth muscle cells. Cell Cycle 2004;3:1050–6.
[8] Walczak K, Dąbrowski W, Langner E, Zgrajka W, Piłat J, Kocki T, et al. Kynurenic acid synthesis and kynurenine aminotransferases expression in colon derived normal and cancer cells. Scand J Gastroenterol 2011;46:903–12.
[9] Litchfield JT, Wilcoxon FA. A simplified method of evaluating dose-effect experiments. J Pharmacol Exp Ther 1949; 96:99–113.
[10] Bertout JA, Patel SA, Simon MC. The impact of O2 availability on human cancer. Nat Rev Cancer 2008;8: 967–75.
[11] Rademakers SE, Span PN, Kaanders JH, Sweep FC, van der Kogel AJ, Bussink J. Molecular aspects of tumour hypoxia. Mol Oncol 2008;2:41–53.
[12] Tennant DA, Gottlieb E. HIF prolyl hydroxylase-3 mediates alpha-ketoglutarate-induced apoptosis and tumor suppression. J Mol Med 2010;88:839–49.
[13] Meerana SM, Kumar Katiyar S. Cell cycle control as a basis for cancer chemoprevention through dietary agents. Front Biosci 2008;13:2191–202.
[14] Xiong Y, Hannon GJ, Zhang H, Casso D, Kobayashi R, Beach D. p21 is a universal inhibitor of cyclin kinases. Nature 1993;366:701–4.
[15] Alkarain A, Jordan R, Slingerland J. p27 deregulation in breast cancer: prognostic significance and implications for therapy. J Mammary Gland Biol Neoplasia 2004; 9:67–80.
[16] Alao JP. The regulation of cyclin D1 degradation: roles in cancer development and the potential for therapeutic invention. Mol Cancer 2007;6:6–24.
[17] Lemons JM, Feng XJ, Bennett BD, Legesse-Miller A, Johnson EL, Raitman I, et al. Quiescent fibroblasts exhibit high metabolic activity. PLoS Biol 2010;8:e1000514.
[18] Hou Y, Wang L, Ding B, Liu Y, Zhu H, Liu J, et al. Dietary alpha-ketoglutarate supplementation ameliorates intestinal injury in lipopolysaccharide-challenged piglets. Amino Acids 2010;39:555–64.
[19] Yao K, Yin Y, Li X, Xi P, Wang J, Lei J, et al. Alpha-ketoglutarate inhibits glutamine degradation and enhances protein synthesis in intestinal porcine epithelial cells. Amino Acids 2011;DOI: 10.1007/s00726-011-1060-6. [Epub ahead of print]
[20] Sarbassov DD, Ali SM, Sabatini DM. Growing roles for the mTOR pathway. Curr Opin Cell Biol 2005;17:596–603.
[21] Vaisman N, Arber N. The role of nutrition and chemoprevention in colorectal cancer: from observations to expectations. Best Pract Res Clin Gastroenterol 2002;16:201–17.
[22] Mukhopadhyay A, Banerjee S, Stafford LJ, Xia CX, Liu M, Aggarwal BB. Curcumin induced suppression of cell proliferation correlates with down regulation of cyclin D1 expression and CDK4-mediated retinoblastoma protein phosphorylation. Oncogene 2002;21:8852–61.
[23] Estrov Z, Shishodia S, Faderl S, Harris D, Van Q, Kantarjian HM, et al. Resveratrol blocks interleukin- 1 [beta]-induced activation of the nuclear transcription factor NF-[kappa]B, inhibits proliferation, causes S-phase arrest and induces apoptosis of acute myeloid leukemia cells. Blood 2003;102:987–95.

Scand J Gastroenterol Downloaded from informahealthcare.com by 87.246.233.207 on 04/10/12
For personal use only.

RIGHTSLINK