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Review

Metabolic changes in cancer: beyond the Warburg effect

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Altered metabolism is one of the hallmarks of cancer cells. The best-known metabolic abnormality in cancer cells is the Warburg effect, which demonstrates an increased glycolysis even in the presence of oxygen. However, tumor-related metabolic abnormalities are not limited to altered balance between glucose fermentation and oxidative phosphorylation. Key tumor genes such as *p53* and *c-myc* are found to be master regulators of metabolism. Metabolic enzymes such as succinate dehydrogenase, fumarate hydratase, pyruvate kinase, and isocitrate dehydrogenase mutations or expressing level alterations are all linked to tumorigenesis. In this review, we introduce some of the cancer-associated metabolic disorders and current understanding of their molecular tumorigenic mechanisms.

Keywords metabolism; signaling pathway; epigenetic; tumorigenesis

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Introduction

Otto Warburg's historic finding on altered metabolism in cancer ushered in an era of study on tumor metabolism, which was mainly focused on the relationship between glycolysis and cellular bioenergetics. Warburg's finding, although mechanistically remains largely unknown, has been exploited clinically by ^{18}F-deoxyglucose positron emission tomography scanning, a widely used technology for solid tumor detection [1]. Tumor cells differ from normal cells by unlimited cell division. It has long been considered that altered metabolism in tumor cells is to facilitate their rapid growth and duplication. In other words, the Warburg effect has been taken for granted a consequence of tumorigenesis. This notion is further fortified by findings that key tumor genes such as *p53* and *myc* are master regulators of metabolism. However, recent progress in studying isocitrate dehydrogenase 1 (IDH1) mutation, pyruvate kinase muscle form 2 (PKM2) alterations, fumarate hydratase (FH) mutations, and succinate dehydrogenase (SDH) mutations have demonstrated that mutation in metabolic enzymes alone is sufficient to initiate tumors, casting doubts to previous belief. Likely, metabolism disorders are direct causes of tumor initiation. Based on inadequate direct evidences, biologists are working actively to build links between altered metabolisms and cancer. We review here types of metabolic disorders that are associated with cancer, in the hope to help drawing a blue print of metabolism disorders and cancer based on current findings from different cancer models.

The Warburg Effect

In 1930s, Otto Warburg observed altered metabolism in cancer cells. In 1956, Otto Warburg [2] originally described his observation that cancer cells exhibit high rates of glucose uptake and lactic acid production. By using Warburg manometer, Warburg and his colleagues found that cancer cells did not consume more oxygen than normal tissue cells, even under normal oxygen circumstances [3], and it seemed that cancer cells preferred to aerobic glycolysis than to oxidative phosphorylation. Warburg [4] initially assumed that cancer cells had an impaired respiration due to the functional defects in mitochondria. However, it was later reported by a number of research groups that cancer cells did not sacrifice their oxidative phosphorylation to the enhanced production of lactate [5,6].

After more than half century's research, the Warburg effect stands true for most types of cancer cells; however, its exact reasons and physiological values remain elusive. People generally think that the Warburg effect will confer growth advantages to tumor cells. Several advantages that cancer cells adapt fermentative glucose metabolism are hypothesized. First, due to uncontrollable growth, the metabolism of cancer cells, like all proliferating cells, have to be adapted to facilitate the uptake and incorporation of nutrients into the biomass that are needed to produce a new cell: amino acids for protein synthesis, nucleic acids for DNA duplication, and lipids for cell biomembrane synthesis. Alternatively, cancer cell adopting glycolysis is to gain growth advantages as compared with normal cells: glycolysis provides acidic environment, which is harmful to normal cells but has no effect to tumor cells [7], underlining the importance of glycolysis as a cellular defense mechanism for cancer cell growth. This hypothesis, mainly based on mathematical models and empirical observations, is supported by the observation that lymphocytes activate glycolysis during fast growing [8]. A third hypothesis is that glycolysis produces less reactive oxygen species (ROS) so that the genome of cancer cells might elude the damage incurred by high concentration of ROS, which would result in apoptosis resistance in tumor issues. That has been verified as one of the defense mechanisms in malignant diseases, and cancer cells gain survival advantage simultaneously [9,10]. Lastly, it is believed that glycolysis can generate ATP faster than oxidative phosphorylation as long as the glucose supply is sufficient. However, this hypothesis is challenged by recent findings that cancer cells, doubling their numbers in days, actually need minimal ATP for proliferation. More than 95% of cancer cells' ATP is used for maintaining cellular function instead of being used for proliferation. The rate of ATP generation, therefore, should not be considered as an advantage.

The cause of the Warburg effect has caught the attention of scientists because people believe that a better understanding of the mechanisms of the Warburg effect may ultimately lead to more effective treatments for cancer. Numerous publications proposed different models, a comprehensive and clear cause of Warburg effect may be on the horizon. We will introduce some of the recent findings and different hypothesizes in the following part of this review.

SDH and FH Mutations

Warburg's hypothesis that cancer cells have defect in mitochondria was not totally unfounded. Indeed, many of the metabolism genes whose mutations can cause cancers are mitochondrial genes. SDH catalyzes the conversion from succinate to fumarate in the reactions of tricarboxylic acid (TCA) cycle, releasing one molecular reduced flavin adenine dinucleotide. SDH composes four subunites, named as SDHA, SDHB, SDHC, and SDHD, which are the classical components of SDH complex [11]. In the last few years, a new factor was identified as a member of SDH complex and was named as SDH5. SDH5 is a participant of the flavination of SDHA [12]. SDH mutations are commonly found in paraganglioma, gastric stromal tumors, and childhood T-cell acute leukemia [13,14]. Germline mutations in SDH seem to be closely associated with human head and neck paragangliomas [14]. These facts suggest that SDH mutations may provide a growth advantage in the initial stages of tumorigenesis. FH, the enzyme next to SDH, catalyzes the reaction from fumarate to malate. FH mutations have been observed in several kind of malignant tumors occurred in different tissues and organs, such as uterine leiomyomatosis, cerebral cavernomas, and breast cancer [15]. Based on these facts, both SDH and FH have been regarded as tumor suppressors.

Recent studies demonstrated that changes in the levels of hypoxia-inducible factor (HIF) were involved in the oncogenicity of SDH and FH mutations [11,16,17]. Hypoxia stress is a common phenomenon in tumor issues, and the predominant regulatory factor in the course of hypoxia response is HIF [18]. Under normal oxygen, HIF1α is degraded through the von Hippel-Lindau (VHL)-mediated ubiquitination pathway. In this reaction, the proline residues of HIF1α need to be hydroxylated before HIF1α could be recognized by VHL [19,20]. The hydroxylation of HIF1α is catalyzed by proline hydroxylases (PHDs). PHDs are a family of α-ketoglutarate (α-KG)-dependent enzymes. During the process of HIF1α hydroxylation, the substrate of α-KG is oxidized accompanying with the generation of succinate as a product [21]. In SDH and FH mutations bearing tumors, activated HIF1α and its target genes amplification, such as vascular endothelial growth factor, and increased angiogenesis are commonly observed; it is hypothesized that SDH and FH mutations induce their tumorigenicity through activating HIF1α pathway. Indeed, mutations of SDH and FH were found to accumulate succinate and fumarate, structural analogs of α-KG that may inhibit PHDs and activate HIF pathway. Subsequent tests verified that fumarate could inhibit PHD2 [16], while succinate could reduce the enzymatic activity of PHD3 [11].

HIF consists of two subunits: α subunit is usually located in cytoplasm (HIF1α) and β subunit located in the nucleus (HIF1β). The inhibition of PHD promotes HIF1α to enter into the nucleus and integrate with HIF1β to form heterodimers, then promotes the expression of a series of HIF target genes, including genes encoding glucose transporters (GLUTs) [22], glycolysis enzymes such as pyruvate dyhydrogenase kinase (PDK) [23,24] and lactate dyhydrogenase A (LDH-A), and myc etc [25]. Amplification of GLUTs may allow transformed cells to compete with normal cells more effectively in the process of glucose uptake. The up-regulation of PDK is able to inhibit the enzymatic activity of pyruvate dehydrogenase (PDH). And the increased levels of LDH-A can accelerate the conversion of pyruvate to lactate. The effect of myc overexpression on cellular metabolism will be discussed in the following section. HIF target genes synergistically promote the Warburg effect, allowing cancer cells to gain growth advantages (**Fig. 1**) [11,17,26].

Although it sounds plausible, the real causes of SDH and FH tumorigenicity remain debatable. For example, among these mutations associated with tumor development, missense mutation is the most frequent mutant type. It has

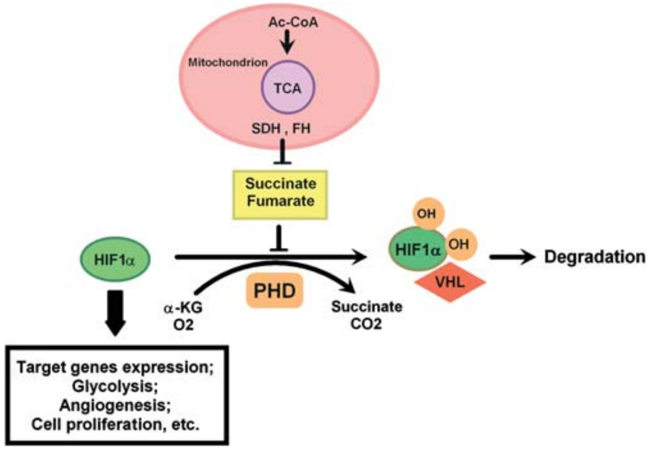

Figure 1 Mutations of SDH and FH increase the accumulation of succinate and/or frumatate Both of these two metabolites can inhibit the enzymatic activity of PHD, resulting in the reduced degradation of HIF1α, and the increased expression of some specific genes such as genes involved in glycolysis, angiogenesis, and cell proliferation, etc. Ac-CoA, acetyl-coenzyme A.

been detected that their contributions to the initiation and development of tumors have nothing to do with their mutant forms; the recessive mutations have equal effects with dominant mutations during the course of tumorigenesis [15]. In addition, cancerous SDH mutations are only detected in SDHB, SDHC, SDHD, and SDH5 but not SDHA, the catalytic subunit [27,28]. These facts suggest that consequences other than succinate and fumarate accumulation may also contribute to the tumorigenicity of SDH and FH mutations.

IDH Mutations

Three isoforms of IDHs are found in humans: IDH1 is mainly located in cytoplasm, while IDH2 and IDH3 in mitochondria. IDH3 uses NAD^+ as a cofactor, suggesting a major role in energy metabolism. IDH1 and IDH2, using $NADP^+$ as a cofactor, may play roles in redox regulation. All three enzymes convert isocitrate to α-KG and have a role in TCA cycle. Over 70% of grade II–III gliomas and most of secondary glioblastomas are detected with IDH1 and IDH2 mutations, especially the mutations of IDH1 [29,30]. IDH mutations also have been observed in acute myeloid leukemias [31] and chondrosarcoma [32]. Wild-type IDHs convert isocitrate into α-KG, while mutant IDHs gain a new enzyme activity of catalyzing α-KG into 2-hydroxyglutarate (2-HG) [30]. Consequently, mutations in IDHs lead to the disruption of mitochondrial oxidative phosphorylation, the reduction of α-KG and the accumulation of 2-HG (**Fig. 2**).

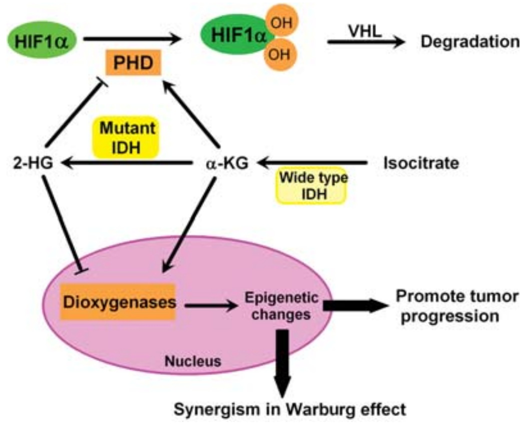

Figure 2 Mutatant IDH can convert α-KG into 2-HG The increased 2-HG could inhibit the activities of α-KG-dependant dioxygenases, leading to wide biological effects.

As we all know, α-KG is not only a key intermediate in the TCA cycle, but also an essential substrate in the reaction of HIF's hydroxylation and degradation. Thus, the reduction of α-KG can result in the stabilization and activation of HIF1. Besides, 2-HG is an analog of α-KG, so it can change the cellular homeostasis of α-KG, and may also inhibit α-KG-dependent enzymatic reactions competitively, leading to a series of cellular biological behavior changes [26]. It has been reported that the accumulation of 2-HG or the reduction of α-KG can both inhibit the activities of dioxygenases [26,29]. Possible downstream targets include: PHDs such as the enzymes involved in the regulation and degradation of HIF1α; histone lysine demethylases such as the superfamily of Jumonji C-terminal domain histone demethylase (JHDM); DNA hydroxylases such as the ten-eleven-translocation (TET) family, etc. [26]. The increased levels of HIF1α play pivotal roles in the promotion of aerobic glycolysis and tumorigenesis. JHDM catalyzes histone demethylation, which leads to the alteration of nucleosome space conformation. TET family catalyzes the hydroxylation of 5′-methylcytosine converting into 5-hydroxymethylcytosine, leading to DNA demethylation [33]. The consequences of IDH1 and IDH2 mutations can result in chromatin remodeling as well as DNA demethylation alteration, both are epigenetic variations [34]. These alterations are powerful enough to induce cell differentiation arrest accordingly and are surely tumorigenic [35]. It is worth pointing out that both decrease of the cellular α-KG level and accumulation of D-2-HG are indispensable for the induction of tumors [26], which has been evidenced by the fact that germline D-2-HG dehydrogenase mutations, which can cause D-2-HG accumulation, are not associated with any type of cancer, while germline L-2-HG dehydrogenase mutations, which can accumulate high levels of L-2-HG, a more potent inhibitor of dioxygenases [26], are associated with several types of tumors [36,37]. It is now become clear that D-2-HG inhibits the activity of dioxygenases and the decreasing levels of α-KG potentiate this inhibition. And when the additive effects are equal to or more intensive than that of L-2-HG, tumor diseases may occur.

Lastly, the red-heated IDH mutation research area generated lots of reports that are even against current opinion. For example, D-2-HG accumulation is even reported to facilitate HIF degradation and an inactivated HIF is proposed as the cause of tumorigenicity [38]. These controversies imply that IDH1 and IDH2 mutations could impose widely influences on the activities in different cellular levels and the progression of disease.

PKM2 Switch

Pyruvate kinase (PK) has four isoforms, and their distributions in tissues and organs in human have certain specificities: PKL mainly located in liver and kidney; PKR mainly in erythrocytes; PKM1 was found in most adult tissues; the expression of PKM2 is specifically related to development, mainly detected in embryonic cells and rapidly dividing cells [39]. In transformed cells PKM2 started to expression again and is thought to be associated with tumor growth. In recent years, increasing number of evidences showed PKM2 expression is tumorigenic [40,41]. However, this concept is challenged recently by a mass spectrometry quantification of PKM1 and PKM2 isoforms in malignant and normal tissues. Bluemlein *et al*. [42] analyzed splice isoforms in 25 human malignant cancers, 6 benign oncocytomas, tissue-matched controls, and several cell lines. PKM2 was indeed the prominent isoform in all cancer samples. However, PKM2 was also the predominant PKM isoform in matched control tissues such as unaffected kidney, lung, liver, and thyroid. Thus, an exchange in PKM1 to PKM2 isoform expression during cancer formation may not be occurring [42], as oppose to current main stream theory. This report, although the only one so far, reminded us to be open minded to the PKM2 functions in tumor initiation and progression.

PKM2 catalyzes the conversion of phosphoenolpyruvate into pyruvate at the last second step of glycolysis. It is one of the rate-limiting enzymes in glucose metabolism and its enzymatic activity can be regulated by conformational change with the binding of allosteric molecules and protein modifications caused by other signaling molecules [39]. For example, metabolic intermediates, fructose-2, 6 -bisphosphate (F-2,6-BP) and fructose-1,6-bisphosphate (F-1,6-BP), are classical well-defined allosteric activators of PKM2. PKM2 exists in two distinct forms in normally rapidly dividing cells: active PKM2 usually is a tetramer in composition of four same subunits; while the inactive PKM2 is dimeric arising from the dissociation of tetrameric PKM2. The equilibrium of the dimer and the tetramer is determined by cellular needs. Cells keep an active balance of PKM2 enzymatic activity to meet the adaption of cell mobilities [39]. It is believed that in tumor cells PKM2 is usually in the form of dimer, which almost inactive in its catalytic activity [42,43]. The outcome of having predominant PKM2 in cancer cells will directly restrain the production of pyruvate and lead to an increase of metabolic intermediates produced in the stage of glycolysis. Eventually, the accumulation of glycolytic products will be precursors of biosynthesis of nucleotide, cholesterol, fatty acids, and other components required for cell proliferation and division through pentose phosphate pathway and other synthetic pathways. A challenge that remains with regard to this theory is that dimeric PKM2 actually keep a certain amount of catalytic activity, based on reports and our own analysis. It is hard to understand that cells will generate a different form of protein, which is both energy consuming and slow in response, to only meet the slowdown of PK enzyme activity, instead of simply regulating PK activity by common means, e.g. allosteric control or post-translational modifications (PTMs). It is, therefore, strongly suggested that the production of PKM2 confers functions other than restraining metabolic flux.

The activity of PKM2 can be regulated by a number of PTM. PKM2 can be phosphorylated at tyrosine residue 105 (Y105) directly mediated by fibroblast growth factor receptor type 1. Further research identified that the phosphorylation of PKM2 at Y105 could disrupt the binding of F-1,6-BP, thus surrender PKM2's allosteric regulation by F-1,6-BP [44]. In cells cultured with a high concentration of glucose, acetylation of PKM2 at the site of lysine 305 (K305), and PKM2 K305 acetylation decreases PKM2 enzyme activity and promotes chaperone-dependent cell autophagy, allowing cells to utilize endogenous macromolecules when deficient in nutrients to ensure cell survival [45]. PKM2 activity can also inhibit by acute increase in intracellular concentrations of ROS through oxidation of Cys358, this has been conformed in human lung cancer cells [46]. Modifications of PKM2 lead to the decrease of enzymatic activity, diverting glucose flux into the pentose phosphate pathway and generating a sufficient reduced form of nicotinamide-adenine dinucleotide phosphate. Both are beneficial to cell proliferation and division. These evidences suggest that PKM2 may acts as an important signaling molecule in the progression of tumor. Besides, PKM2 can be induced to relocate into nuclear by the activation of signaling pathway and function as transcriptional coactivator. Nuclear PKM2 is able to interact with transcription factor (TIF) HIF to promote the expression of HIF target genes [47]. With tyrosine 333-phosphorylated β-catenin, nuclear PKM2 promotes the acetylation of histone H3 and the expression of cyclin D1 [48]. Again, these results force us to come to the conclusion that PKM2 alters cancer cell metabolism through multiple pathways instead of only a door keeper for glycolytic flux (**Fig. 3**).

The multifunctional roles hypothesis of PKM2 got a solid proof most recently. Yang *et al*. showed that PKM2 directly binds to histone H3 and phosphorylates histone H3 at threonine 11 upon endothelial growth factor (EGF) receptor activation. This phosphorylation is essential for the dissociation of HDAC3 from the CCND1 and MYC promoter regions, which is required for acetylation of histone H3 at K9. PKM2-dependent histone H3 modifications, triggered initially by PKM2-mediated phosphorylation, are fundamental in EGF-induced expression of cyclin D1 and c-myc, tumor cell proliferation, cell-cycle progression, and brain tumorigenesis (**Fig. 4**) [49]. These findings indicate

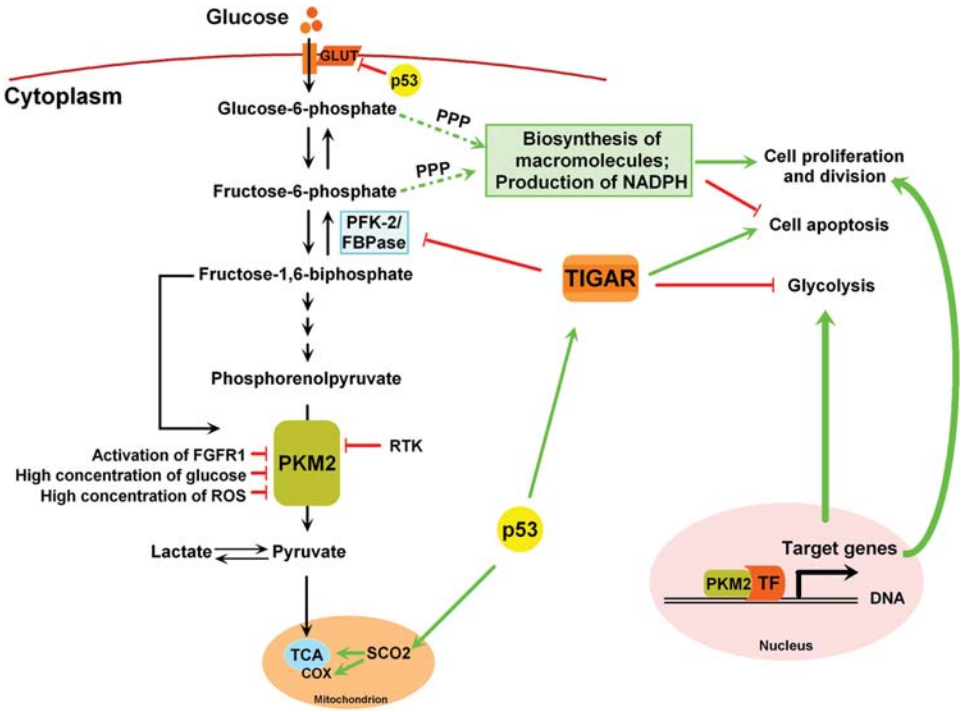
Glucose
GLUT
p53
Cytoplasm
Glucose-6-phosphate
PPP
Biosynthesis of macromolecules; Production of NADPH
Cell proliferation and division
PPP
Fructose-6-phosphate
PFK-2/ FBPase
Cell apoptosis
TIGAR
Glycolysis
Fructose-1,6-biphosphate
Phosphorenolpyruvate
Activation of FGFR1
High concentration of glucose
High concentration of ROS
PKM2
RTK
p53
Lactate
Pyruvate
Target genes
PKM2
TF
DNA
Nucleus
TCA
COX
SCO2
Mitochondrion

Figure 3 Interactions between PKM2 and cellular signaling pathway PFK-2/FBPase, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase; PPP, pentose phosphate pathway; RTK, receptor tyrosine kinase; SCO2, synthesis of cytochrome C oxidative 2; COX, cytochrome oxidase C complex; TIGAR, TP53-induced glycolysis and apoptosis regulator; TF, transcription factor.

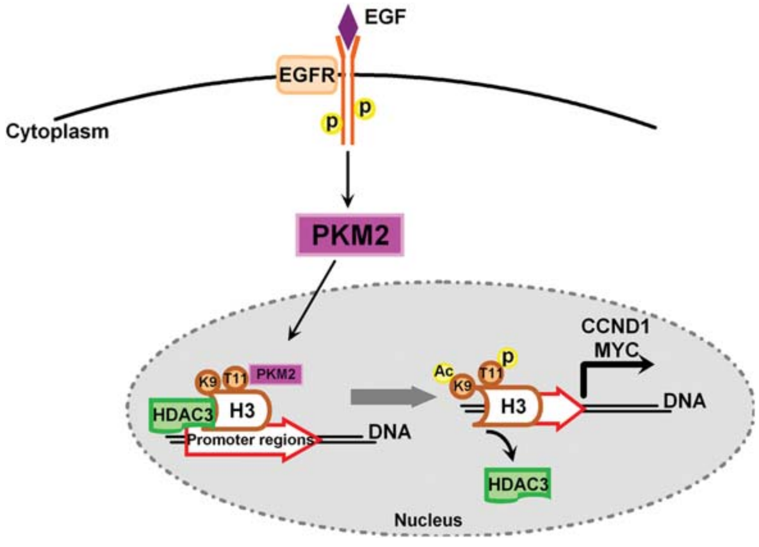
EGF
EGFR
Cytoplasm
p
p
PKM2
K9
T11
PKM2
HDAC3
H3
Promoter regions
DNA
Ac
K9
T11
p
H3
CCND1
MYC
DNA
HDAC3
Nucleus

Figure 4 PKM2 catalyzes the phosphorylation of histone H3 and promotes gene transcription EGF, epidermal growth factor; EGFR, epidermal growth factor receptor.

that PKM2 is also a protein kinase, in addition to its metabolic functions, extending PKM2's function to gene transcriptional regulation and signal transduction. The remaining important questions are what are other substrates, if there is any, of PKM2 in cells and what signaling pathways are directly regulated by PKM2?

p53 and Metabolism

p53 is one of the most important tumor suppressor proteins and plays significant roles in normal growth and development, including the induction of apoptosis, regulation of cell cycle, DNA repair, and maintenance of genome stability. Its mutation or depletion is associated with most cancers [50]. p53 exerts its regulations via a complex network. Cellular functions that are regulated by p53 involve ROS, DNA damage and repair, cell cycle, authophagy, and, most recently, metabolism.

The role of p53 as a central component of the stress response machinery is well established. Levels of intracellular ROS, metabolic stress, hypoxia, DNA damage can all activate p53 [51,52]. Take ROS as an example, cells continuously release ROS during metabolism and other cellular processes. Cells respond to different levels of ROS, and usually result in different outcomes. Under low-ROS condition, p53 directs cells to proliferation, and under high ROS conditions, p53 activates genes that lead to cell apoptosis [53,54].

Increasing evidences have shown that p53 plays an important role in the regulation of both glycolysis and oxidative phosphorylation, implying a coordinating role of p53 in these two metabolic pathways and a key regulator for the Warburg effect. Metabolic enzymes including glucose transporters, glycolytic enzymes, and TCA cycle enzymes are downstream targets of p53. p53-responsive elements exist in the promoters of PGM55 and hexokinase II genes, suggesting that p53 can regulate at least some steps in glycolysis. p53 can slow glycolysis and therefore reduce the increase in glycolysis that is characteristic of cancers [50]. p53 can inhibit the expression of the glucose transporters, especially GLUT1 and GLUT4 [55], resulting in reduced glucose uptake and increase the levels of tumor protein 53-induced glycolysis and apoptosis regulator (TIGAR) [56]. TIGAR expression causes the down-regulation of FBPase because of the similarity of functional domain with bifunctional enzymes 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphatase (PFK-2/FBPase) [50,57]. FBPase can promote the degradation of F-2,6-BP. The decrease of F-2,6-BP is of great benefit to the formation of fructose-6-phosphate, then make for the metabolic intermediates into the pentose phosphate pathway to anabolic metabolism. With this assistance, tumor cells survive the stress. Similar to p53 response to the levels of ROS, the effects of TIGAR expression on cell survival are also likely to be cell- and context-dependent. The inhibition of glycolysis is also achieved by p53-dependent transcriptional activation of synthesis of cytochrome C oxidative 2, resulting in enhanced mitochondrial respiration through downstream effectors cytochrome oxidase C complex (COX) [58]. COX is the main site of oxygen utilization in human cells. Through these pathways, the mode of energy production in cancer cells is similar but not identical with non-transformed cells. In this way, p53 exerts some effects of inhibition on tumor growth.

The universal roles of p53 in metabolic regulation make it difficult to summarize how p53 mutations cause metabolic reprogramming in cancer cells. The findings that metabolic stresses actually activate p53 make it even impossible to conclude that altered metabolism is the cause or outcome of p53 mutation. The bottom line is that profound metabolic alterations had occurred in the process of cancer initiation.

c-myc and Metabolism

The amplification of oncogenic TIF c-myc is universal in tumors arising from different tissues and organs [59]. As a TF, c-myc cooperates with other TFs and exerts its function in the regulation of cell proliferation and differentiation. Many of c-myc target genes are involved in the maintenance of stem cell self-renewal ability and tumorigenesis [60,61], which have been well documented. It is worth noting that activation of c-myc induces glycolysis and glutaminolysis, two typical metabolism alterations in cancer cells [62].

Like that of p53, activation of c-myc increases the levels of glucose transporters as well as glycolytic enzymes. One of the outstanding function is that c-myc induces the splicing factors to produce PKM2, one of the hallmarks of tumor metabolism. In PKM splicing, three heterogeneous nuclear ribonucleoprotein (hnRNP) proteins, polypyrimidine tract-binding protein (PTB or hnRNPI), hnRNPA1 and hnRNPA2, bind repressively to sequences flanking exon 9 of PKM2, resulting in exon 10 inclusion. c-myc up-regulates transcription of PTB, hnRNPA1, and hnRNPA2, ensuring a high PKM2/PKM1 ratio. In human gliomas, overexpression of c-myc, PTB, hnRNPA1, and hnRNPA2 correlates with PKM2 expression [63], these findings augment the role of c-myc in aerobic glycolysis. The deregulated c-myc can also increase the expression of LDH-A, hastening the conversion of pyruvate to lactate [64], and up-regulate the activity of PDK1. The enhanced PDK1 leads to the inhibition of PDH, causing the inhibition of oxidative phosphorylation. Interestingly, c-myc-induced metabolic changes mimic hypoxia effects while cells are actually under normal oxygen environments, suggesting that c-myc can facilitate the Warburg effect or aerobic glycolysis. Moreover, c-myc interacts with TIF HIF to promote HIF expression and inhibit the degradation of HIF1α, the increased levels of HIF imposes positive feedback on c-myc, which potentiates the effect of aerobic glycolysis [59].

Glutamine can be converted to α-KG after a series of enzymatic reactions and can enters TCA cycle as energy fuel in cancer cells. Glutamine-generated α-KG goes through part of the TCA cycle, generates malate, which is then transported out of mitochondria into cytoplasm and oxidized with the production of lactate. The process is termed by [65]. Glutaminolysis was originally named by Mckeehan to describe the partial oxidation of glutamine. Glutaminolysis was found to promote DNA synthesis by through [^{3}H] thymine incorporation experiment with lymphocytes cultured in a glutamine-deficiency medium [6], suggesting glutaminolysis is likely a protective mechanism in rapidly dividing cells. Besides, c-myc positively regulates the expression of glutamine transporters as well as the enzyme glutaminase (GLS). c-myc interacts with some transcription factors displaying repression effects of some target genes. For example, c-myc suppression of miR-23a/b is able to enhance glutamine catabolism through increased expression of GLS [66,67]. These findings demonstrate that c-myc is an important regulator in the balance of energy metabolism and biosynthetic metabolism required in rapidly dividing transformed cells (**Fig. 5**).

Perspectives

Metabolism reprogram is universally accepted as one of the hallmarks of cancer. However, we are still facing more challenges than answers to how reprogrammed metabolism is related to cancer. One of the most important questions needed to be answered is that which one occurs first, cancer cells or altered metabolism? This chicken and egg question is still not easy to answer. On one hand, mutations in oncogenes or tumor suppressor genes such as c-myc and p53 are known direct causes of cancer, on the other hand, mutations in metabolic genes such as IDH1, IDH2, SDH, and FH also cause certain types of cancers. Moreover, metabolic stresses cause tumor-associated genes, such as c-myc and p53, alterations, and changes in tumor-associated genes is now known to result in metabolic deregulations. Therefore, besides traditional concept that cell signaling disorder is the direct cause of cancer initiation, metabolic alterations could be the real causes of cancers. Two models can be proposed based on current facts about cancers. First, tumor-associated gene mutations likely cause metabolic changes first and the altered metabolism, which has a new homeostasis of metabolites, has the ability to reprogram epigenetics as well as signaling networks and to cause cancer. The second model is that altered metabolism, either caused by metabolic gene mutations or by environmental factors, can reprogram epigenetics as well as signaling networks and cause cancer; while tumor-associated gene mutations are consequences of activated gene expression. The second model, although sounds more controversial, gets some support from recent

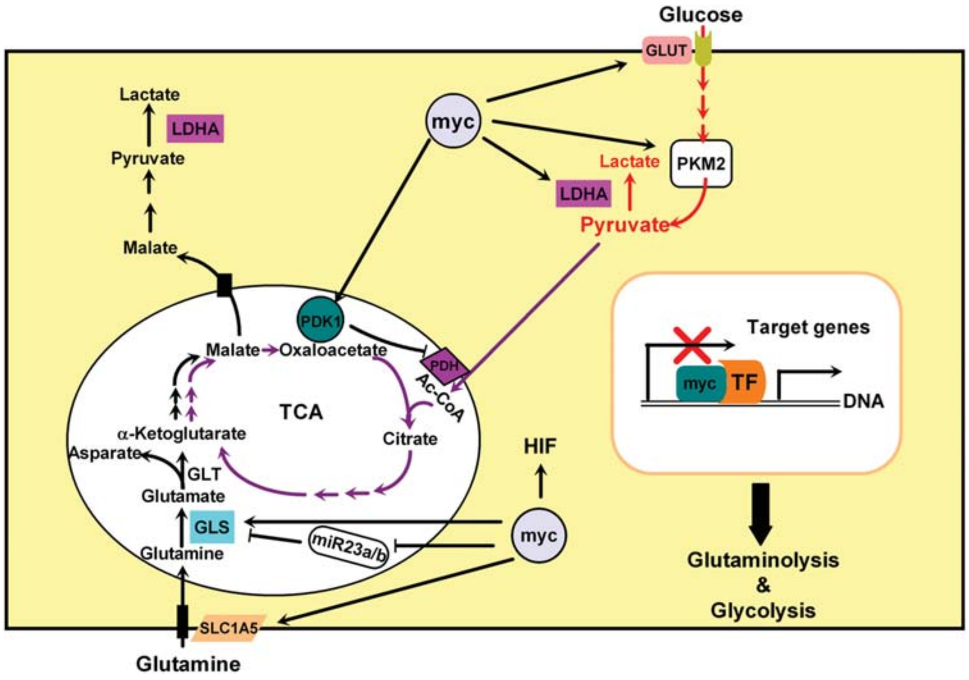

Figure 5 Schematic diagram demonstrates the contributions of c-myc to glucolysis (red arrow lines) and glutaminolysis (black arrow lines) GLT, glutamate transaminase.

findings. In glioma, IDH1 mutations seem to happen in the early stage of disease onset, even before p53 mutation was detected in patients [68]. Regardless of which model is more reasonable, metabolism seems to be taking center stage of cancer research. The elucidation of how metabolism changes cause cancers will shed light on future novel cancer treatment development.

Funding

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References

1 Ter-Pogossian MM, Phelps ME, Hoffman EJ and Mullani NA. A positron-emission transaxial tomograph for nuclear imaging (PETT). Radiology 1975, 114: 89–98.

2 Warburg O. On the origin of cancer cells. Science 1956, 123: 309–314.

3 Koppenol WH, Bounds PL and Dang CV. Otto Warburg’s contributions to current concepts of cancer metabolism. Nat Rev Cancer 2011, 11: 325–337.

4 Warburg O. On respiratory impairment in cancer cells. Science 1956, 124: 269–270.

5 Weinhouse S. On respiratory impairment in cancer cells. Science 1956, 124: 267–269.

6 Newsholme EA, Crabtree B and Ardawi MS. The role of high rates of glycolysis and glutamine utilization in rapidly dividing cells. Biosci Rep 1985, 5: 393–400.

7 Gatenby RA and Gillies RJ. Why do cancers have high aerobic glycolysis? Nat Rev Cancer 2004, 4: 891–899.

8 Fox CJ, Hammerman PS and Thompson CB. Fuel feeds function: energy metabolism and the T-cell response. Nat Rev Immunol 2005, 5: 844–852.

9 Brand KA and Hermfisse U. Aerobic glycolysis by proliferating cells: a protective strategy against reactive oxygen species. FASEB J 1997, 11: 388–395.

10 Spitz DR, Sim JE, Ridnour LA, Galoforo SS and Lee YJ. Glucose deprivation-induced oxidative stress in human tumor cells. A fundamental defect in metabolism? Ann N Y Acad Sci 2000, 899: 349–362.

11 King A, Selak MA and Gottlieb E. Succinate dehydrogenase and fumarate hydratase: linking mitochondrial dysfunction and cancer. Oncogene 2006, 25: 4675–4682.

12 Hao HX, Khalimonchuk O, Schraders M, Dephoure N, Bayley JP, Kunst H and Devilee P, *et al*. SDH5, a gene required for flavination of succinate dehydrogenase, is mutated in paraganglioma. Science 2009, 325: 1139–1142.

13 Baysal BE, Willett-Brozick JE, Lawrence EC, Drovdlic CM, Savul SA, McLeod DR and Yee HA, *et al*. Prevalence of SDHB, SDHC, and SDHD germline mutations in clinic patients with head and neck paragangliomas. J Med Genet 2002, 39: 178–183.

14 Baysal BE. A recurrent stop-codon mutation in succinate dehydrogenase subunit B gene in normal peripheral blood and childhood T-cell acute leukemia. PLoS One 2007, 2: e436.

15 Tomlinson IP, Alam NA, Rowan AJ, Barclay E, Jaeger EE, Kelsell D and Leigh I, *et al*. Germline mutations in FH predispose to dominantly inherited uterine fibroids, skin leiomyomata and papillary renal cell cancer. Nat Genet 2002, 30: 406–410.

16 Isaacs JS, Jung YJ, Mole DR, Lee S, Torres-Cabala C, Chung YL and Merino M, *et al*. HIF overexpression correlates with biallelic loss of fumarate hydratase in renal cancer: novel role of fumarate in regulation of HIF stability. Cancer Cell 2005, 8: 143–153.

17 Selak MA, Armour SM, MacKenzie ED, Boulahbel H, Watson DG, Mansfield KD and Pan Y, *et al*. Succinate links TCA cycle dysfunction to oncogenesis by inhibiting HIF-alpha prolyl hydroxylase. Cancer Cell 2005, 7: 77–85.

18 Wang GL, Jiang BH, Rue EA and Semenza GL. Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O_2 tension. Proc Natl Acad Sci USA 1995, 92: 5510–5514.

19 Semenza GL. Hypoxia-inducible factors in physiology and medicine. Cell 2012, 148: 399–408.

20 Martín-Puig S, Temes E, Olmos G, Jones DR, Aragonés J and Landázuri MO. Role of iron (II)-2-oxoglutarate-dependent dioxygenases in the generation of hypoxia-induced phosphatidic acid through HIF-1/2 and von Hippel-Lindau-independent mechanisms. J Biol Chem 2004, 279: 9504–9511.

21 Chen H and Costa M. Iron- and 2-oxoglutarate-dependent dioxygenases: an emerging group of molecular targets for nickel toxicity and carcinogenicity. Biometals 2009, 22: 191–196.

22 Goda N and Kanai M. Hypoxia-inducible factors and their roles in energy metabolism. Int J Hematol 2012, 95: 457–463.

23 Kim JW, Tchernyshyov I, Semenza GL and Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. Cell Metab 2006, 3: 177–185.

24 Semenza GL, Roth PH, Fang HM and Wang GL. Transcriptional regulation of genes encoding glycolytic enzymes by hypoxia-inducible factor 1. J Biol Chem 1994, 269: 23757–23763.

25 Gordan JD, Thompson CB and Simon MC. HIF and c-Myc: sibling rivals for control of cancer cell metabolism and proliferation. Cancer Cell 2007, 12: 108–113.

26 Xu W, Yang H, Liu Y, Yang Y, Wang P, Kim SH and Ito S, *et al*. Oncometabolite 2-hydroxyglutarate is a competitive inhibitor of alpha-ketoglutarate-dependent dioxygenases. Cancer Cell 2011, 19: 17–30.

27 Pasini B and Stratakis CA. SDH mutations in tumorigenesis and inherited endocrine tumours: lesson from the phaeochromocytoma-paraganglioma syndromes. J Intern Med 2009, 266: 19–42.

28 Bardella C, Pollard PJ and Tomlinson I. SDH mutations in cancer. Biochim Biophys Acta 2011, 1807: 1432–1443.

29 Zhao S, Lin Y, Xu W, Jiang W, Zha Z, Wang P and Yu W, *et al*. Glioma-derived mutations in IDH1 dominantly inhibit IDH1 catalytic activity and induce HIF-1alpha. Science 2009, 324: 261–265.

30 Dang L, White DW, Gross S, Bennett BD, Bittinger MA, Driggers EM and Fantin VR, *et al*. Cancer-associated IDH1 mutations produce 2-hydroxyglutarate. Nature 2009, 462: 739–744.

31 Figueroa ME, Abdel-Wahab O, Lu C, Ward PS, Patel J, Shih A and Li Y, *et al*. Leukemic IDH1 and IDH2 mutations result in a hypermethylation phenotype, disrupt TET2 function, and impair hematopoietic differentiation. Cancer Cell 2010, 18: 553–567.

32 Amary MF, Bacsi K, Maggiani F, Damato S, Halai D, Berisha F and Pollock R, *et al*. IDH1 and IDH2 mutations are frequent events in central chondrosarcoma and central and periosteal chondromas but not in other mesenchymal tumours. J Pathol 2011, 224: 334–343.

33 Gu TP, Guo F, Yang H, Wu HP, Xu GF, Liu W and Xie ZG, *et al*. The role of Tet3 DNA dioxygenase in epigenetic reprogramming by oocytes. Nature 2011, 477: 606–610.

34 Lu C and Thompson CB. Metabolic regulation of epigenetics. Cell Metab 2012, 16: 9–17.

35 Lu C, Ward PS, Kapoor GS, Rohle D, Turcan S, Abdel-Wahab O and Edwards CR, *et al*. IDH mutation impairs histone demethylation and results in a block to cell differentiation. Nature 2012, 483: 474–478.

36 Struys EA, Salomons GS, Achouri Y, Van Schaftingen E, Grosso S, Craigen WJ and Verhoeven NM, *et al*. Mutations in the D-2-hydroxyglutarate dehydrogenase gene cause D-2-hydroxyglutaric aciduria. Am J Hum Genet 2005, 76: 358–360.

37 Suhs KW, Erdmann P, Shamdeen MG, Papanagiotou P and Dillmann U. Adult manifestation of L-2-hydroxyglutarate dehydrogenase deficiency by a novel mutation. Neurology 2012, 78: 1186–1187.

38 Koivunen P, Lee S, Duncan CG, Lopez G, Lu G, Ramkissoon S and Losman JA, *et al*. Transformation by the (R)-enantiomer of 2-hydroxyglutarate linked to EGLN activation. Nature 2012, 483: 484–488.

39 Gupta V and Bamezai RN. Human pyruvate kinase M2: a multifunctional protein. Protein Sci 2010, 19: 2031–2044.

40 Christofk HR, Vander Heiden MG, Harris MH, Ramanathan A, Gerszten RE, Wei R and Fleming MD, *et al*. The M2 splice isoform of pyruvate kinase is important for cancer metabolism and tumour growth. Nature 2008, 452: 230–233.

41 Christofk HR, Vander Heiden MG, Wu N, Asara JM and Cantley LC. Pyruvate kinase M2 is a phosphotyrosine-binding protein. Nature 2008, 452: 181–186.

42 Bluemlein K, Grüning NM, Feichtinger RG, Lehrach H, Kofler B and Ralser M. No evidence for a shift in pyruvate kinase PKM1 to PKM2 expression during tumorigenesis. Oncotarget 2011, 2: 393–400.

43 Anastasiou D, Yu Y, Israelsen WJ, Jiang JK, Boxer MB, Hong BS and Tempel W, *et al*. Pyruvate kinase M2 activators promote tetramer formation and suppress tumorigenesis. Nat Chem Biol 2012, doi: 10.1038/nchembio.1060 [Epub ahead of print].

44 Hitosugi T, Kang SK, Heiden MGV, Chung TW, Elf S, Lythgoe K and Dong SZ, *et al*. Tyrosine phosphorylation inhibits PKM2 to promote the Warburg effect and tumor growth. Sci Signal 2009, 2: ra73.

45 Lv L, Li D, Zhao D, Lin R, Chu Y, Zhang H and Zha Z, *et al*. Acetylation targets the M2 isoform of pyruvate kinase for degradation through chaperone-mediated autophagy and promotes tumor growth. Mol Cell 2011, 42: 719–730.

46 Anastasiou D, Poulogiannis G, Asara JM, Boxer MB, Jiang JK, Shen M and Bellinger G, *et al*. Inhibition of pyruvate kinase M2 by reactive oxygen species contributes to cellular antioxidant responses. Science 2011, 334: 1278–1283.

47 Luo W, Hu H, Chang R, Zhong J, Knabel M, O'Meally R and Cole RN, *et al*. Pyruvate kinase M2 is a PHD3-stimulated coactivator for hypoxia-inducible factor 1. Cell 2011, 145: 732–744.

48 Yang W, Xia Y, Ji H, Zheng Y, Liang J, Huang W and Gao X, *et al*. Nuclear PKM2 regulates beta-catenin transactivation upon EGFR activation. Nature 2011, 480: 118–122.

49 Yang W, Xia Y, Hawke D, Li X, Liang J, Xing D and Aldape K, *et al*. PKM2 phosphorylates histone H3 and promotes gene transcription and tumorigenesis. Cell 2012, 150: 685–696.

50 Vousden KH and Ryan KM. p53 and metabolism. Nat Rev Cancer 2009, 9: 691–700.

51 Bensaad K, Cheung EC and Vousden KH. Modulation of intracellular ROS levels by TIGAR controls autophagy. EMBO J 2009, 28: 3015–3026.

52 Nakano K, Balint E, Ashcroft M and Vousden KH. A ribonucleotide reductase gene is a transcriptional target of p53 and p73. Oncogene 2000, 19: 4283–4289.

53 Polyak K, Xia Y, Zweier JL, Kinzler KW and Vogelstein B. A model for p53-induced apoptosis. Nature 1997, 389: 300–305.

54 Chen K, Albano A, Ho A and Keaney JF, Jr. Activation of p53 by oxidative stress involves platelet-derived growth factor-beta receptor-mediated ataxia telangiectasia mutated (ATM) kinase activation. J Biol Chem 2003, 278: 39527–39533.

55 Schwartzenberg-Bar-Yoseph F, Armoni M and Karnieli E. The tumor suppressor p53 down-regulates glucose transporters GLUT1 and GLUT4 gene expression. Cancer Res 2004, 64: 2627–2633.

56 Bensaad K, Tsuruta A, Selak MA, Vidal MN, Nakano K, Bartrons R and Gottlieb E, *et al*. TIGAR, a p53-inducible regulator of glycolysis and apoptosis. Cell 2006, 126: 107–120.

57 Okar DA, Manzano A, Navarro-Sabatè A, Riera L, Bartrons R and Lange AJ. PFK-2/FBPase-2: maker and breaker of the essential biofactor fructose-2,6-bisphosphate. Trends Biochem Sci 2001, 26: 30–35.

58 Matoba S, Kang JG, Patino WD, Wragg A, Boehm M, Gavrilova O and Hurley PJ, *et al*. p53 regulates mitochondrial respiration. Science 2006, 312: 1650–1653.

59 Dang CV, Kim JW, Gao P and Yustein J. The interplay between MYC and HIF in cancer. Nat Rev Cancer 2008, 8: 51–56.

60 Dang CV. MYC on the path to cancer. Cell 2012, 149: 22–35.

61 Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T and Okita K, *et al*. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. Nat Biotechnol 2008, 26: 101–106.

62 Kaadige MR, Elgort MG and Ayer DE. Coordination of glucose and glutamine utilization by an expanded Myc network. Transcription 2010, 1: 36–40.

63 David CJ, Chen M, Assanah M, Canoll P and Manley JL. HnRNP proteins controlled by c-Myc deregulate pyruvate kinase mRNA splicing in cancer. Nature 2010, 463: 364–368.

64 Shim H, Dolde C, Lewis BC, Wu CS, Dang G, Jungmann RA and Dalla-Favera R, *et al*. c-Myc transactivation of LDH-A: implications for tumor metabolism and growth. Proc Natl Acad Sci USA 1997, 94: 6658–6663.

65 Reitzer LJ, Wice BM and Kennell D. Evidence that glutamine, not sugar, is the major energy source for cultured HeLa cells. J Biol Chem 1979, 254: 2669–2676.

66 McKeehan WL. Glycolysis, glutaminolysis and cell proliferation. Cell Biol Int Rep 1982, 6: 635–650.

67 Gao P, Tchernyshyov I, Chang TC, Lee YS, Kita K, Ochi T and Zeller KI, *et al*. c-Myc suppression of miR-23a/b enhances mitochondrial glutaminase expression and glutamine metabolism. Nature 2009, 458: 762–765.

68 Ichimura K, Pearson DM, Kocialkowski S, Bäcklund LM, Chan R, Jones DT and Collins VP. IDH1 mutations are present in the majority of common adult gliomas but rare in primary glioblastomas. Neuro Oncol 2009, 11: 341–347.