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The Crabtree and Warburg effects: Do metabolite-induced regulations participate in their induction?☆

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ABSTRACT

The Crabtree and Warburg effects are two well-known deviations of cell energy metabolism that will be described herein. A number of hypotheses have been formulated regarding the molecular mechanisms leading to these cellular energy metabolism deviations. In this review, we will focus on the emerging notion that metabolite-induced regulations participate in the induction of these effects. All throughout this review, it should be kept in mind that no regulatory mechanism is exclusive and that it may vary in cancer cells owing to different cell types or oncogenic background. This article is part of a Special Issue entitled 'EBEC 2016: 19th European Bioenergetics Conference, Riva del Garda, Italy, July 2–6, 2016', edited by Prof. Paolo Bernardi.

1. The Crabtree effect [1]

A careful review of the recent literature did not show that this field has much evolved since our previous review [1]. Consequently, the paragraph below is largely taken from our previous work [1] with permission.

The Crabtree effect is defined as the glucose-induced repression of respiratory flux [2]. The addition of external glucose to Crabtree-sensitive cells triggers in a few seconds the partial inhibition of O_2 consumption, which discards the involvement of gene expression and *de novo* protein synthesis. Even though a number of hypotheses have been formulated, its triggering mechanisms are still unknown. It is also possible that its induction is due to a combination of several factors [3]. One of the most accepted hypothesis is that two glycolysis enzymes (phosphoglycerate kinase and pyruvate kinase) and mitochondria compete for free cytosolic ADP [3,4]. If glycolysis is overactive it could, in theory, override mitochondria regarding ADP uptake. As the latter is one of the substrates of oxidative phosphorylation, this would limit one of the substrates of the ATP synthase and consequently respiration would be decreased. Nonetheless, it is unlikely that this could occur *in vivo* as the Km for the mitochondrial adenine nucleotide translocase (ANT) is almost 100-times lower than that of the glycolysis enzymes [5]. This led authors to conclude that mitochondria would still use cytosolic ADP even if the glycolysis enzymes increase their activity. However, it should be stressed here that glycolysis enzymes use Mg^{2+}-chelated nucleotides as substrates whereas the actual substrates for ANT are non-chelated nucleotides. Thus this eventual competition for free ADP between glycolysis and mitochondria will highly depend on the ratio between chelated and non-chelated ADP.

The Crabtree effect on some tumor cells could be eliminated by adding an excess of phosphate (Pi) *in vitro*. It thus has been proposed that a decrease in Pi was the actual trigger of this metabolic phenomenon [6]. This seems in accordance with the dramatic decrease in Pi levels observed after glucose addition in tumor cells [3]. The thermodynamic phosphate potential (*i.e.* [ATP/ADP·Pi]) [5], may be crucial during the

Abbreviations: 2HG, 2-hydroxyglutarate; 6PGD, 6-phosphogluconate dehydrogenase; ACC, acetyl-CoA carboxylase; ANT, adenine nucleotide translocase; ADP, adenosine diphosphate; AMP, adenosine monophosphate; AMPK, AMPKinase; ATP, adenosine triphosphate; Fru6P, fructose 6 phosphate; Fru1,6BP, fructose 1,6-biphosphate; FH, fumarate hydratase; G6PDH, glucose 6 phosphate dehydrogenase; Glc6P, glucose 6 phosphate; HIF, hypoxia inducible factor; Hxk2, type 2 hexokinase; IDH, isocitrate dehydrogenase; LKB1, tumor suppressor liver kinase B1; PKM2, M2 isoform of pyruvate kinase; MO25, scaffolding-like adaptor protein mouse protein 25; MPC, mitochondrial pyruvate carrier; NADPH, nicotinamide adenine nucleotide phosphate reduced form; PPP, oxidative pentose phosphate pathway; PTP, permeability transition pore; PKM1, M1 isoform of pyruvate kinase; PHDs, prolyl hydroxylase enzymes; Ru-5-P, Ribulose-5-phosphate; ROS, reactive oxygen species; STRAD, pseudokinase Ste20-related adaptor; SDH, succinate dehydrogenase; TCA, tricarboxylic acid cycle.

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Crabtree effect [7], changes in this parameter have been detected in response to glucose addition to sarcoma ascites tumor cells [7]. Further, Ca^{2+} has been proposed to be involved in the induction of the Crabtree effect. Cytosolic Ca^{2+} levels could increase depending on physiological conditions and in response to specific stimuli. One study showed an increased mitochondrial Ca^{2+} uptake in response to glucose [8]. In these conditions, this cation inhibited the mitochondrial ATP synthase inducing a decrease of respiration [9]. However, this could not be taken as a common mechanism for Crabtree-positive cells as (i) Ca^{2+} levels were shown to be constant in response to glucose in an hepatoma cell line [3] (ii) *Saccharomyces cerevisiae* cells are well known to lack a mitochondrial Ca^{2+} uniporter [10]. Another proposed mechanism is the permeability of the mitochondrial outer membrane through the porin channel. Indeed this channel regulates the access of substrates to the intermembrane space and thus it could regulate oxidative phosphorylation [11]. If ADP or the respiratory substrates were kept in the cytoplasm this would induce a decreased respiratory flux. This possibility has been overlooked, although it has been suggested that respiratory substrate availability may decrease upon induction of the Crabtree effect [12]. However, it has also been shown that in normal adult cardiomyocytes and HL-1 cardiac cell line, intracellular local restrictions of diffusion of adenine nucleotides and metabolic feedback regulation of respiration *via* phosphotransfer networks are different, most probably related to differences in structural organization of these cells [13]. Contrary to cardiomyocytes where mitochondria and CaMgATPases are organized into tight complexes which ensure effective energy transfer and feedback signaling mediated by Creatine Kinase and Adenylate Kinase isoforms, in HL-1 cells energy metabolism is less organized [14]. In these cells the permeability of the outer membrane for ADP and other substrates is increased and the mitochondrial compartment is very dynamic leading to an increase in ATP consumption [15,16].

Our laboratory has shown that one metabolite that links glycolysis to the inhibition of respiration is fructose 1,6-biphosphate (Fru1,6BP) [17]. Indeed, we have shown that three glycolysis hexoses phosphates, namely Glc6P, Fru6P and Fru1,6BP regulate the respiratory flux. At low, physiological concentrations, whereas Glc6P and Fru6P slightly stimulated the respiratory flux, Fru1,6BP inhibits mitochondrial respiratory rate at the level of respiratory complexes III and IV. Moreover, when added in the presence of Glc6P, Fru1,6BP strongly antagonized the Glc6P-induced increase in respiration. In permeabilized spheroplasts and at physiological concentrations of hexoses phosphates, the only observed effect was an inhibition of respiration by Fru1,6BP. The Fru1,6BP mediated inhibition of mitochondrial respiration was observed in mitochondria isolated from Crabtree positive yeast whereas no inhibition was observed in mitochondria isolated from Crabtree negative yeast. Last but not least, we were able to show that this inhibition is observed in isolated rat liver mitochondria, which shows that this process is conserved and extends to mammalian cells. Altogether, these results led us to propose that Fru1,6BP participates in the establishment of the Crabtree effect. This regulation points to the fact that an impairment of mitochondrial function is not a prerequisite for Crabtree effect induction since the same decrease of respiration caused by Fru1,6BP can be observed in mitochondria obtained from a non-tumor source. In addition a protective role of Fru1,6BP was observed in the brain [18], liver and heart in ischemia reperfusion injury [19] and in hypoxic astrocytes [20]. In these studies the authors proposed that Fru1,6BP could be metabolized and buffer the ATP ratio, as well as decrease the ROS production through activation of G6PDH, which increases the levels of NADPH leading to an increase in reduced glutathione ratio (Fig. 1). Further studies are needed in order to know if these effects are related with an inhibition of respiration. Recently it has been shown that Fru1,6BP inhibits the permeability transition pore (PTP) in *S. cerevisiae* [21], but its influence on the PTP of mammalian cells has not yet been investigated. Last, an antiapoptotic effect of Fru1,6BP through NO overproduction was proposed [22]. Glc6P is another hexose phosphate that seems to have an impact on mitochondrial function. The presence of this hexose-monophosphate is important for the release of mitochondria-bound hexokinase and to enhance Bax-mediated release of cytochrome c [23]. The detachment of mitochondrial Hxk2 by Glc6P overproduction may play an important role in the promotion of apoptosis and respiration [24]. In addition it was proposed that in oocytes of *Xenopus laevis*, Glc6P inhibits apoptotic signals through overproduction of NADPH, which prevents the activation of caspase II [25].

As a conclusion, from the above-mentioned data (Fig. 1), we propose that the participation of hexose phosphates as metabolic messengers and their influence on tumor cell oxidative metabolism needs to be further evaluated.

2. The Warburg effect

The Warburg effect in cancer cells may require several hours or even days to develop, and hence transcriptional/translational regulations are necessarily involved. However, we will focus here on what seems to be an overlooked mechanism involved in the phenotypic expression of this effect, which is metabolite-induced regulations.

In order to proliferate, cells must comply with the energy demand imposed by vital processes such as macromolecule biosynthesis, DNA replication, ion gradients generation and cell structure maintenance. Mitochondria play an important role in the response to this energy demand as they synthesize most of the cellular ATP through oxidative phosphorylation. However, it was observed early on by Otto Warburg that cancer cells exhibit a decreased mitochondrial oxidative metabolism [26]. The early discoveries from O. Warburg pointed out that cancer cells display a decreased respiration along with an enhanced lactate production, whose respective rates correlate with the increase in cellular proliferation *i.e.* the faster the cell proliferate the higher the lactate production rate, suggesting that cancer cells depend mainly on fermentative metabolism for ATP generation [26]. Despite the decrease in energy yield as a consequence of the "glycolytic phenotype" this seems to allow an increase in cell proliferation rate and be applicable to other fast growing cells [27]. Because the repression of oxidative metabolism occurs even in the presence of oxygen, this metabolic phenomenon is known as "aerobic glycolysis" or the "Warburg effect". The specific advantages that cancer cells acquire by undergoing this metabolic switch have been suggested to be an acceleration through anabolic pathways, most of which arise from glycolysis intermediates. It is also possible that cells use this mechanism in order to proliferate in hypoxic environments, such as conditions prevailing within solid tumors [28]. A correlation between the glycolytic phenotype and tumor invasiveness has also been suggested [4]. Nonetheless, there is a considerable body of evidence that challenges the paradigm of the purely "glycolytic" cancer cell [29]. It has been demonstrated that some glioma, hepatoma and breast cancer cell lines possess functional mitochondria and that they obtain their ATP mainly from oxidative phosphorylation [30,31,32,33]. Moreover, it has been demonstrated that some cancer cells can reversibly switch between fermentation and oxidative metabolism, depending on the absence (where glucose is replaced by galactose) or the presence of glucose and the environmental conditions [34,35,36]. This suggests that the metabolic plasticity observed *in vitro* may have an impact on tumor physiology *in vivo*. Therefore, it is crucial to understand the molecular mechanisms by which cancer cells can reversibly regulate their energy metabolism.

A major mitochondrial metabolic pathway that is modified in cancer cells is glutamine metabolism. Glutamine is the most abundant free alpha-amino acid in plasma (0.6–0.9 mmol/l) and skeletal muscle. This nutrient plays an important role in regulating gene expression, protein turnover, anti-oxidative function, nutrient metabolism, and acid–base balance. In proliferating cells, where anabolic pathways are very active, the tricarboxylic acid (TCA) cycle furnishes intermediates for biosynthesis pathways [37]. In this scheme, glutamine plays a major anaplerotic role since its degradation to α-ketoglutarate allows the maintenance of intermediates in the TCA cycle [38]. Moreover, it has

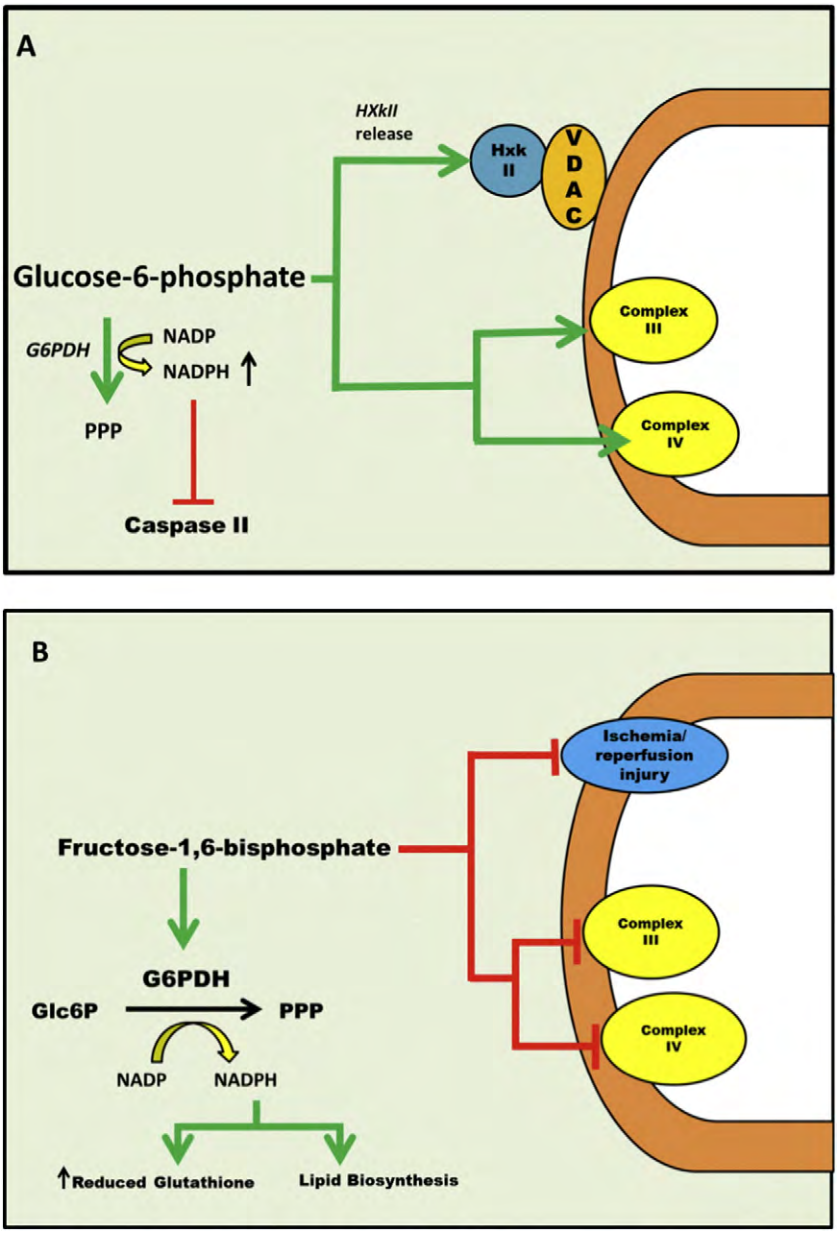

Fig. 1. (A) scheme illustrating the glucose 6 phosphate-induced regulations on mitochondria and PPP. Physiological concentrations of glucose 6-phosphate stimulate the respiratory flux. Glucose 6 phosphate is important for the release of mitochondria-bound hexokinase and to enhance Bax-mediated release of cytochrome c. In oocytes of *Xenopus laevis*, G6P inhibits apoptotic signals through overproduction of NADPH, which prevents the activation of caspase II (B) scheme illustrating the fructose-1,6-bisphosphate-induced regulations on metabolism. PPP: pentose phosphate pathway, G6PDH, glucose-6-phosphatedehydrogenase. Fru1,6BP inhibits mitochondrial respiration. Fru16BP has a protective role in ischemia reperfusion injury.

been shown that NADPH levels can be a controlling factor for cell proliferation [39]. Using ^{13}C NMR spectroscopy, DeBerardinis et al. [37] studied the metabolism of glioblastoma cells that exhibit aerobic glycolysis. They were able to show that in these cells, the TCA cycle was active and that intermediates of this cycle were used in biosynthetic pathways such as fatty acid synthesis. Moreover, they clearly showed that this process, that requires both NADPH and oxaloacetate, was ascribable to a high rate of glutamine metabolism. [3-^{13}C] glutamine consumption (glutaminolysis) showed oxidation of glutamine-derived malate by malic enzyme, which produces NADPH. Moreover, they were able to show that the glutaminolytic flux was at least as high as the G6PGH flux (the other major source of NADPH). Since this flux is higher than what is needed for fatty acid synthesis, they proposed that glutaminolysis flux is enough to provide the NADPH required for fatty acid synthesis. Moreover, in their conditions, anaplerotic acetate is derived from glutamine. In conclusion, their ^{13}C NMR study clearly shows that glutamine catabolism provides a carbon source that supports cell proliferation. Glutamine can also be the main source of acetyl-coA and citrate in proliferating cells when pyruvate transport in the mitochondria is reduced. Indeed, pyruvate enters the mitochondrial matrix through to the MPC carrier [40,41]. This carrier is a heterodimer composed of two subunits: MPC1 and MPC2. Whenever one of these subunits is dysfunctional, matricial citrate decreases [40,41]. This decrease can be reversed by synthesis of acetyl-CoA and citrate from glutamine [37]. Consequently, it should be taken in account that (i) glutamine metabolism might be a significant source of Krebs cycle intermediates in cells proliferating at a high rate (ii) lactate might not only arise from glucose metabolism but also from glutamine metabolism, thus, lactate production flux is not necessarily representative of the flux through glycolysis.

As stated above, cancer cells mainly depend on fermentative metabolism for ATP generation. This occurs even in the presence of oxygen (the Warburg effect). Below, we will review the metabolite-induced regulations that lead to a modulation of fermentative metabolism to achieve ATP production. First, it has been shown that the M2 isoform of pyruvate kinase (PKM2) is positively regulated by serine. Consequently, in the presence of high levels of serine (that is necessary for biomass production), PKM2 is fully active and glycolysis rate is high –a hallmark of the Warburg effect-. Moreover, the activity of the master regulator of cell energy homeostasis, AMPK is negatively regulated by ribulose 5P which diverts carbons away from glycolysis – "a counter Warburg effect"-. Phosphofructokinase, that is a major glycolysis controlling enzyme in non-transformed cells is expressed and modulated in such a way in cancer cells that its allosteric inhibition by citrate is alleviated. This allows an increase in the flux through this enzyme, a decrease in its control over the glycolytic flux and thus an increase in glycolytic rate –a hallmark of the Warburg effect-. Last, oncometabolites have been shown to inhibit competitively α-ketoglutarate dependent dehydrogenases. This leads to a stabilization of the transcription factor HIF1α and induces the changes in gene expression in response to hypoxia (regarding energy metabolism: mainly an increase in glycolysis and a decrease in oxidative phosphorylation). Consequently the build-up of these metabolites elicits a hypoxia-like response in the presence of oxygen –the Warburg effect-.

2.1. Pyruvate kinase M2 is allosterically activated by metabolic intermediates [42]

Pyruvate kinase catalyzes the final step of glycolysis, the synthesis of ATP and pyruvate from phosphoenol pyruvate and ADP. In mammals, there are four PK isoforms (PKM1, PKM2, PKL — liver, and PKR — red blood cells). PKM1 is present in many terminally differentiated tissues, whereas PKM2 is present in tissues with anabolic functions, proliferating cells, and cancer cells. It has been shown that cancer cells engineered to express PKM1 fail to support tumor growth [39,43]. However, a conflicting report shows that M2 isoform of pyruvate kinase is dispensable for tumor maintenance and growth [44]. PKM1 and PKM2 are alternative splice isoforms of the Pykm gene and differ only by the inclusion of one mutually exclusive exon. Despite their substantial sequence similarity, PKM1 and PKM2 have very different catalytic and regulatory properties. PKM1 has constitutively high catalytic activity whereas PKM2 activity is subject to complex allosteric regulation. Thus, pyruvate kinase catalyzes a step in glucose metabolism that can be crucial for controlling cell proliferation. The regulation of PKM2 allows cells access to the ATP producing function of the enzyme when it is in the active state and allows the use of glycolysis to support biosynthesis when in the off state (increase in glycolysis intermediates concentrations). PKM1 is constitutively active because it forms a stable tetramer whereas PKM2 can exist either as an active tetramer or an inactive non-tetramer.

Several post translational modifications of PKM2 have been described [45]. However, we will focus here on metabolite-induced modifications. A well-known activator of PKM2 is the glycolytic intermediate fructose 1,6 biphosphate (Fru1,6BP) that promotes the tetramerization of the enzyme in its active conformation. Consequently, PMK2 exists in a monomer–tetramer equilibrium that can be altered by the presence of Fru1,6BP. PKM2 activators may impair tumor cell proliferation by interfering with anabolic metabolism indeed, activator treatment *in vitro* and *in vivo* results in decreased pools of ribose-phosphate and serine, which are key precursors for nucleotide and amino acid metabolism [43]. Consequently, an activation of PKM2 through Fru1,6BP alleviates the building up of glycolytic intermediates and their engulfment in anabolic pathways.

The amino acid L-serine, one of the so-called non-essential amino acids, plays a central role in cellular proliferation. L-Serine is the predominant source of one-carbon groups for *de novo* synthesis of purine nucleotides and deoxythymidine monophosphate. Its metabolism is an anabolic pathway required for growth and proliferation [46]. It recruits carbon away from the energy production pathway of glucose utilization. While investigating the relationship between glycolysis and the serine biosynthesis pathway (that arises from glycolysis), Chaneton et al. [47] were able to show that serine directly activates PKM2, independently of Fru1,6BP mediated activation. This activation occurs within the physiological range of serine concentration. Further, this activation is specific of PKM2 since PKM1, that exhibits a high basal activity is refractory to both serine and Fru1,6BP activation. When serine is abundant PKM2 is fully active enabling the maximal use of glucose through glycolysis and when the steady state level of serine drops an attenuation of PKM2 activity occurs which redirects glucose derived carbons back in the serine biosynthetic pathway (Fig. 2).

Last, it was shown that SAICAR (succinylaminoimidazolecarboxamide ribose-5′-phosphate, an intermediate of the *de novo* purine nucleotide synthesis pathway) specifically stimulates PKM2 [48]. Using liquid-chromatography mass spectrometry, the authors were able to show that SAICAR is a metabolite that interacts with PKM2. Moreover, assessment of PKM2 *in vitro* activity in the presence or absence of SAICAR showed that the binding of SAICAR to this enzyme activates it in such a way that its activity became close to that of PKM1. They were able to show that this regulation has an *in vivo* relevance since in cancer cells, metabolic flux and cellular energy balance were altered by changes in SAICAR concentration.

2.2. Role of Ru-5-P concentration in the balance between fatty acid degradation and synthesis through AMPK inactivation; link between the pentose-phosphate pathway and lipogenesis

In cancer cells, the high activity of aerobic glycolysis is associated to a rise in different intermediates of this pathway leading to an increase in some precursors for anabolic biosynthesis of macromolecules that allow rapid cell proliferation and an activation of the oxidative pentose phosphate pathway (PPP) – through glucose-6-phosphate – that produces ribose-5-phosphate (a precursor for nucleotide synthesis) and NADPH which is required for biosynthesis of lipids, appropriate redox status and antioxidant defense (Fig. 1A). Thus PPP plays an important role in the metabolic reorganization that favors tumor cell proliferation and disease development. A molecular mechanism that links PPP activation and the increase in lipogenesis has been recently evidenced [49]. At high concentration, Ribulose-5-phosphate (Ru-5-P) produced by the 6-phosphogluconate dehydrogenase (6PGD) disrupts the association between LKB1 (tumor suppressor liver kinase B1), MO25 (scaffolding-like adaptor protein mouse protein 25) and STRAD (pseudokinase Ste20-related adaptor) that is required for full AMPK (AMPKinase) activation. Such a decrease in AMPK activity leads to an increase in fatty-acid and cholesterol synthesis and inhibition of two main metabolic enzymes that participate in fatty-acids degradation *i.e.* acetyl-CoA carboxylase (ACC) 1 and 2. The above-mentioned study suggests that 6PGD provides an additional link between the oxidative PPP and lipogenesis through Ru-5-P-dependent inhibition of LKB1/AMPK signaling. Although it was shown in that study that the intracellular level of Ru-5-P decreased in 6PGD knockdown cells, which suggests an important role for 6PGD in maintaining the intracellular Ru-5-P level that cannot be compensated by other pathways. This does not exclude the potential contribution of non-oxidative PPP in maintaining physiological Ru5-P levels to regulate lipogenesis. In addition, these findings regarding a commonly important role of 6PGD in the oxidative PPP in cancer cells are different from the previous report using H1975 lung cancer cells suggesting that 6PGD functions may vary in cancer cells owing to different cell types or oncogenic background [50].

2.3. Regulation of phosphofructokinase (PFK) activity by citrate and fructose 2,6-biphosphate (Fru2,6BP)

In the carbohydrate catabolism pathway, PFK is one of the most regulated enzymes and it has been shown that, in different types of cancer,

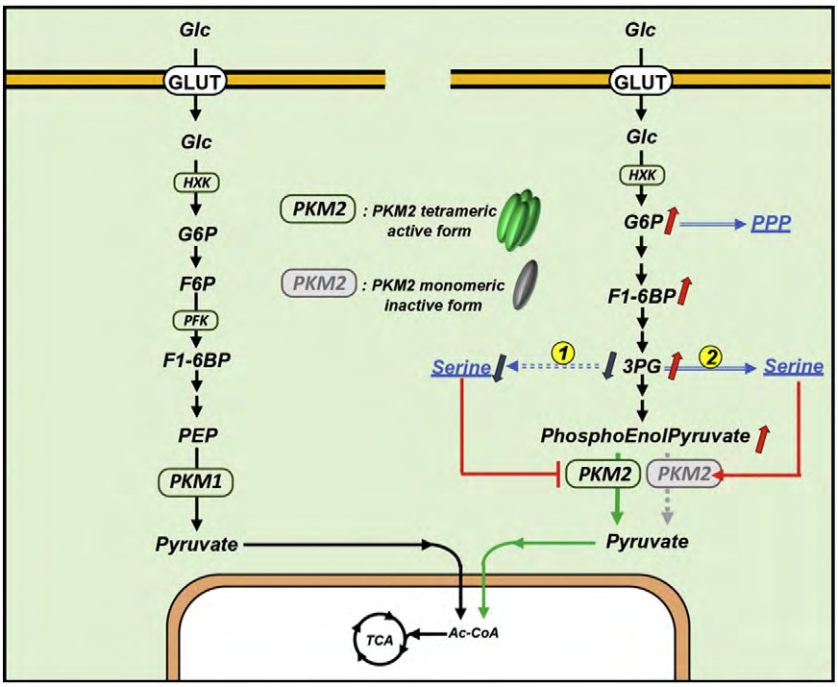

Fig. 2. Scheme illustrating the PKM regulations and the consequences of these regulations on anabolic pathways. PKM1 has constitutively high catalytic activity. PKM2 activity is subject to allosteric regulation. PKM1 is constitutively active because it forms a stable tetramer whereas PKM2 can exist either as an active tetramer or an inactive non-tetramer. When serine is abundant PKM2 is fully active enabling the maximal use of glucose through glycolysis. When the steady state level of serine drops an attenuation of PKM2 activity occurs which redirects glucose-derived carbons back in the serine biosynthetic pathway.

the rate of glycolysis is, at least in part, determined by PFK activity. The allosteric regulation of PFK is essentially due to 5 metabolites: AMP, ADP and Fru2,6BP are activators while ATP and citrate are inhibitors. In non-transformed cells one of the most important regulation concerns the allosteric response of PFK activity to citrate, a metabolite that is at the crossroad of the Krebs cycle and lipid metabolism. Increase in this metabolite concentration strongly inhibits glycolysis through PFK inhibition and favors fatty acid beta-oxidation. In many kinds of tumors, it has been shown that PFK allosteric regulation is largely altered in such a way that this enzyme is more sensitive to Fru2,6BP and less sensitive to citrate. Indeed, in mammalian cells, PFK is an homo or hetero-tetramer of L, M and C isoforms which are differently expressed in various tissues *i.e.* L is the main isoform expressed in liver and kidney, M is the sole isoform found in skeletal muscle while C is predominantly expressed is platelets. These isoforms possess different kinetic properties, particularly concerning the sensitivity towards allosteric effectors (for a detailed description of these kinetic properties of PFK isoforms, see [51]. For instance, the M isoform is the most sensitive to Fru2,6BP and the L isoform is the less sensitive to the citrate induced-inhibition. In cancer cells, the over-expressed isoforms are preferentially the ones for which the sensitivity to citrate is low (L isoform essentially). It has been proposed that in some human gliomas this L isoform may be specifically phosphorylated [52]. However this phosphorylation has not been associated to any significant modification in the kinetic properties of PFK.

Another process leading to a large decrease in PFK sensitivity to ATP and citrate and to an increase in Fru2,6BP induced PFK activation has been described in a tumor tissue developed in mice after subcutaneous infection with tumorigenic B16-F10 cells. In this case, a posttranslational modification of M isoform of PFK induced by proteolysis leads to a short fragment (47 kD instead 87 kD) in which the regulatory properties are changed: its activity is insensitive to citrate, and compared to the native isoform it presents a low sensitivity to ATP and is strongly activated by Fru2,6BP [53]. Such regulations of PFK activity in rapidly proliferating cells allow an increase in the flux through glycolysis. Indeed, it has been shown that the regulations of PFK in cancer cells are such that this enzyme does not exert significant control over the glycolytic flux [54,55].

2.4. Oncometabolites and their role in metabolism deviation

The term oncometabolite has recently been coined and assigned with confidence to 2-hydroxyglutarate, the reduced form of 2-oxoglutarate (2OG). An oncometabolite is a small molecule component (or enantiomer) of normal metabolism whose accumulation causes metabolic dysregulation and consequently primes cells allowing future progression to cancer. We will here focus on the dysregulations induced by these oncometabolites that arise from the Krebs cycle.

2.4.1. α-ketoglutarate (αKG)-dependent dioxygenases

α-ketoglutarate (αKG)-dependent dioxygenases are important modulators of both the oxygen-sensing machinery and the epigenome. Regarding the oxygen-sensing machinery, the majority of the changes in gene expression observed in response to hypoxia are due to HIFs, heterodimeric transcription factors that are tightly linked to tumorigenesis [56]. HIF transcription factors consist of an α- and a β-subunit that are constitutively expressed. However, under normoxia the α-subunit is highly labile [57], [58] and the rapid degradation of HIFα is regulated by oxygen-dependent prolyl hydroxylase enzymes (PHDs), which hydroxylate two defined prolyl residues in its oxygen-dependent degradation (ODD) domain. This in turn recruits an E3 ubiquitin ligase complex containing pVHL, resulting in HIFα ubiquitylation and degradation by the proteasome. Consequently, any dysregulation of the hydroxylation process would lead to a stabilization of HIF. Recently, metabolic changes driven by mutations in genes related to the TCA cycle, have indicated an alternative role, that of the 'oncometabolite' [59]. A particular metabolite builds up within the cell and contributes to the tumorigenic process. Three enzymes of the TCA cycle have been shown to be involved in this process: fumarate hydratase, succinate dehydrogenase and isocitrate dehydrogenase. Mutations in fumarate hydratase (FH) and succinate dehydrogenase (SDH) subunits lead to loss of gene function, and

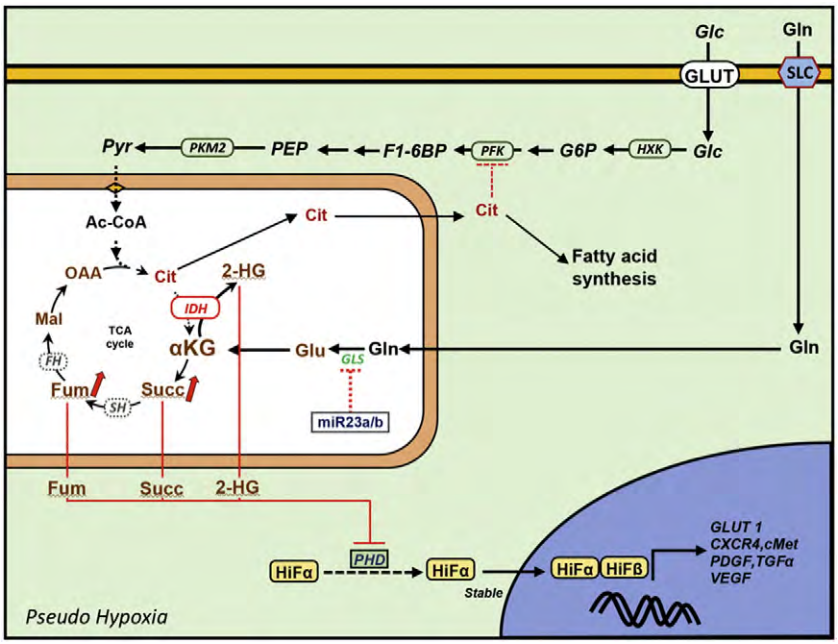

Fig. 3. Scheme illustrating the regulations induced by oncometabolites. The majority of the changes in gene expression observed in response to hypoxia are due to HIFs, heterodimeric transcription factors that are tightly linked to tumorigenesis. The rapid degradation of HIFα is down-regulated by the competitive inhibition of oxygen-dependent prolyl hydroxylase enzymes (PHDs) by the three oncometabolites succinate, fumarate and 2-HG. The build up of these metabolites is due to mutations in fumarate hydratase, succinate dehydrogenase and isocitrate dehydrogenase. The inhibition of these enzymes leads to the stabilization of HIF1α through a decrease in its hydroxylation rate and induce the majority of the changes in gene expression observed in response to hypoxia. This allows a "hypoxia-like" cellular response even in the presence of oxygen.

accumulation of the substrates fumarate and succinate, respectively. Conversely, for isocitrate dehydrogenase (IDH), a single-allele mutation confers a gain of function, producing an excess of a new metabolite, 2-hydroxyglutarate (2HG) [59]. These three oncometabolites inhibit competitively the α-ketoglutarate (αKG)-dependent dioxygenases. Succinate and fumarate, which are structurally similar, inhibit these enzymes through product inhibition, since it has been shown that the effects of both metabolites can be reversed by the addition of excess αKG *in vitro* and *in vivo* [60]. 2HG is structurally very similar to αKG and glutamate, the only difference is the presence of a hydroxyl group instead of a ketone or amine group, respectively. It is this similarity that results in the competitive inhibition of αKG-dependent dioxygenases by 2HG, as it occupies the same binding site as αKG [61]. As stated above, the inhibition of these enzymes leads to the stabilization of HIF1α through a decrease in its hydroxylation rate and induce the majority of the changes in gene expression observed in response to hypoxia. This allows a "hypoxia-like" cellular response even in the presence of oxygen (Fig. 3).

2.4.2. Histone methylation [62]

Recent studies have shown that Krebs cycle intermediates can shape the epigenetic landscape of chromatin by regulating DNA and histone methylation. α-ketoglutarate is a key metabolite in the Krebs cycle and is also an obligatory substrate for α-oxoglutarate-dependent dioxygenases. This enzyme family includes the major enzymes of DNA and histone demethylation, *i.e.* Ten-Eleven Translocation and Jumonji C domain containing demethylases. As stated above, succinate and fumarate, are potent inhibitors of α-ketoglutarate (αKG)-dependent dioxygenases enzymes, *i.e.* the balance of the Krebs cycle reactions can affect the level of DNA and histone methylation and thus control gene expression.

3. Conclusion

Metabolites *i.e.*, the intermediaries from diverse metabolic pathways, are demonstrating their role as metabolic messengers that regulate the activity of diverse enzymes and impinge on the half-life of enzymes. This concept opens a whole new field on the control of cell survival and metabolism.

Transparency Document

The Transparency document associated with this article can be found in online version.

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