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Review

The Warburg and Crabtree effects: On the origin of cancer cell energy metabolism and of yeast glucose repression☆

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ABSTRACT

During the last decades a considerable amount of research has been focused on cancer. Recently, tumor cell metabolism has been considered as a possible target for cancer therapy. It is widely accepted that tumors display enhanced glycolytic activity and impaired oxidative phosphorylation (Warburg effect). Therefore, it seems reasonable that disruption of glycolysis might be a promising candidate for specific anti-cancer therapy. Nevertheless, the concept of aerobic glycolysis as the paradigm of tumor cell metabolism has been challenged, as some tumor cells exhibit high rates of oxidative phosphorylation. Mitochondrial physiology in cancer cells is linked to the Warburg effect. Besides, its central role in apoptosis makes this organelle a promising "dual hit target" to selectively eliminate tumor cells. From a metabolic point of view, the fermenting yeast *Saccharomyces cerevisiae* and tumor cells share several features. In this paper we will review these common metabolic properties as well as the possible origins of the Crabtree and Warburg effects. This article is part of a Special Issue entitled: Bioenergetics of Cancer.

1. The hallmarks of cancer cell energy metabolism: The Warburg effect and the Crabtree effect

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 energy metabolism as they synthesize most of the cellular ATP through oxidative phosphorylation. However, it was suggested that cancer cells suppress mitochondrial metabolism [1]. The early discoveries from O. Warburg pointed out that cancer cells display a decreased respiration along with an enhanced lactate production, suggesting that they depend mainly on fermentative metabolism for ATP generation [1]. In spite of 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 [2].

Because the repression of oxidative metabolism occurs even in the presence of oxygen, this metabolic phenomenon is known as "aerobic glycolysis", also known as the "Warburg effect". The specific advantages that cancer cells acquire by undergoing this metabolic switch are unknown. Although it is possible that these cells use this mechanism in order to proliferate in hypoxic environments, such as conditions prevailing within solid tumors [3]. Another hypothesis is that the down-regulation of oxidative metabolism could help these cells to escape from apoptosis [4–6]. A correlation between the glycolytic phenotype and tumor invasiveness has also been suggested [7].

Nonetheless, there is a considerable body of evidence that challenges the paradigm of the purely "glycolytic" cancer cell [8]. 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 [9–12]. Moreover, it has been demonstrated that some cancer cells can reversibly switch between fermentation and oxidative metabolism, depending on the absence or the presence of glucose and the environmental conditions [13–15]. Interestingly, a recent model proposed that "glycolytic" cells could establish a metabolic symbiosis with the "oxidative" ones through lactate shuttling [16]. This points out that the metabolic plasticity observed *in vitro* may have an impact on tumor physiology *in vivo*. Therefore, it is crucial to understand the mechanisms by which cancer cells can reversibly regulate their energy metabolism. Regarding this, a well-defined feature of some cancer cells is the glucose-induced suppression of respiration and oxidative phosphorylation [17,18]. This is a short-term and reversible event and is referred to as the "Crabtree effect". This reversible shift might represent an advantage of cancer cells *in vivo*, as it would allow them to adapt their metabolism to the rather heterogeneous microenvironments in malignant solid overgrowths.

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Recently, cancer cell energy metabolism has been suggested as a possible target in therapy [10,18] and much of the actual research in the field is being addressed to this particular issue. It is therefore crucial to clearly understand the long-term metabolic reprogramming of cancer cells (the Warburg effect) and the short-term adaptation mechanisms (the Crabtree effect) as the targeting of both would lead to much more effective therapeutic strategies.

2. Molecular mechanisms that may give rise to the Warburg effect

It is assumed that the Warburg effect originates from an increase in glucose uptake and glycolysis and/or a down-regulation of mitochondrial metabolism (reviewed in [18]). There is experimental evidence that fit into each one of these scenarios (Fig. 1). Here we briefly summarize some of the molecular mechanisms that may give rise to the aerobic glycolysis phenomenon. Nevertheless, care must be taken regarding interpretation of the data, as each particular cancer cell carries its own mutations and there is no unique mechanism that could explain the Warburg effect.

2.1. Glucose transport and glycolysis

Commonly, the controlling step of a metabolic pathway is the substrate supply [19]. This has been shown to be the case for some cancer cell lines [20]. According to this, the overexpression of glucose transporters has been proposed as one of the main determinants for the Warburg effect [21]. Results obtained using the positron emission tomography (PET scan) seem to support this proposal. This technique is based on the use of glucose derivatives such as ^{18}fluoro-deoxyglucose as a tracer for imaging its uptake in tissues in vivo [22]. Theoretically, as cancer cells have high glycolytic rates, they will avidly trap glucose and thus they could be visualized and distinguished from normal tissue. FDG-PET has been used for diagnostic procedures and it has been found to be applicable in a variety of cancers [22]. However, high glucose uptake does not necessarily mean that mitochondrial metabolism is down-regulated and this must be taken into account for in vitro studies.

The high affinity glucose transporters (Glut1 and Glut3) are overexpressed in several cancer cell lines [23]. The inhibition of the Glut transporters has been used to impair the growth of tumor cells *in vitro* [24]. Furthermore, it has been demonstrated that glucose transport could effectively be the rate-controlling step of glycolysis in hepatocarcinoma and HeLa cell lines and therefore it may be a suitable target for pharmacological anti-tumor agents [20].

High glycolytic fluxes may also be explained by the increased activity of the enzymes participating in the pathway. In this regard, it has been found that each one of them is overexpressed and/or deregulated in several cancer cell lines [25]. Although an enhanced expression cannot be strictly correlated with an increase of the metabolic flux, some studies have demonstrated that the activity of each one of the enzymes in the pathway is several-fold increased compared to their normal counterparts [26]. It is also important to consider that the contribution to the overall metabolic flux is different for each enzyme [27]. Consequently, only the stimulation of the steps that kinetically control the glycolytic flux would have an impact for the quantitative increase in glycolysis. Hexokinase (HK), phosphofructokinase (PFK) and pyruvate kinase (PK) may be the rate-controlling steps and their regulation and expression patterns change in some tumors.

Hexokinase II (HKII) is the main isoform expressed in some tumors [28]. It is found to be associated with the mitochondrial outer membrane interacting with the voltage-dependent anionic channel (VDAC) [29]. This localization would allow HKII to immediately use the ATP generated by oxidative phosphorylation and also it would help it to overcome the product-mediated inhibition that is normally observed for this enzyme [29]. In hepatoma and Ehrlich ascites, HKII is indeed the slowest step in the glycolytic sequence [26,30]. Thus, the HKII–mitochondria interaction would offer an explanation for the

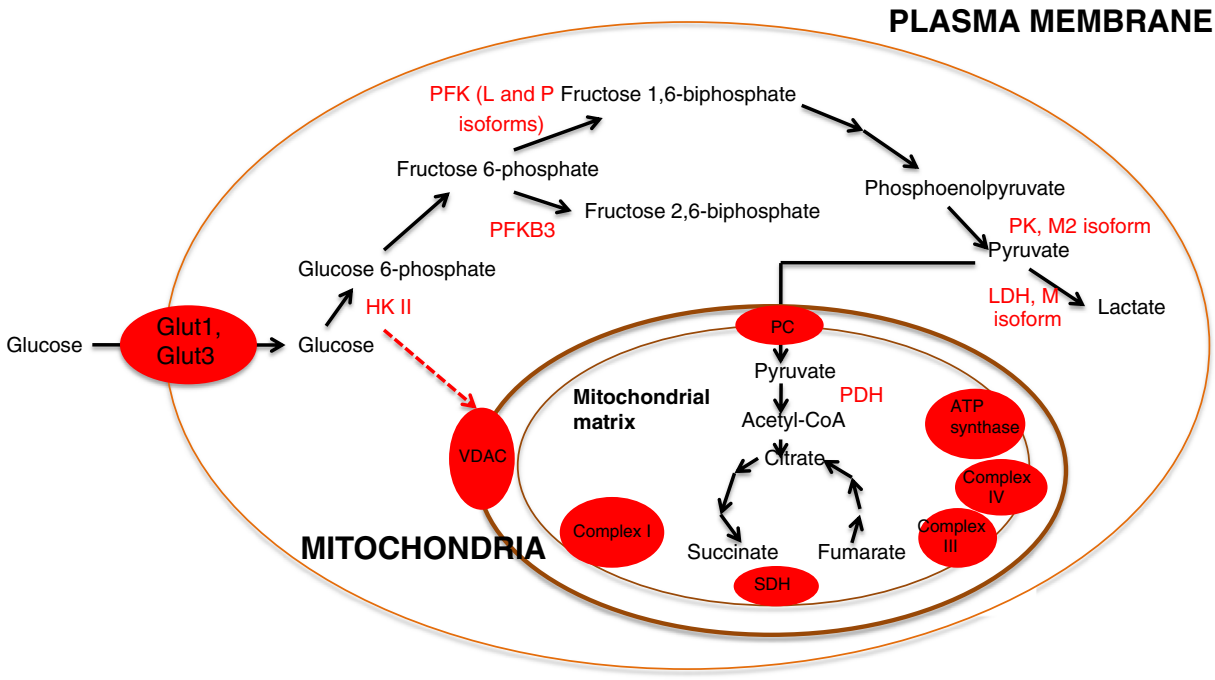

Fig. 1. The mechanisms proposed to explain the aerobic glycolysis (Warburg effect) of cancer cells. The high-affinity glucose transporters (Glut1 and Glut3) in the plasma membrane are overexpressed. Hexokinase isoform II (HK II) interacts with mitochondria through the voltage-dependent anionic channel (VDAC). Phosphofructokinase (PFK) is overactive because the predominance of its L- and P-isoforms. An overexpressed PFKB3 maintains higher levels of fructose 2,6-biphosphate that further activate PFK. The M2 isoform of pyruvate kinase (PK) regulates the glycolytic flux and promotes metabolite accumulation in order to fulfill the biosynthetic needs of the cell. The M-isoform of lactate dehydrogenase (LDH) is overexpressed. Pyruvate transport into the mitochondrial matrix through its specific carrier (PC) is decreased. Pyruvate dehydrogenase complex is inhibited in a phosphorylation-dependent mechanism mediated by an overexpressed pyruvate dehydrogenase kinase (PDHK). Mutations in succinate dehydrogenase (SDH) impair its activity. The levels of the mitochondrial respiratory complexes I, III and IV are decreased. Mitochondria ATP synthase activity is restricted by the overexpression of its inhibitory subunit.

elevated lactate production observed in these cases. However, this would not be the case for all tumors as it has been demonstrated that glucose 6-phosphate effectively inhibits the mitochondria-bound HKII [20,26]. Another drawback of this model is that the high glycolytic flux would be completely dependent on a functional mitochondrial metabolism, which is assumed to be downregulated or impaired in cancer cells (see below).

PFK activity is regulated according to the energy state of the cell, being inhibited when ATP demand decreases. Some alterations on the activity of this enzyme have also been reported [31]. For instance, in leukemia and lymphoma cell lines, the L- and P-isoforms (mainly expressed in liver and platelets, respectively) are the predominant forms [32]. Interestingly, the allosteric properties of these isoforms allow the maximal activity of the enzyme even in low energy demand conditions, i.e. they respond less effectively to their inhibitors (citrate and ATP) while they are highly activated by lower concentrations of fructose 2,6-biphosphate (F26bP) [31,32].

High levels of F26bP are also found on cancer cells [33]. It has been shown that in malignant cells the levels of this intermediate depend on PFKB3 (ATP-dependent phosphofructokinase) [34]. This enzyme belongs to a family of enzymes that have a dual activity: they phosphorylate fructose 6-phosphate (F6P) to F26bP and they are also able to revert this by dephosphorylation [34]. PFKB3 is an isoform that has a low phosphatase activity and is overexpressed in cancer [35]. This may explain the high activity of PFK that has been detected in some tumors [26].

Pyruvate kinase alterations have also been identified, as isoform M2 predominates in some cancer cell lines [35]. PK-M2 activity depends on its quaternary structure: its tetrameric form is more active than the dimer [36]. Some evidences point out that this enzyme is present in its inactive form in tumors [35]. This is probably mediated by PK-M2 phosphorylation, elicited by the activation of the tyrosine kinase signaling triggered by growth factors [37]. The phosphorylated dimeric form does not allow the binding of the allosteric activator of the enzyme (fructose 1,6-biphosphate) thereby leading to a lower activity of PK [38,39]. However, the inhibition of PKM2 seems contradictory with the high glycolytic flux measured in cancer cells. Furthermore, an inactive PK would severely impair cell energy production in the cells that depends mainly on glycolysis for ATP synthesis. It is proposed that the low activity of PKM2 would allow the accumulation of the glycolysis metabolites that would eventually serve as precursors for biosynthesis [35].

2.2. The pyruvate crossroad

Pyruvate is located at the intersection between two of the main catabolic pathways of the cell: glycolysis and the Krebs cycle. This metabolite is transported into the mitochondrial matrix in order to be converted to acetyl CoA and afterwards enters the Krebs cycle to yield reducing equivalents that will be used by the respiratory chain to drive oxidative phosphorylation. Alternatively, pyruvate can remain in the cytosol and be reduced by lactate dehydrogenase.

To explain the aerobic glycolysis, it has been proposed that pyruvate could not be efficiently metabolized by mitochondria thereby deviating the metabolic flux into lactate production. Three events would account for this: a) the restriction of pyruvate transport into mitochondrial matrix, b) the inhibition of the pyruvate dehydrogenase complex (PDH) activity, and c) the overactivation of lactate dehydrogenase.

Pyruvate can enter to mitochondrial matrix through a specific carrier located in the inner membrane of this organelle [40]. Pyruvate transport has been shown to be decreased in mitochondria isolated from a hepatoma cell line [41,42]. Although this may well explain the aerobic glycolysis, there is evidence that showed an enhanced pyruvate uptake and oxidation in a different hepatoma cell line [43].

The pyruvate dehydrogenase complex (PDH) catalyzes the decarboxylation of pyruvate and its condensation with coenzyme A to produce acetyl-CoA. Its activity is regulated according to the energy availability in the cell, being inactive when cellular energy supply is high [44]. PDH activity can also be modulated by reversible phosphorylation. In this regard, the pyruvate dehydrogenase kinases (Pdhk) phosphorylate and inactivate the complex while the pyruvate dehydrogenase phosphatases (PDP) revert this inhibition [44]. A balance between both processes would define the activity of PDH and hence the substrate supply to Krebs cycle. Disruption of the expression of Pdhk1 decreased lactate production in head and neck squamous cancer cells suggesting an elimination of the Warburg effect [45,46]. Moreover, in these cells the expression of Pdhk1 is mediated by the Hypoxia-Induced transcription Factor 1 (HIF-1) [45,46], which is also implicated in the overexpression of the glycolysis enzymes and the glucose transporters in cancer.

An alternative model for the inhibition of pyruvate dehydrogenase activity is the production of acetoin, a by-product from the non-oxidative decarboxylation of pyruvate [47]. This compound inhibits pyruvate oxidation in isolated mitochondria [48]. Acetoin is found in significant amounts in tumors but not in normal cells, pointing out a restriction of pyruvate influx into the Krebs cycle in cancer cells [47]. Nonetheless, the acetoin-mediated inhibition of PDH could be completely reverted in vitro by ADP and other respiratory substrates as α-ketoglutarate, malate and glutamate [48]. Thus, as these other substrates are normally present in the cell, it is unlikely that the entry of pyruvate to the Krebs cycle be completely inhibited by acetoin.

Pyruvate is reduced in the cell cytosol by lactate dehydrogenase in order to keep a constant supply of NAD^+ required to drive glycolysis. This enzyme is also overexpressed in a variety of cancer cell lines [49] and the disruption of its expression stimulates respiration and decreases tumor cell viability in hypoxic conditions [50,51]. Lactate dehydrogenase is a homo- or hetero-tetramer composed of H or M subunits. The latter is the predominant form in muscle [52]. In several human tumors, an overexpression of the M subunit was detected [49].

2.3. Krebs cycle and oxidative phosphorylation defects

Defects in the tricarboxylic acid cycle have also been proposed as high citrate efflux is detected on mitochondria isolated from a hepatoma cell line [53,54]. This seems to correlate with the lack of state 3 respiration in these mitochondria [53,54]. As citrate is oxidized by isocitrate dehydrogenase in the cell cytoplasm in order to produce NADPH, these results could be interpreted as a deviation of carbon flux towards lipid synthesis as a consequence of an impaired Krebs cycle. This feature seems to be restricted to this cell line as another study that used a different hepatoma cell line revealed no citrate efflux [43]. Another impairment of the Krebs cycle may be at the level of succinate dehydrogenase (SDH), which also participates in the mitochondrial respiratory chain as the complex II. Mutations on SDH commonly occur in phaeochromocytomas and paragangliomas [55].

A decrease of ADP translocation to the mitochondrial matrix has been reported to occur, as well as the inhibition of the ATP synthase [56,57]. Both events would evidently restrict ATP production in mitochondria and lead to a diminished respiratory rate and consequently the cell would have to rely mostly on glycolysis-derived ATP. Reduced content and/or activity of the respiratory chain components may occur. There are some reports demonstrating the down-regulation of complexes I, III and IV [55,58,59]. As mentioned above, these features could be particular for a specific cell line and they could not be extrapolated to other tumors. In fact, other cell lines possess fully functional oxidative phosphorylation [9–12].

Another interesting feature of cancer cell energy metabolism is their extensive consumption of glutamine [60]. Glutaminolysis is

indeed highly increased in cancer cells [61,62]. Glutamine is involved in numerous anabolic pathways (such as nucleic acids) and can be degraded in the Krebs cycle thereby generating ATP [63,64] through both substrate level phosphorylation and oxidative phosphorylation. Glutaminolysis generates i) malate which, through the malic enzyme, will give rise to NADPH that can be used to fuel lipid biosynthesis; ii) oxaloacetate, which will generate citrate, which is necessary for lipid biosynthesis [65]. Consequently, glutaminolysis is an important pathway mandatory for cancer cell proliferation and could be a good therapeutic target [66]. It has been shown that glutamine metabolism can be targeted in humans using the glutamine analogue DON (6-Diazo-5-oxo-L-norleucine) [67]. However, toxicity can be an issue in attempts to target glutamine metabolism using DON [68]. Recent studies suggest that the green tea polyphenol (EGCG) could target glutamine metabolism by inhibiting glutamate dehydrogenase under low glucose conditions [69].

3. The Crabtree effect and its induction

Some cancer cells, in spite of possessing functional mitochondria, can switch between glycolytic and oxidative metabolism in a reversible fashion (the Crabtree effect) [17,18]. Regarding the novel therapies based on the inhibition of tumor cell energy metabolism, the sole inhibition of glycolysis would not be sufficient to eliminate all malignant cells some of them may easily overcome the inhibition of the fermentative metabolism. This would bring as a consequence the incomplete elimination of cancer cells and it also would increase the probability of reoccurrence after treatment. Because of this, it is important to precisely know the Crabtree effect and its underlying causes in order to properly target the cancer cells.

The mechanism by which the Crabtree effect is triggered is unknown. Moreover, it is probable that its induction may be due to a combination of several factors [70] (Fig. 2). The most accepted hypothesis is that the glycolysis enzymes (phosphoglycerate kinase and pyruvate kinase) and mitochondria compete for free cytoplasmic ADP [7,71]. 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 [72]. This implies that mitochondria would still use the cytosolic ADP even if the glycolysis enzymes increase their activity.

The Crabtree effect on tumor cells could be eliminated by adding an excess of phosphate (Pi) *in vitro* and, because of that, it has been proposed as the actual trigger of this metabolic phenomenon [73]. This seems in accordance with the dramatic decrease in Pi levels observed after glucose addition in tumor cells [70]. The thermodynamic phosphate potential (i.e. [ATP/ADP·Pi]), which quantitatively determines the metabolic flux in the cell [72], may be crucial during the Crabtree effect [74]. Regarding this, changes in this parameter have been detected in response to glucose addition to sarcoma ascites tumor cells [74]. Other studies based on the phosphate potential and the redox potential ([NAD(P)H/NAD(P)$^+$]) need to be done in order to confirm the possible implication of the thermodynamic forces.

Cytoplasmic 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 [75]. In these conditions, this cation inhibited the mitochondrial ATP synthase inducing a decrease of respiration [76]. However, this could not be taken as a common event of the Crabtree-positive cells as Ca^{2+} levels were shown to be constant in response to glucose in a different hepatoma cell line [70].

Mitochondrial outer membrane regulates the access of substrates to the intermembrane space and thus it could regulate oxidative phosphorylation [77]. 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 in these

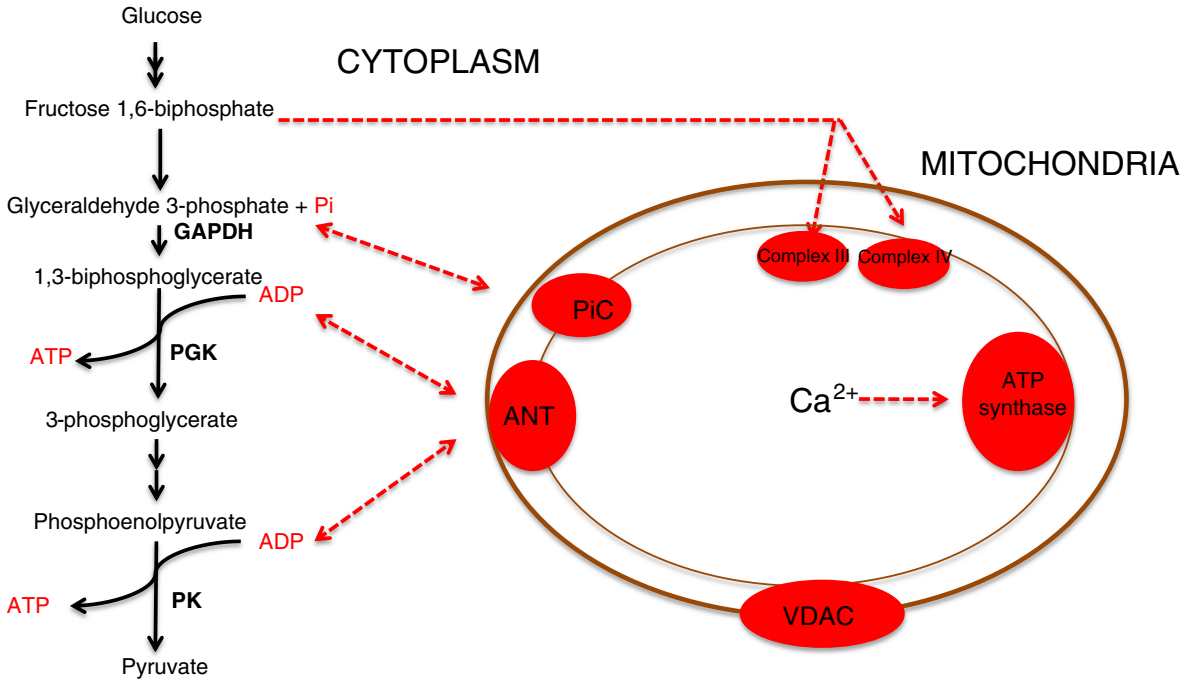

Fig. 2. The mechanisms that could explain the Crabtree effect induction. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and the mitochondrial phosphate carrier (PiC) compete for the free cytoplasmic phosphate pool (Pi). A similar competition for free ADP occurs between the mitochondrial adenine nucleotide translocase (ANT) and two of the glycolysis enzymes: Phosphoglycerate kinase (PGK) and pyruvate kinase (PK). Fructose 1,6-biphosphate inhibits the mitochondrial respiratory complexes III and IV. Increased Ca^{2+} accumulation in the mitochondrial matrix lead to the inhibition of the ATP synthase. Another possible mechanism is a modified diffusion of ADP through the mitochondrial outer membrane.

conditions [78]. 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 [79]. Moreover, contrary to cardiomyocytes where mitochondria and CaMgATPases are organized into tight complexes which ensure effective energy transfer and feedback signaling between these structures via specialized pathways mediated by CK and AK isoforms and direct adenine nucleotide channeling, these complexes do not exist in HL-1 cells due to less organized energy metabolism [80]. In such 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 [81,82].

One mechanism that links glycolysis acceleration to the inhibition of respiration is fructose 1,6-biphosphate (F16bP) [83]. At physiological levels, F16bP induce a decrease on the activity of mitochondrial complexes III and IV [83]. An important finding was that the Crabtree effect could be induced on mitochondria isolated from normal rat liver by incubating them in the presence of F16bP concentrations similar to those measured in hepatoma cells [83]. This demonstrates that the impairment of the mitochondrial oxidative metabolism is not a requisite for the Crabtree effect induction and may explain its reversible nature.

4. Cancer cell and yeast metabolism: Metabolic similarities

The budding yeast *Saccharomyces cerevisiae* possesses the ability to adapt its metabolism to the environmental conditions. When glucose supply is high, the yeast use fermentation as its main metabolic pathway and when this carbon source is scarce it can switch to oxidative metabolism. This is regulated by short- and long-term events [84].

Regarding energy metabolism, there are similarities between the glucose-induced repression of oxidative metabolism of yeast and the "aerobic glycolysis" of tumor cells (Fig. 3). In both cell types, the downregulation of oxidative metabolism is observed along with an enhanced fermentation despite the presence of oxygen (reviewed in [18]). Moreover, *S. cerevisiae* is a Crabtree-positive cell [85]. This points out *S. cerevisiae* as an amenable metabolic model for the screening of metabolism-targeted drugs employed for anti-tumor therapy [18].

Yeast shares with cancer cells the metabolic features that are identified as the underlying causes of the Warburg effect (see above) [18]. For instance, as cancer cells, fermenting yeast over-express all glycolysis enzymes in response to glucose [86,87]. The activity and/or expression pattern of the glycolysis key enzymes (hexokinase, phosphofructokinase and pyruvate kinase) are also modified in yeast. During growth on glucose, only hexokinase II is expressed [87,88]. The latter is a high affinity isoform that is insensitive to

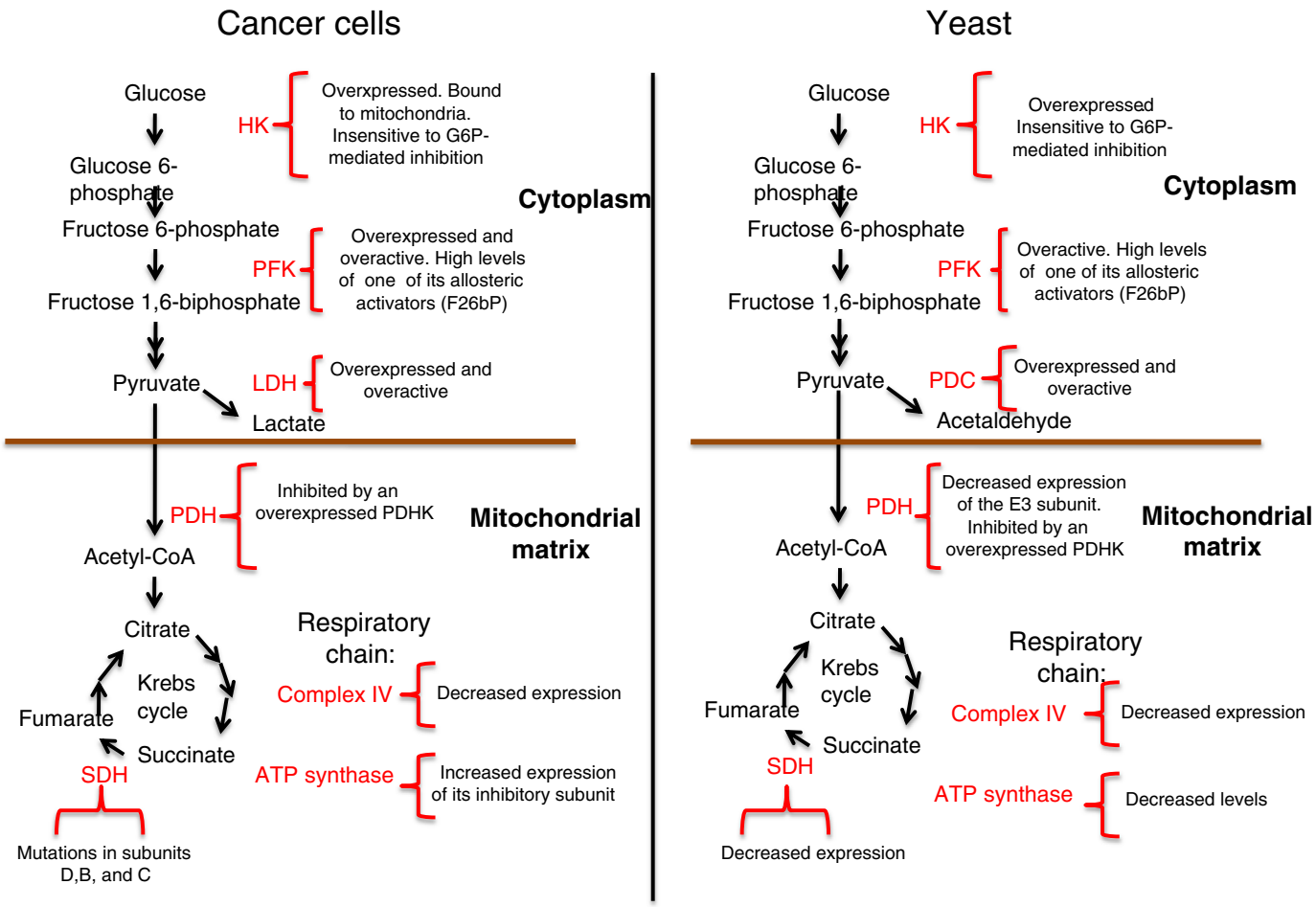

Fig. 3. Metabolic similarities between cancer cells and yeast. Hexokinase (HK) isoforms insensitive to glucose 6-phosphate-mediated inhibition are overexpressed. Phosphofructokinase (PFK) is activated by higher levels of one of its allosteric activators: fructose 2,6-biphosphate (F26bP). The enzymes that metabolize pyruvate in the cell cytoplasm are overexpressed: Lactate dehydrogenase (LDH) in the case of cancer cells and pyruvate decarboxylase in yeast (PDC). Pyruvate dehydrogenase complex (PDH) is inhibited through phosphorylation by an overexpressed pyruvate dehydrogenase kinase (PDHK). Succinate dehydrogenase (SDH) has mutations in different subunits in cancer cells and its expression is down-regulated in yeast. In both cases, a decreased expression of mitochondrial complex IV is observed as well as a down-regulation of the ATP synthase.

product-mediated inhibition [88,89]. Although there seem to be no changes in the expression of phosphofructokinase [90], it is highly active because of the increased levels of its allosteric activator (fructose 2,6-biphosphate) [91]. In fermentative conditions a pyruvate kinase isoform (Pyk1) is expressed, that responds more effectively to the allosteric activation mediated by fructose 1,6-biphosphate [92].

As in tumors, the regulation at the pyruvate crossroad level was demonstrated to occur in yeast. During fermentation, the entry of pyruvate to the Krebs cycle seems to be limited by the down-regulation of its oxidation while its cytoplasmic metabolism is highly active (see below).

S. cerevisiae also has a pyruvate transport that translocates it into the mitochondrial matrix [93]. As for the mammalian homologue, little is known about the regulation of this carrier and the gene sequence coding for the yeast homologue has just recently been identified [94].

The yeast also expresses homologues of the mammalian pyruvate dehydrogenase kinase (Pdhk) and the respective phosphatases (Pdp) (see above) [95,96]. The activity of the yeast pyruvate dehydrogenase is restricted by the phosphorylation of the enzyme [97]. The dephosphorylated form has been detected in yeast growing on non-fermentable carbon sources, i.e. when these cells obtain their energy mainly through oxidative metabolism [95]. Pyruvate dehydrogenase complex is also submitted to transcriptional regulation, as the expression of its subunit E3 (lipoamide dehydrogenase) is decreased during fermentation [98].

Lactate dehydrogenase is also present in yeast: two isoforms are located in the mitochondrial inner membrane [99,100]. The mitochondrial forms irreversibly oxidize lactate to pyruvate and participate in the respiratory chain [99,101] and the role of the cytoplasmic isoform is still unknown. In yeast, the reduction of pyruvate metabolism in cytosol depends mainly on pyruvate decarboxylase (Pdc) [102]. This enzyme produces acetaldehyde that is either oxidized in mitochondrial matrix [103] or reduced to ethanol by alcohol dehydrogenase (Adh) in a reaction that reoxidize the cytoplasmic NADH [104]. Even though the cytoplasmic pyruvate metabolism is not strictly the same as that of tumor cells, an increased flow through this pathway inhibits the substrate supply to oxidative metabolism, just as seen with the increased activity of lactate dehydrogenase in cancer cells. According to this, during the onset of fermentation the activation of Pdc is concomitant with a decrease in respiration [85].

Krebs cycle down-regulation has also been identified in fermenting yeast. In these conditions the enzymatic activities of aconitase, isocitrate dehydrogenase and malate dehydrogenase are decreased [105]. The activity of the mitochondrial respiratory complexes II and IV is also lower in fermentation. This may originate by negative regulation at the transcriptional level [86,105].

As mentioned above, *S. cerevisiae* is a Crabtree-positive: after glucose addition, respiration is inhibited [83]. The same mechanisms that could explain the Crabtree effect induction in tumor cells (see above) may equally apply to the yeast model: limitations in ADP and Pi levels [106], Ca^{2+}-induced decrease in respiration [107], reduced permeability of the mitochondrial outer membrane [108] and fructose 1,6-biphosphate mediated inhibition of the respiratory chain [83]. There are yeasts other than *S. cerevisiae* that are classified as Crabtree-negative, such as *Candida utilis* and *Kluyveromyces lactis* [85]. The characterization of the differences between both yeast types may provide some insight into the precise mechanisms by which the Crabtree effect is induced. In this regard, it has been demonstrated that *C. utilis* is insensitive to the effect exerted by fructose 1,6-biphosphate [83]. This indicates that this mechanism is a particular feature of the Crabtree-positive cells.

Although it may be argued that fermenting yeast lack the genetic defects identified in cancer cells, *S. cerevisiae* possesses homologues of cancer-related genes (such as p53, BRCA1, BRCA2, Cyclin D and Ras) [109]. An interesting approach would be to introduce mutations in these genes and use this "tumorized yeasts" as a model for anti-cancer drug screening and for metabolism studies in order to determine how each one of these mutations would contribute to the Warburg effect in cancer.

5. Metabolism regulation by oncogenes-homologues in yeast

A cell must generate enough energy and acquire or synthesize biomolecules at a sufficient rate to meet proliferation demands. Cancer is essentially a disease in which cells have lost their usual checks on cell proliferation. Consequently, tumor cells often display fundamental changes in pathways of energy metabolism and nutrient uptake [110]. Furthermore, several of the mutations that lead to cancer also drive the altered metabolism of tumor cells [111]. This fundamental metabolic switch may confer a selective growth advantage and/or resistance to apoptosis to allow cancer cells to maintain mitochondrial bioenergetics and integrity during cell growth and proliferation. How oncogene and tumors suppressor networks influence cellular metabolism and bioenergetics to support growth and proliferation in mammalian cells has been extensively reviewed [112]. We will focus here on some of the yeast homologues of cancer-related genes and their influence on yeast cell metabolism and proliferation.

5.1. The Ras oncogene

The Ras subfamily is a protein subfamily of small GTPases involved in cellular signal transduction, and is also used to designate gene subfamily of the genes encoding those proteins. Activation of Ras signaling causes cell growth, differentiation and survival. Ras is the prototypical member of the Ras superfamily of proteins which are all related in structure and regulate diverse cell behaviors. In yeast, the Ras proteins (Ras1 and Ras2) regulate the cAMP/PKA-signaling pathway which is involved in many physiological adaptations of cells in response to environmental changes. This includes the diauxic shift, responses to nutrient starvation, oxidative stress, and heat shock [113–118]. In the yeast Ras signaling cascade, CDC25 catalyzes the conversion of GDP-Ras1 and GDP-Ras2 to GTP-Ras1 and GTP-Ras2, which are the activators of CYR1, the adenylate cyclase [119] which catalyzes cAMP synthesis. The cAMP intracellular concentration thus depends on the respective activities of CYR1 and the phosphodiesterases PDE1 and PDE2. High cAMP concentrations promote the dissociation of the regulatory subunit (BCY1) [120] from the catalytic subunits (TPK1, TPK2, and TPK3) [121], thus activating the catalytic subunits of protein kinase A (PKA), which phosphorylates a variety of substrates.

The homologue of the RasV12 mammalian oncogene is the RasV19 protein in yeast. The yeast strain carrying this mutation has been extensively studied and harbors profound modification in its energy metabolism. Indeed, the Ras2V19 strain is affected in its ability to store glycogen [122] and presents a hyperinvasive growth phenotype. Further, mitochondrial metabolism is profoundly modified and quantitative measurements show that there is an increase in mitochondrial amount in the cells carrying the Ras2V19 allele. Both cellular respiratory rate and mitochondrial cytochromes are increased (×2) [123]. This phenomenon leads to uncoupling between biomass synthesis and catabolism and generates a decrease in the enthalpy growth yield [124]. This study showed that mitochondria by themselves are a major heat dissipative system in a fully aerobic metabolism and that a subtle adaptation of the amount of mitochondria to the growth rate is necessary to maintain the enthalpy growth yield. Further studies with yeast mutants of the Ras/cAMP cascade have shown that this pathway is a major regulator of mitochondrial energy metabolism [123–128] in such a way that an overactivation of this cascade induces an increase in this metabolism whereas an underactivation of this cascade induces a decrease in this metabolism.

Interestingly, it has been shown that the oncoprotein H-RasV12 increases mitochondrial metabolism [129], further strengthening the fact that there is strong similarities between the regulation of yeast and mammalian cells energy metabolism by oncogene/oncogene homologues.

5.2. Sch9, the yeast homologue of Akt

Growth factor-independent activation of the PI3K/Akt (a known oncogene) pathway drives changes in cellular metabolism to promote cancer cell growth and proliferation [112]. The SCH9 gene of the yeast *S. cerevisiae* encodes a serine–threonine protein kinase with a catalytic domain very similar to that of human Akt1 [130]. There are several lines of evidence that Sch9p plays an important role in glucose signaling in the budding yeast. Early work showed parallelism and complementarity of SCH9 signaling with the cyclic AMP-dependent protein kinase (PKA) pathway, which signals hexose abundance [131–134]. It was also shown that Sch9p integrates nutrient signals with cell size regulation. In fact, the Δsch9 mutation was one of the most potent modifiers of cell size identified in a genome-wide screen for pathways coupling cell growth and division in yeast [135]. Sch9p is an activator of ribosomal protein and ribosomal biogenesis regulons and is required for carbon source modulation of cell size [136]. Furthermore, it was recently shown that the Δsch9 mutation upregulates electron transport chain gene expression and that this is associated with an increase in mitochondrial respiration [137], further strengthening the fact that yeast homologues of oncogene play a role in the regulation of cell metabolism.

It has recently been shown that yeast Sch9 is a central component of a network that controls a common set of genes implicated in a metabolic switch from the TCA cycle and respiration to glycolysis and glycerol biosynthesis. During chronological survival, mutants lacking SCH9 depleted extracellular ethanol and reduced stored lipids, but synthesized and released glycerol. Deletion of the glycerol biosynthesis genes GPD1, GPD2, or RHR2, among the most up-regulated in long-lived Δsch9, Δtor1, and Δras2 mutants, was sufficient to reverse chronological life span extension in Δsch9 mutants, suggesting that glycerol production, in addition to the regulation of stress resistance systems, optimizes life span extension [138].

5.2.1. Conclusion

The emergence of metabolic enzymes as important regulator of cancer cell growth suggests that metabolic control is a key element of tumor progression. Understanding the pathways that regulate cancer cell metabolism may lead to greater understanding of cancer cell development and progression, and has the potential to open a new vista of metabolic therapy for cancer treatment. As shown in the last paragraph of this review, there are strong similarities between mammalian and yeast cell metabolism regulation by oncogenes/oncogenes homologues. An interesting approach would be to use “tumorized yeasts” as a model for anti-cancer drug screening and for metabolism studies in order to determine how each one of these mutations would contribute to the profound metabolic alterations in cancer.

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