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ORIGINAL ARTICLE

Increased expression of transglutaminase 2 drives glycolytic metabolism in renal carcinoma cells

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Abstract Transglutaminase 2 (TGase 2) expression and glycolysis are increased in most renal cell carcinoma (RCC) cell lines compared to the HEK293 kidney cell line. Although increased glycolysis and altered tricarboxylic acid cycle are common in RCC, the detailed mechanism by which this phenomenon occurs remains to be elucidated. In the present study, TGase 2 siRNA treatment lowered glucose consumption and lactate levels by about 20–30 % in RCC cells; conversely, high expression of TGase 2 increased glucose consumption and lactate production together with decreased mitochondrial aconitase (Aco 2) levels. In addition, TGase 2 siRNA increased mitochondrial membrane potential and ATP levels by about 20–30 % and restored Aco 2 levels in RCC cells. Similarly, Aco 2 levels and ATP production decreased significantly upon TGase 2 overexpression in HEK293 cells. Therefore, TGase 2 leads to depletion of Aco 2, which promotes glycolytic metabolism in RCC cells.

Keywords Transglutaminase 2 · Aconitase 2 · Glycolysis · Renal cell carcinoma

Introduction

A recent GC–MS metabolomic study revealed that renal cancer tissues had a different metabolic profile from normal tissues (Catchpole et al. 2011): renal cell carcinoma (RCC) tissues displayed the Warburg glycolytic phenotype and higher glucose levels than normal tissues. The Warburg effect in RCC is related to von Hippel–Lindau (VHL) alterations, which are common in sporadic clear cell RCC (Kim and Kaelin 2004) and can occur through gene mutation (Moore et al. 2011), gene silencing via methylation (Moore et al. 2011), or post-translational polymerization by transglutaminase 2 (TGase 2) (Kim et al. 2011). VHL loss increases hypoxia inducible factor 1 (HIF1), which affects several metabolic pathways (Alberghini et al. 2005). HIF1 increases the expression of glucose transporters (Chen et al. 2001), hexokinase (Mathupala et al. 2001), lactate dehydrogenase (LDH), which drives the conversion of pyruvate to lactate (Firth et al. 1995), and pyruvate dehydrogenase kinase 1 (PDK1), which inhibits the pyruvate dehydrogenase-driven conversion of pyruvate to acetyl-coA (Kim et al. 2006a). Therefore, HIF-mediated induction of LDH and PDK1 is associated with TCA cycle inhibition in RCC.

Conditionally knockdown of VHL under normoxia induces specific target proteins such as aminopeptidase A, collagen type V, alpha 1, cyclin G2, DEC1/Stra13, endothelin 1, low-density lipoprotein receptor-related protein 1, MIC2/CD99, and TGase 2 in RCCs (Wykoff et al. 2000). Therefore, TGase 2 is a VHL repressible gene. In HEK293 cells, hypoxia induces TGase 2 expression through an HIF-1-dependent pathway (Jang et al. 2010). Therefore, TGase 2 is an HIF-1 inducible gene, which gives advantages to RCC cells through modulating both apoptosis via caspase 3 and cathepsin D depletion (Yamaguchi and Wang 2006; Kim et al. 2013) and survival pathways via NF-κB activation (Lee et al. 2004; Kim et al. 2006b; Mehta et al. 2010).

Recently, we found that TGase 2 contributes to the development of RCC. TGase 2 (E.C. 2.3.2.13, protein-

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glutamine γ-glutamyltransferase) is a calcium-dependent cross-linking enzyme that catalyzes the formation of an isopeptide bond between protein-bound glutamine and protein-bound lysine (Kim 2011). Although TGase 2 was found to be cross-linking enzyme, there are reports that TGase 2 participates in multiple enzymatic functions (Fesus and Piacentini 2002). We found that RCC commonly shows high expression of TGase 2 and high levels of autophagy (Ku et al. 2013). We also reported that high expression of TGase 2 was responsible for mitochondrial dysfunction in Huntington's disease through depletion of Aco 2 (aconitate hydratase; EC 4.2.1.3) via protein cross-linking in neuron (Kim et al. 2005). Overexpression of TGase 2 promotes the translocation of TGase 2 into mitochondria and the subsequent targeting of Aco 2 (Kim et al. 2005). In vitro, TGase 2 polymerizes Aco 2 in a time-dependent manner using mitochondrial fraction (Kim et al. 2005). Aco 2 is an enzyme that catalyzes the stereo-specific isomerization of citrate to isocitrate via *cis*-aconitate in the tricarboxylic acid cycle, a non-redox-active process. Therefore, decreased Aco 2 activity causes energy metabolism dysfunction (Cheng et al. 2013). Interestingly, a report suggests that the increase in citrate upon Aco 2 inhibition induces amino acid synthesis in citrus fruit callus (Degu et al. 2011). Increased citrate also restores oxaloacetate and acetyl-CoA, which promotes de novo lipid synthesis in cancer cells if it is exported from the mitochondria (Icard et al. 2012). Therefore, we investigated whether TGase 2 expression contributes to Aco 2 regulation in RCC, which may promote glycolytic metabolism.

Materials and methods

Antibodies

Antibodies against β-actin and LDH were obtained from Santa Cruz Biotechnology; Antibodies against Aco 2 and COX IV were obtained from Cell Signaling Technologies. The antibody to TGase 2 was obtained from Lab Vision (clone CUB 7402 + TG100).

Cell culture and treatment

The HEK293 cell was obtained from the American Type Culture Collection. Caki-1 and ACHN cells were obtained from NCI (MTA Number: 2702-09). Cells were cultured in complete DMEM or RPMI 1640 containing 10 % fetal bovine serum under 5 % CO_2 at 37 °C. A small quantity of interfering RNAs (siRNAs) targeting human TGase 2 (Invitrogen) or Aco 2 (Santa Cruz) were introduced into cells using Lipofectamine RNAiMAX (Invitrogen), according to the manufacturer's instructions. As negative controls, cells were incubated with Lipofectamine RNAiMAX and a universal negative siRNA (Invitrogen). TGase 2 was cloned in pcDNA Plasmid and transfection was done with Lipofectamine 2000 (Invitrogen). For glucose starvation, cells were incubated in glucose-free RPMI (GIBCO).

Measurements of glucose, lactate, ATP, and Aco 2 activity

Glucose consumption, lactate secretion and intracellular ATP level assays were carried out at 12, 24, and 48 h after transfection in normal medium. Briefly, cells were transfected with siRNA or TGase 2 plasmid, media were changed 4 h later and cells were cultured in normal medium. At each time point, glucose and lactate concentrations were measured in the culture media using the Glucose Assay Kit (#K606-100) and the Lactate Assay kit II (#K627-100) (Biovision) according to the manufacturer's instructions. Calculated lactate production ranged from 2 to 8 mmol per 1×10^4 cells. Glucose consumption was extrapolated by subtracting the measured glucose concentrations in the media (3–9 mmol/1×10^4 cells) from the original glucose concentration (DMEM; 25 mmol, RPMI 1640; 11 mmol). Intracellular ATP levels were determined in cell lysates using the ATP Assay Kit (Biovision; #K354-100), according to the manufacturer's instructions. Measured intracellular ATP ranged from 10 to 30 nmol per 1×10^6 cells. The activity of Aco 2 (mitochondrial aconitase) was measured using the Aconitase Activity Assay Kit (Biovision; #K716-100). For measuring Aco 2 activity, samples were processed according to the manufacturer's mitochondrial aconitase assay protocol. Calculated Aco 2 activity ranged from 0.8 to 10 nmol/min/ml.

Preparation of a mitochondrial fraction

Mitochondrial fraction was prepared using Mitochondria Isolation Kit for profiling cultured cells (Sigma) according to the manufacturer's instructions.

Western blotting

For Western blot analysis, cells were lysed and protein assays were carried out to normalize the proteins using a BCA protein assay kit (Pierce). Protein samples were separated on 10 % polyacrylamide gels and then transferred to polyvinylidene difluoride membranes. After blocking non-specific bindings, the membranes were incubated with indicated antibodies. For detecting Aco 2 polymer, insoluble fraction was prepared as described previously. Briefly, cells were lysed in buffer containing 50-mM Tris HCl (pH 7.5), 150-mM NaCl, 0.5 % Nonidet

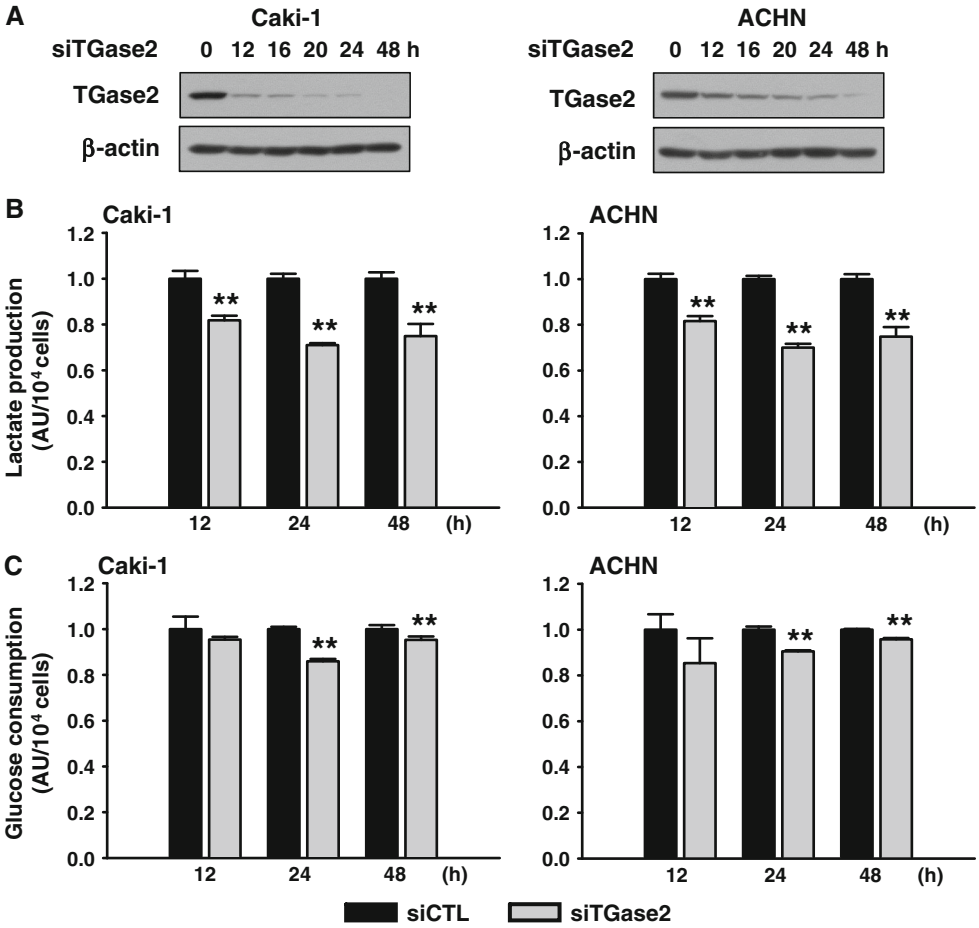

Fig. 1 TGase 2 regulates glycolytic metabolism. **a** Cells were transfected with TGase 2-specific siRNA for the indicated time course. Expression of TGase 2 was detected using western blotting. **b** Lactate production and **c** glucose consumption in TGase 2 knockdown RCC cells including Caki-1 and ACHN under normal culture conditions. Data are presented as mean ± SE in three separate experiments in triplicates (all three replicates of each of three experiments has been plotted, i.e., $n = 9$). *$p < 0.05$; **$p < 0.01$

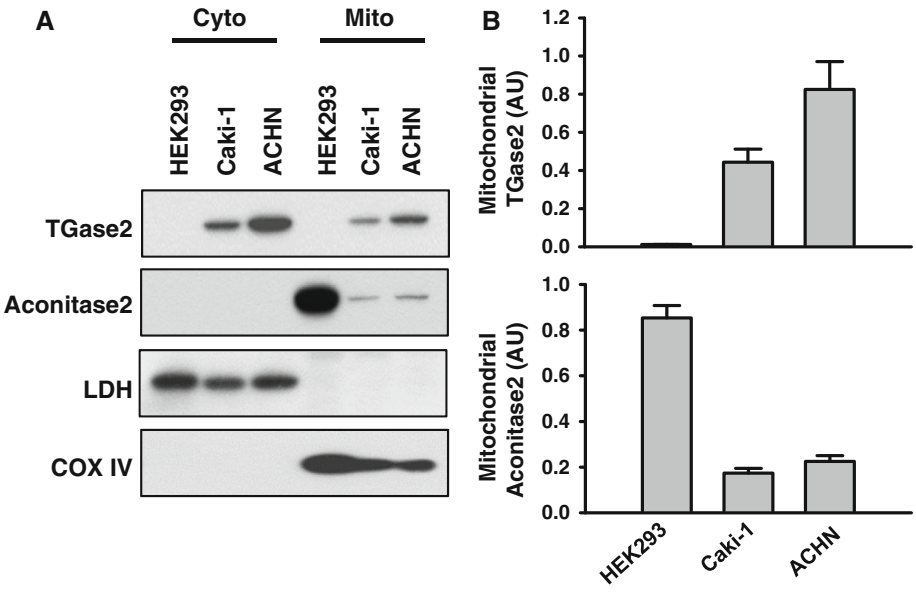

Fig. 2 Aco 2 is reduced in RCC cells. **a** Cytosol (Cyto) or mitochondrial (Mito) fractions from HEK293, Caki-1 and ACHN cell lines were analyzed by western blotting. LDH and COX IV were used as cytosolic or mitochondrial marker, respectively. **b** Relative mitochondrial TGase 2 and Aco 2 signal intensities were measured by densitometry and indicated as arbitrary unit (AU). Values are mean ± SE for three independent experiments

P40, 5-mM EDTA, and a protease inhibitor cocktail, and centrifuged at 16,000 g at 4 °C for 20 min. After centrifugation, the pellet (insoluble fraction) was dissolved in sample buffer containing 20 % mercaptoethanol, 1 % Triton X-100, 1 % deoxycholate and 1 % SDS, and boiled for 5 min.

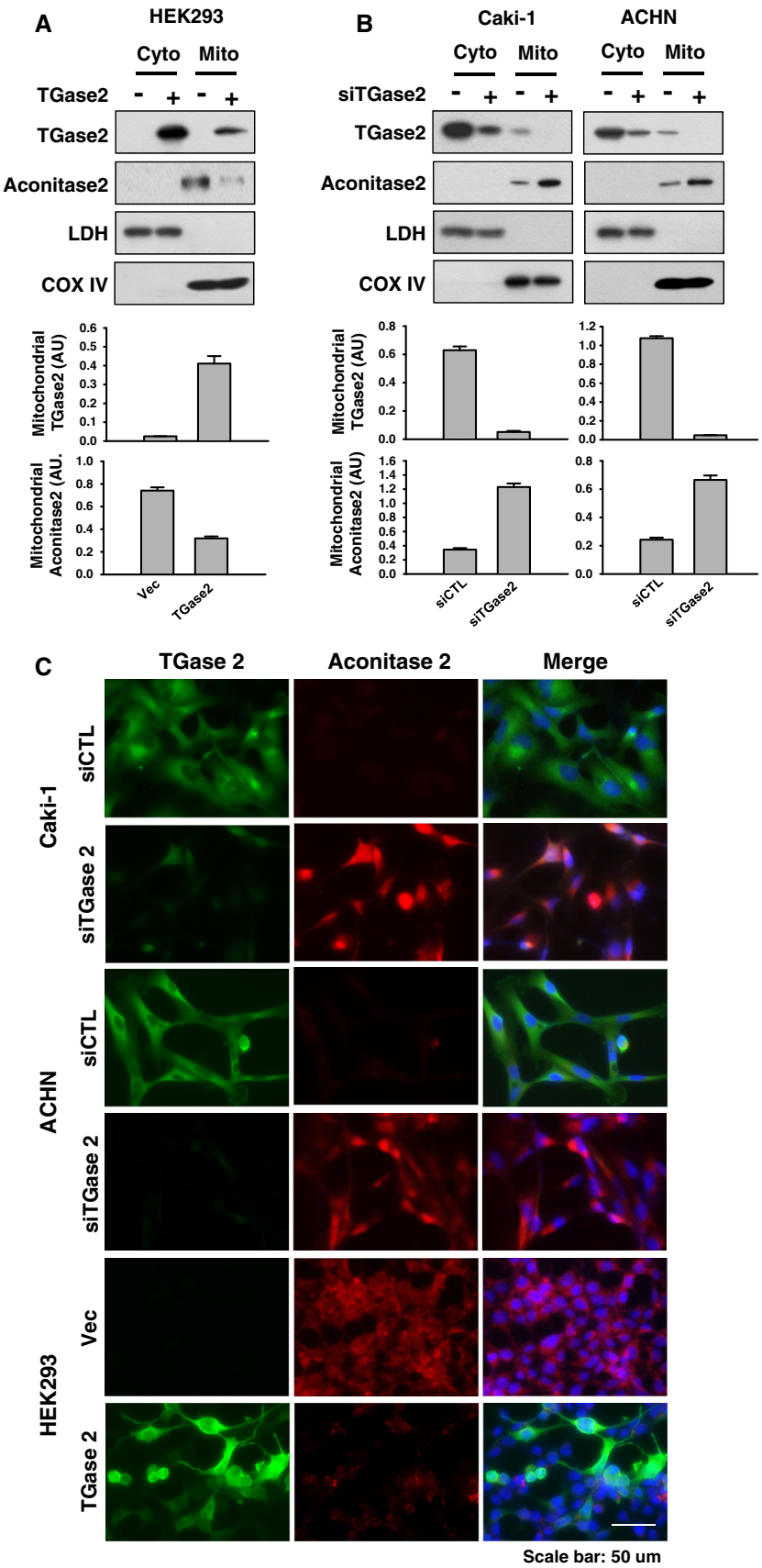

Fig. 3 Aco 2 is downregulated by TGase 2. **a** At 48 h after HEK293 cells were transfected with control or with TGase 2 expression vector, isolated cytosol or mitochondrial fraction were analyzed by western blotting. **b** At 48 h after Caki-1 and ACHN cells were transfected with TGase 2 siRNA, isolated factions were analyzed by western blotting. Relative mitochondrial TGase 2 and Aco 2 signal intensities were measured by densitometry and indicated as arbitrary unit (AU). Values are mean ± S.E. for three independent experiments. **c** Immunofluorescence staining of TGase 2 and Aco 2 in indicated cells. Nuclei were stained with DAPI. *Scale bar* 50 μm

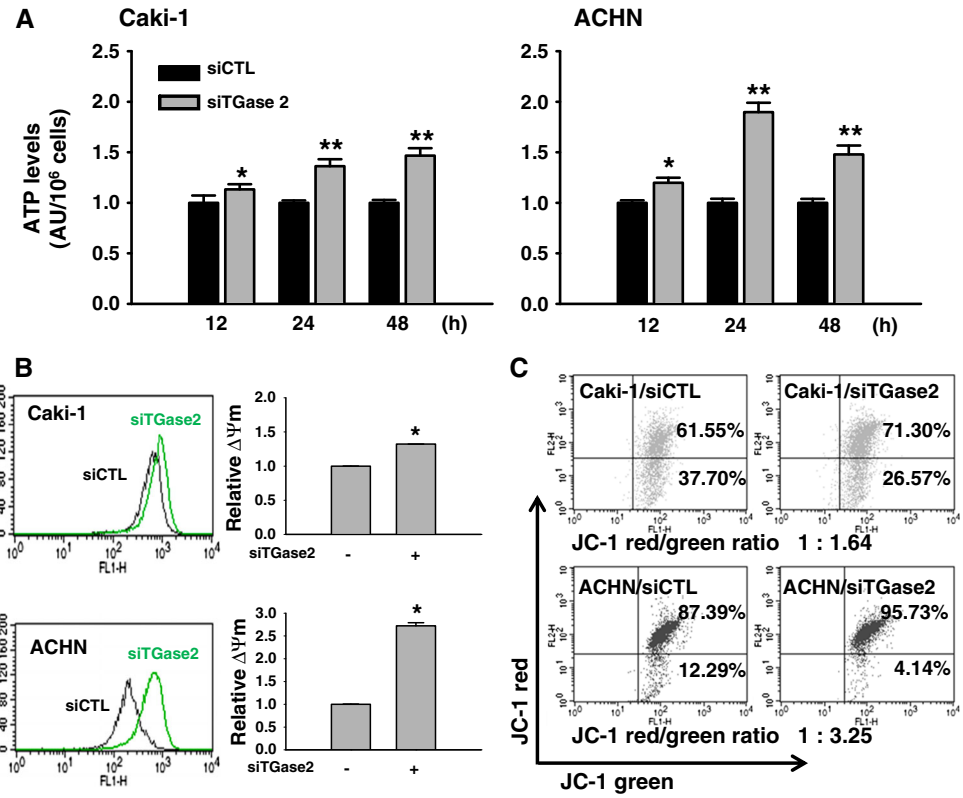

Fig. 4 TGase 2 regulates mitochondrial function. **a** ATP assay. Total cell extracts prepared from indicated cells were subjected to ATP assay using ATP Assay Kit. **b** $\Delta\Psi_m$ assay of TGase 2-siRNA-transfected cells. Indicated cells were stained with $DiOC_6$ and then analyzed using a flow cytometry. **c** $\Delta\Psi_m$ was analyzed by JC-1 staining. $\Delta\Psi_m$ change was demonstrated by the changes in JC-1 fluorescence from red (JC-1 aggregates) to green (JC-1 monomers). Data are presented as mean ± SE in three separate experiments in triplicates (all three replicates of each of three experiments has been plotted, i.e., $n = 9$). $^*p < 0.05$; $^{**}p < 0.01$

Polymerization of Aco 2 by human transglutaminase 2

Purified and concentrated human TGase 2 was incubated with mitochondrial fraction of HEK293 cells at 37 °C for 2 h in reaction buffer (100 mM Tris–HCl [pH 7.5], 150 mM NaCl, 1 mM EDTA, 10 mM $CaCl_2$, 5 mM DTT), and then subjected to Western blotting.

Measurement of mitochondrial transmembrane potential ($\Delta\Psi_m$)

Changes in mitochondrial transmembrane potential ($\Delta\Psi_m$) were evaluated by staining with 40 nM $DiOC_6$ (3,3′-dihexiloxacarbocyanine iodide, Sigma) or 2.5 μg/ml JC-1(5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolcarbocyanine iodide, Sigma). Cells were incubated with $DiOC_6$ or JC-1 for 15 min at 37 °C, washed, and resuspended in PBS. The cells were then analyzed using a FACSCalibur™ flow cytometer (Beckton Dickinson). In the case of DiOC6, a high $\Delta\Psi_m$ was attributed to cells with a high fluorescence signal; in the case of JC-1, cells with a high $\Delta\Psi_m$ were those forming J aggregates. Since JC-1 exhibits potential-dependent accumulation in mitochondria, indicated by a fluorescence emission shift from green to red, the ratios of red:green fluorescence intensity values were calculated.

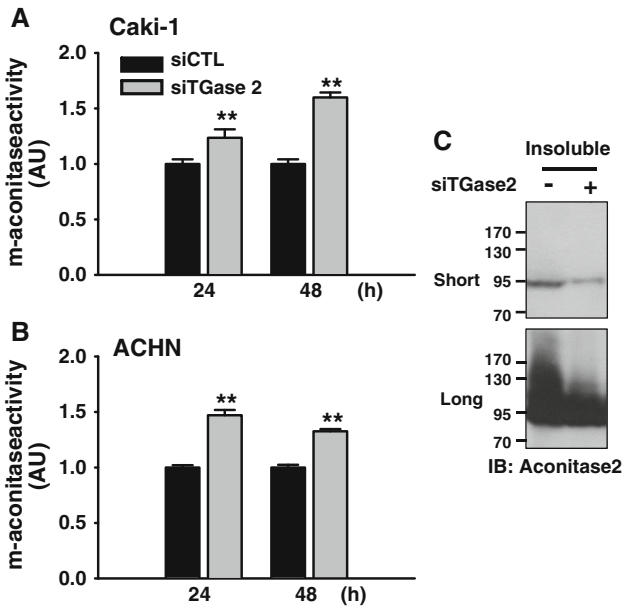

Fig. 5 TGase 2 suppresses Aco 2 activity. Aco 2 activity of Caki-1 (**a**) and ACHN (**b**) was measured using the Aconitase Activity Assay Kit. Indicated time after transfection, cells were processed according to the manufacturer's assay protocol. **c** Caki-1 cells were transfected with a control siRNA or a TGase 2 siRNA. TGase 2-induced Aco 2 polymers were detected in insoluble fraction by western blotting. Data are presented as mean ± SE in three separate experiments in triplicates (all three replicates of each of three experiments has been plotted, i.e., $n = 9$). $^*p < 0.05$; $^{**}p < 0.01$

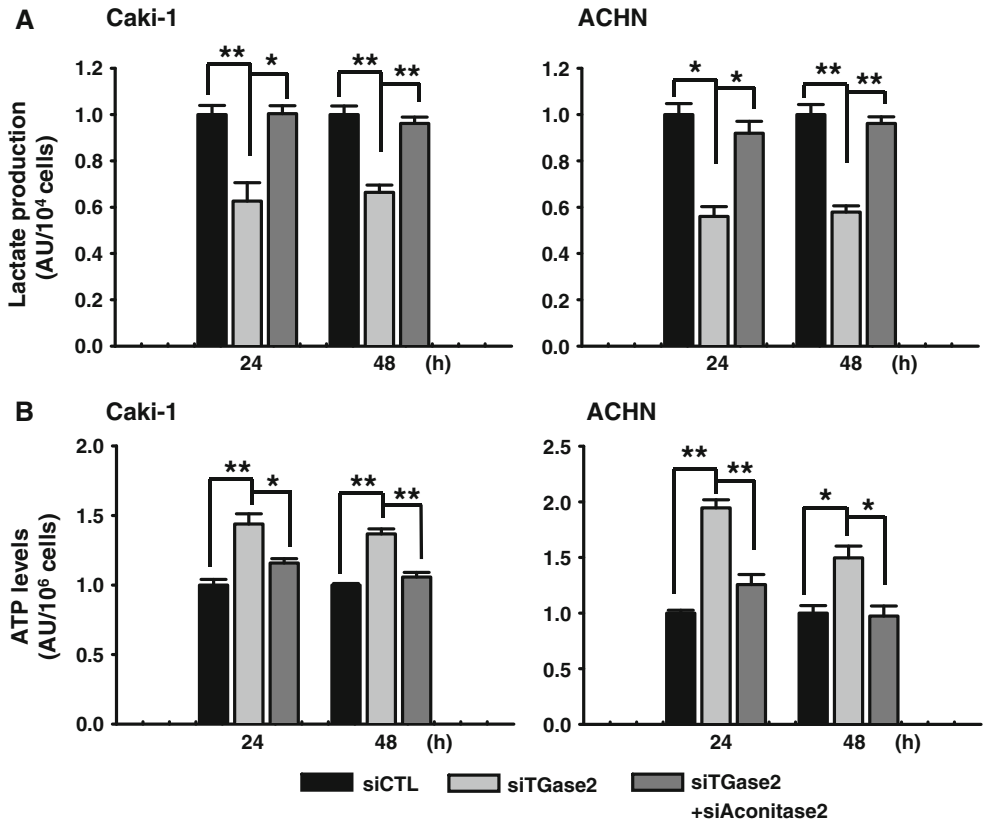

Fig. 6 The TGase 2 mediated Aco 2 regulation contributes to the increase of glycolytic metabolism. RCC cells were transfected with TGase 2 siRNA alone or TGase 2 siRNA and Aco 2 siRNA together. Changes in lactate production (**a**) and intracellular ATP levels (**b**) in response to each transfection were measured at indicated time points. Data are presented as mean ± SE in three separate experiments in triplicates (all three replicates of each of three experiments has been plotted, i.e., $n = 9$). *$p < 0.05$; **$p < 0.01$

Immunofluorescence analysis

After transfection with TGase 2 siRNA, cells were grown on coverslips and fixed in 4 % paraformaldehyde for 10 min at room temperature. Fixed cells were stained with indicated antibodies. Alexa 488 (green)- or Alexa 546 (red)-conjugated secondary antibodies were used for visualization. DAPI was used to co-stain the nuclei (blue). Images were obtained using a Zeiss Axiovert 200 M (Carl Zeiss).

Statistical analysis

Results are expressed as mean ± SE of at least three independent experiments. Student's *t* test was used to determine statistical significance.

Results

TGase 2 knockdown decreases glucose uptake and downregulates glycolytic metabolism in RCC

To test whether TGase 2 affects metabolism in RCC, glucose uptake and lactate production were measured 12, 24, 48 h after TGase 2 knockdown by siRNA (Fig. 1). TGase 2 knockdown decreased lactate production: a 20–30 % decrease was observed in Caki-1 and ACHN under normal culture conditions (Fig. 1a). TGase 2 knockdown also downregulated glycolytic metabolism by about 20–30 % in RCC (Fig. 1b).

Aco 2 is downregulated by TGase 2 in RCC

Aco 2 catalyzes the conversion of citrate to isocitrate in the tricarboxylic acid as a key player in the TCA cycle since Aco 2 knock out fly was lethal (Cheng et al. 2013). Previously, we found that Aco 2 was one of the targets of TGase 2 in mitochondria (Kim et al. 2005). Depletion of Aco 2 is closely associated with mitochondrial dysfunction in degenerative neuronal diseases (Kim et al. 2005; Schapira 1999). Aco 2 levels were decreased in the mitochondrial fraction of RCC cells compared to the mitochondrial fraction of HEK293 cells (Fig. 2a, b). LDH and COX IV were used as cytosolic and mitochondrial markers, respectively. To test whether reduced expression of Aco 2 in RCC relies on TGase 2 expression, HEK293 cells were transfected with control or TGase 2 expression vector, and

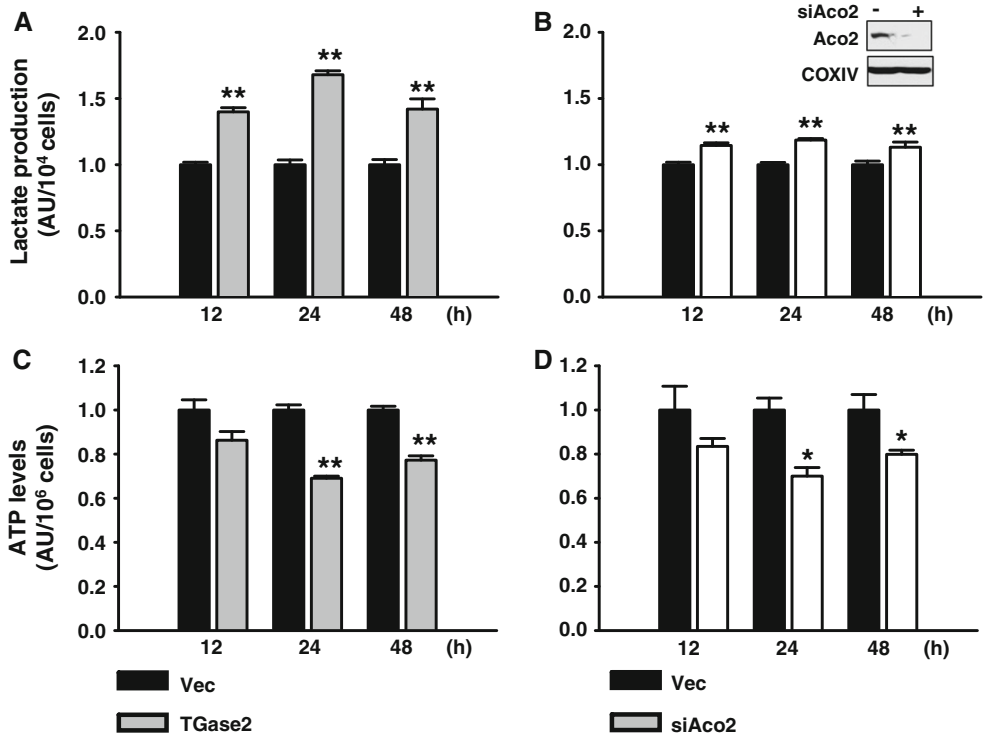

Fig. 7 TGase 2 facilitates glycolytic metabolism via Aco 2 inhibition. HEK293 cells were transfected with TGase 2 expression vector or Aco 2-siRNA. **a**, **b** Lactate assay, **c**, **d** ATP assay were done indicated time after transfection. Data are presented as mean ± SE in three separate experiments in triplicates (all three replicates of each of three experiments has been plotted, i.e., $n = 9$). $^{*}p < 0.05$; $^{**}p < 0.01$

the cytosol and mitochondrial fractions were analyzed by Western blotting at 48 h (Fig. 3a). In the mitochondrial fraction, TGase 2 expression was increased while Aco 2 expression was decreased (Fig. 3a). To determine whether the reduction in Aco 2 levels in mitochondria depended on TGase 2 expression, Caki-1 and ACHN cells were transfected with TGase 2 siRNA and subcellular factions were analyzed by Western blotting for Aco 2 at 48 h (Fig. 3b). Aco 2 levels were restored by TGase 2 siRNA (Fig. 3b). Immunofluorescence staining of Aco 2 and TGase 2 showed that TGase 2 knockdown restored Aco 2 levels in Caki-1 and ACHN, and TGase 2 transfection depleted Aco 2 level in HEK293 cells (Fig. 3c).

TGase 2 regulates mitochondrial function

To test whether TGase 2 regulates mitochondrial function through depletion of Aco 2, intracellular ATP level and mitochondrial membrane potential ($\Delta\Psi_m$) were measured with or without TGase 2 knockdown. 48 h treatment of TGase 2 siRNA increased ATP production by about 50 % (Fig. 4a). To evaluate $\Delta\Psi_m$, cells were stained with $DiOC_6$ or JC-1 and then analyzed by flow cytometry. In $DiOC_6$ staining, $\Delta\Psi_m$ was increased by about 40 % in CAKI-1 cells and by about 170 % in ACHN cells upon TGase 2 siRNA transfection (Fig. 4b). $\Delta\Psi_m$ was also analyzed by JC-1 staining. $\Delta\Psi_m$ change was observed by the changes in JC-1 fluorescence from red (JC-1 aggregates) to green (JC-1 monomers). TGase 2 knockdown increased green JC-1 staining, which implies increase of mitochondrial membrane potential (Fig. 4c).

TGase 2 suppresses Aco 2 activity in RCC

To test whether TGase 2 expression functionally regulates Aco 2 activity in RCC, Aco 2 activity was tested with TGase 2 siRNA transfection (Fig. 4). Aco 2 activity of Caki-1 (Fig. 5a) and ACHN (Fig. 5b) was measured using the Aconitase Activity Assay Kit. 24 and 48 h after TGase 2 siRNA transfection, Aco 2 activity showed increased up to 50 % to compare to control. Immuno blotting of Aco 2 using insoluble fraction of Caki-1 cell showed high molecular weight proteins, which has been clearly reduced by TGase 2 siRNA treatment (Fig. 5c).

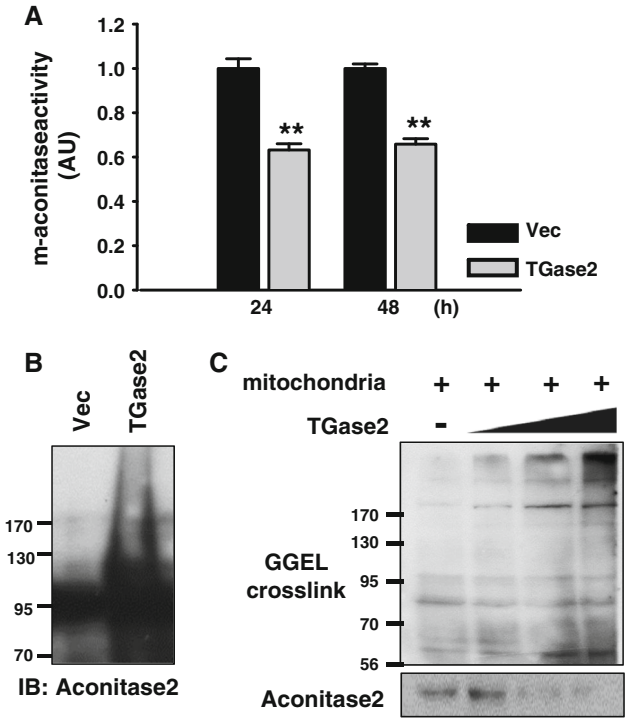

Fig. 8 TGase 2 inhibits Aco 2 through cross-linking. **a** HEK293 cells were transfected with TGase 2 expression vector and Aco 2 activity assay were done indicated time after transfection. **b** TGasae 2-induced Aco 2 polymers were detected in insoluble fraction by western blotting. **c** TGase 2-induced cross-linking was detected in vitro by immunoblotting using γ-glutamyl-ε-lysine (GGEL) cross-link specific antibody

TGase 2 facilitates aerobic glycolysis via Aco 2 inhibition

To test whether TGase 2 expression increases aerobic glycolysis by depleting Aco 2, Caki-1 and ACHN cells were transfected with TGase 2 siRNA in combination with Aco 2 siRNA. Lactate production was decreased by about 40–50 % (Fig. 6a) while ATP production was increased by 40–80 % (Fig. 6b) upon TGase 2 siRNA transfection. However, co-transfection of TGase 2 siRNA and Aco 2 siRNA almost completely reversed TGase 2 siRNA mediated metabolic changes including lactate production and ATP level (Fig. 6a, b).

TGase 2 facilitates aerobic glycolysis

To test whether TGase 2 overexpression increases glycolytic metabolism, lactate production, ATP level and Aco 2 activity assay were measured after HEK293 cells were transfected with TGase 2 expression vector. Data showed that TGase 2 expression induced lactate production (Fig. 7a), decrease of ATP production (Fig. 7c) and reduced Aco 2 activity (Fig. 8a). We proposed that TGase 2-mediated metabolic change may be based on the direct regulation of Aco 2. Therefore, we tested whether Aco 2 siRNA-transfected HEK293 cells may present the similar metabolic changes. Lactate production and ATP level were measured after HEK293 cells were transfected with Aco 2 siRNA for 48 h. Data showed that Aco 2 siRNA transfection for 48 h increased lactate production (Fig. 7b) and decreased ATP production (Fig. 7d) as well. TGase 2-induced Aco 2 polymers were also detected in insoluble fraction of HEK293 transfected with TGase 2 for 48 h by western blotting (Fig. 8b). TGase 2-induced cross-linking was detected in vitro by immunoblotting using γ-glutamyl-ε-lysine (GGEL) cross-link-specific antibody (Fig. 8c).

Discussion

Although TGase 2 is not a metabolic enzyme, however, TGase 2 is responsible for the regulation of metabolic enzyme mitochondrial Aco 2. Previously, we found that increase of TGase 2 in the neuronal mitochondria from Huntington's disease (HD) is generally associated with decrease of Aco 2 level (Kim et al. 2005). One of the representative characters of HD is mitochondrial metabolic dysfunction (Gu et al. 1996) (Browne et al. 1997) that is interestingly correlated with high expression of TGase 2 (Kim et al. 2005). Furthermore, mass analysis showed that TGase 2 specifically targets Aco 2 in the mitochondrial fraction (Kim et al. 2005).

TGase 2 expression promotes glycolytic metabolism via Aco 2 depletion in RCC. In this study, TGase 2 expression induced lactate production and secretion in RCC. TGase 2-mediated mitochondrial dysfunction may drive lactate production from pyruvate. TGase 2 induces HIF-1α via VHL depletion (Kim et al. 2011) in the cytosol. TGase 2-mediated HIF-1α induction (Kim et al. 2011) may promote lactate production because LDH (Firth et al. 1995) and MCT4 (Ullah et al. 2006) are induced by HIF-1α. Therefore, increased expression of TGase 2 contributes to glycolytic metabolism directly by Aco 2 depletion as well as indirectly by HIF-1α induction.

In contrast to the small changes in glucose consumption and lactate production observed 12, 24 and 48 h after transfection of TGase 2 siRNA (Fig. 1), the change in mitochondrial membrane potential was dramatic (Fig. 4). This change may suggest that TGase 2-mediated Aco 2 depletion (Figs. 2, 3) will shift the metabolism to a more glycolytic state (Fig. 1). These results are consistent with the data showing that Aco 2 siRNA induces an increase in lactate production of less than 20 % in HEK293 cells (Fig. 7) and ATP production was reduced by Aco 2 siRNA by about 20 % compared to control siRNA (Fig. 7).

In summary, increased TGase 2 expression induces Aco 2 depletion and mitochondrial dysfunction, thereby inhibiting ATP production through the TCA cycle and promoting glycolytic metabolism.

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Conflict of interest We declare that none of the authors have any financial interest related to this work, and none of the authors have any financial support beyond the research grant mentioned above.

References

Alberghini A, Recalcati S, Tacchini L, Santambrogio P, Campanella A, Cairo G (2005) Loss of the von Hippel Lindau tumor suppressor disrupts iron homeostasis in renal carcinoma cells. J Biol Chem 280(34):30120–30128. doi:10.1074/jbc.M500971200

Browne SE, Bowling AC, MacGarvey U, Baik MJ, Berger SC, Muqit MM, Bird ED, Beal MF (1997) Oxidative damage and metabolic dysfunction in Huntington's disease: selective vulnerability of the basal ganglia. Ann Neurol 41(5):646–653. doi:10.1002/ana.410410514

Catchpole G, Platzer A, Weikert C, Kempkensteffen C, Johannsen M, Krause H, Jung K, Miller K, Willmitzer L, Selbig J, Weikert S (2011) Metabolic profiling reveals key metabolic features of renal cell carcinoma. J Cell Mol Med 15(1):109–118. doi:10.1111/j.1582-4934.2009.00939.x

Chen C, Pore N, Behrooz A, Ismail-Beigi F, Maity A (2001) Regulation of glut1 mRNA by hypoxia-inducible factor-1. Interaction between H-ras and hypoxia. J Biol Chem 276(12):9519–9525. doi:10.1074/jbc.M010144200

Cheng Z, Tsuda M, Kishita Y, Sato Y, Aigaki T (2013) Impaired energy metabolism in a *Drosophila* model of mitochondrial aconitase deficiency. Biochem Biophys Res Commun 433(1):145–150. doi:10.1016/j.bbrc.2013.02.040

Degu A, Hatew B, Nunes-Nesi A, Shlizerman L, Zur N, Katz E, Fernie AR, Blumwald E, Sadka A (2011) Inhibition of aconitase in citrus fruit callus results in a metabolic shift towards amino acid biosynthesis. Planta 234(3):501–513. doi:10.1007/s00425-011-1411-2

Fesus L, Piacentini M (2002) Transglutaminase 2: an enigmatic enzyme with diverse functions. Trends Biochem Sci 27(10):534–539

Firth JD, Ebert BL, Ratcliffe PJ (1995) Hypoxic regulation of lactate dehydrogenase A. Interaction between hypoxia-inducible factor 1 and cAMP response elements. J Biol Chem 270(36):21021–21027

Gu M, Gash MT, Mann VM, Javoy-Agid F, Cooper JM, Schapira AH (1996) Mitochondrial defect in Huntington's disease caudate nucleus. Ann Neurol 39(3):385–389. doi:10.1002/ana.410390317

Icard P, Poulain L, Lincet H (2012) Understanding the central role of citrate in the metabolism of cancer cells. Biochim Biophys Acta. 1825(1):111–6. doi:10.1016/j.bbcan.2011.10.007

Jang GY, Jeon JH, Cho SY, Shin DM, Kim CW, Jeong EM, Bae HC, Kim TW, Lee SH, Choi Y, Lee DS, Park SC, Kim IG (2010) Transglutaminase 2 suppresses apoptosis by modulating caspase 3 and NF-kappaB activity in hypoxic tumor cells. Oncogene 29(3):356–367. doi:10.1038/onc.2009.342

Kim SY (2011) Transglutaminase 2: a new paradigm for NF-kappaB involvement in disease. Adv Enzymol Relat Areas Mol Biol 78:161–195

Kim WY, Kaelin WG (2004) Role of VHL gene mutation in human cancer. J Clin Oncol 22(24):4991–5004. doi:10.1200/JCO.2004.05.061

Kim SY, Marekov L, Bubber P, Browne SE, Stavrovskaya I, Lee J, Steinert PM, Blass JP, Beal MF, Gibson GE, Cooper AJ (2005) Mitochondrial aconitase is a transglutaminase 2 substrate: transglutamination is a probable mechanism contributing to high-molecular-weight aggregates of aconitase and loss of aconitase activity in Huntington disease brain. Neurochem Res 30(10):1245–1255. doi:10.1007/s11064-005-8796-x

Kim JW, Tchernyshyov I, Semenza GL, Dang CV (2006a) HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 3(3):177–185. doi:10.1016/j.cmet.2006.02.002

Kim WY, Safran M, Buckley MR, Ebert BL, Glickman J, Bosenberg M, Regan M, Kaelin WG Jr (2006b) Failure to prolyl hydroxylate hypoxia-inducible factor alpha phenocopies VHL inactivation in vivo. EMBO J 25(19):4650–4662. doi:10.1038/sj.emboj.7601300

Kim DS, Choi YB, Han BG, Park SY, Jeon Y, Kim DH, Ahn ER, Shin JE, Lee BI, Lee H, Hong KM, Kim SY (2011) Cancer cells promote survival through depletion of the von Hippel–Lindau tumor suppressor by protein crosslinking. Oncogene 30(48):4780–4790. doi:10.1038/onc.2011.183

Kim SJ, Kim KH, Ahn ER, Yoo BC, Kim SY (2013) Depletion of cathepsin D by transglutaminase 2 through protein cross-linking promotes cell survival. Amino Acids 44(1):73–80. doi:10.1007/s00726-011-1089-6

Ku BM, Kim DS, Kim KH, Yoo BC, Kim SH, Gong YD, Kim SY (2013) Transglutaminase 2 inhibition found to induce p53 mediated apoptosis in renal cell carcinoma. FASEB J Off Publ Fed Am Soc Exp Biol 27(9):3487–3495. doi:10.1096/fj.12-224220

Lee J, Kim YS, Choi DH, Bang MS, Han TR, Joh TH, Kim SY (2004) Transglutaminase 2 induces nuclear factor-kappaB activation via a novel pathway in BV-2 microglia. J Biol Chem 279(51):53725–53735. doi:10.1074/jbc.M407627200

Mathupala SP, Rempel A, Pedersen PL (2001) Glucose catabolism in cancer cells: identification and characterization of a marked activation response of the type II hexokinase gene to hypoxic conditions. J Biol Chem 276(46):43407–43412. doi:10.1074/jbc.M108181200

Mehta K, Kumar A, Kim HI (2010) Transglutaminase 2: a multi-tasking protein in the complex circuitry of inflammation and cancer. Biochem Pharmacol 80(12):1921–1929. doi:10.1016/j.bcp.2010.06.029

Moore LE, Nickerson ML, Brennan P, Toro JR, Jaeger E, Rinsky J, Han SS, Zaridze D, Matveev V, Janout V, Kollarova H, Bencko V, Navratilova M, Szeszenia-Dabrowska N, Mates D, Schmidt LS, Lenz P, Karami S, Linehan WM, Merino M, Chanock S, Boffetta P, Chow WH, Waldman FM, Rothman N (2011) Von Hippel–Lindau (VHL) inactivation in sporadic clear cell renal cancer: associations with germline VHL polymorphisms and etiologic risk factors. PLoS Genet 7(10):e1002312. doi:10.1371/journal.pgen.1002312

Schapira AH (1999) Mitochondrial involvement in Parkinson's disease, Huntington's disease, hereditary spastic paraplegia and Friedreich's ataxia. Biochim Biophys Acta 1410(2):159–170

Ullah MS, Davies AJ, Halestrap AP (2006) The plasma membrane lactate transporter MCT4, but not MCT1, is up-regulated by hypoxia through a HIF-1alpha-dependent mechanism. J Biol Chem 281(14):9030–9037. doi:10.1074/jbc.M511397200

Wykoff CC, Pugh CW, Maxwell PH, Harris AL, Ratcliffe PJ (2000) Identification of novel hypoxia dependent and independent target genes of the von Hippel–Lindau (VHL) tumour suppressor by mRNA differential expression profiling. Oncogene 19(54):6297–6305. doi:10.1038/sj.onc.1204012

Yamaguchi H, Wang HG (2006) Tissue transglutaminase serves as an inhibitor of apoptosis by cross-linking caspase 3 in thapsigargin-treated cells. Mol Cell Biol 26(2):569–579. doi:10.1128/MCB.26.2.569-579.2006