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Review

Mitochondria in cancer: Not just innocent bystanders

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ABSTRACT

The first half of the 20th century produced substantial breakthroughs in bioenergetics and mitochondria research. During that time, Otto Warburg observed abnormally high glycolysis and lactate production in oxygenated cancer cells, leading him to suggest that defects in mitochondrial functions are at the heart of malignant cell transformation. Warburg's hypothesis profoundly influenced the present perception of cancer metabolism, positioning what is termed aerobic glycolysis in the mainstream of clinical oncology. While some of his ideas stood the test of time, they also frequently generated misconceptions regarding the biochemical mechanisms of cell transformation. This review examines experimental evidence which supports or refutes the Warburg effect and discusses the possible advantages conferred on cancer cells by 'metabolic transformation'.

1. Introduction

Mitochondria are cellular organelles bounded by two distinct membranes. They hold about one tenth of the cellular proteins, their own DNA and comparing weight to weight, convert over 10,000 times more energy per second than the sun [1]. The Swiss anatomist Rudolf Albrecht von Koelliker first described mitochondria in 1857, calling them sarcosomes. In 1890, the German pathologist Richard Altman proposed that they were intracellular parasites and 8 years later the German microbiologist Carl Benda finally gave them the name "mitochondria". In 1945, the Belgian-American biochemist Albert Claude isolated them by centrifugation from disrupted cells and showed that they catalyzed respiration and since then mitochondria have been defined as the powerhouse of the cell. In the following decades, biochemists tracked down the different components of this powerhouse and characterized them (reviewed in Refs. [2,3]). Among the forceful fruits of a very intense period during the first half of the 20th century were the uncovering of the electron transport chain by Henrich Otto Weiland, David Keilin and Otto Warburg, the mechanism of complete oxidation of nutrients into carbon dioxide through the tricarboxylic acid cycle (TCA) by Hans Krebs, and the mechanism of oxidative phosphorylation by Peter Mitchell's chemiosmotic theory.

Thereafter, bioenergetics and 'mitochondriology' languished until increasing evidence indicated that mitochondria are involved in various cellular processes, from regulation of metabolic flux to programmed cell death (apoptosis). The importance of mitochondria in cell physiology supported a pioneering observation made by Otto Warburg, back in the 1920s. Warburg took advantage of new techniques for monitoring gas exchanges in isolated tissues and simultaneously measured oxygen consumption and lactate production in tumour slices either in the presence or absence of oxygen. In the presence of oxygen, the rapidly growing tumour cells consumed glucose at a surprisingly high rate compared to normal cells and secreted most of the glucose-derived carbon in the form of lactate. Warburg put forward that this phenomenon, termed 'aerobic glycolysis', was provoked by mitochondrial impairment and was the origin of cancer cell transformation [4].

Warburg's hypothesis profoundly influenced the present perception of cancer metabolism, moving aerobic glycolysis into the mainstream of clinical oncology. While some of his ideas stood the test of time, they frequently generated misconceptions about the underlying biochemical mechanisms of cancer cell transformation. In fact, the first incontestable examples of causality between mitochondrial dysfunction and tumorigenesis were only discovered less than a decade ago when mutations in succinate dehydrogenase (SDH) or fumarate hydratase (FH), both enzymes of the TCA cycle, were found to be the initiating events of familial paraganglioma or leiomyoma and of papillary renal cell cancer, respectively [5,6]. Thus, except for very few known forms of cancer, the mitochondrial impairment observed in many tumours could be the consequence of complex metabolic shifts that, while conferring survival and replicative advantages to cancer cells, have no direct effect on cancer formation. This review is devoted to the analysis of mitochondrial function in cancer cells. In particular, we examined the experimental evidence supporting or refuting the Warburg effect and the potential benefit that 'metabolic transformation' has for cancer cells.

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2. The metabolic transformation of cancer cells: dissecting the Warburg effect

In order to engage in fast replicative division, a cancer cell must duplicate its genome, synthesize proteins and lipids and assemble these components to form daughter cells. These activities require the uptake of extracellular nutrients and their arduous and rigorous conversion into biosynthetic precursors rather than processing them as catabolites. Through changes in the expression and activity of enzymes that determine the rate of metabolic fluxes, including nutrient uptake and utilization, tumour cells can achieve this metabolic transformation. Research over the past few years has pursued this aspect of tumorigenesis, revealing metabolic activities in diverse tumour types and proving that oncogenic mutations can promote metabolic autonomy by driving nutrient uptake to levels that often exceed those required for cell growth and proliferation [7].

The most evident and most studied aspect of metabolic transformation is the dependence on glucose as a source for carbon intermediates for anabolic pathways and for ATP synthesis. Most of the anabolic processes required for accelerated growth rate are accomplished by increased glycolysis, which is supported by replenishing TCA cycle intermediates (anaplerosis) [7,8] (Fig. 1). To generate ribose 5-phosphate for nucleotide biosynthesis, cells divert carbon from glycolysis into either the oxidative or non-oxidative arm of the pentose phosphate pathway [9]. Consistent with the need for robust *de novo* lipid synthesis, tumour cells express high levels of the lipogenic enzymes ATP-citrate lyase, acetyl-CoA carboxylase and fatty acid synthase [10–13]. Importantly, TCA cycle-derived citrate is the only source for the cytosolic acetyl-CoA required for lipid biosynthesis (Fig. 1). Increased protein production involves *de novo* synthesis of non-essential amino acids. Both glucose and glutamine, heavily consumed by cancer cells, are early precursors of non-essential amino acids (Fig. 1). Two glycolytic intermediates, 3-phosphoglycerate and pyruvate, are directly needed for the biosynthesis of serine and alanine (serine is further metabolised into glycine or cysteine). In addition, TCA cycle intermediates are used to synthesise aspartate, asparagine, glutamate, proline, arginine and glutamine (Fig. 1). Clearly, anaplerosis sustains TCA cycle function under conditions of substantial biosynthesis of non-essential amino acids and fatty acids. It is achieved through either converting pyruvate to oxaloacetate by pyruvate carboxylase or breaking down glutamine into α-ketoglutarate (glutaminolysis) (Fig. 1). The latter process predominates in cancer cells [14–17] rendering glutamine an essential amino acid and the source of TCA cycle-derived anabolic metabolites.

It is remarkable that the tight connection between cellular metabolism, mitochondrial bioenergetics, and tumorigenesis was envisaged more than seven decades ago by Otto Warburg. Warburg however, focused on the bioenergetic arm of the metabolic transformation phenomenon. He suggested that defects in mitochondrial oxidative phosphorylation lead to increased glycolytic flow as the only alternative for ATP production. Today, the spotlight of metabolic transformation research has shifted to anabolic processes [18]. There is however an unresolved discrepancy between Warburg's original observation and anabolic transformation as it is viewed today: if indeed tumour cells produce and secrete high amounts of lactate, derived either from glucose as Warburg proposed, or partially from glutamine as was recently suggested [14], these are wasted carbon atoms that cannot be used for anabolism. Therefore, while it is expected that rapidly growing tumours will take up and process carbon to a high degree for anabolism, the high rate of lactate production may indeed be due to bioenergetic causes. The pivotal biochemical decision point is pyruvate. Pyruvate can be either reduced to lactate and shuttled out of the cell or transported to the mitochondria to be further oxidized to CO_2 and/or other anabolic precursors (Fig. 1).

3. Evidence for increased glycolysis in cancer

Recently, interest in tumour metabolism has rekindled mainly thanks to the widespread clinical applications of the Positron Emission Tomography (PET) imaging technique which can use the glucose analogue tracer 18-fluorodeoxyglucose (FDG). FDG–PET imaging of thousands of patients has shown that most primary and metastatic human cancers display significantly increased glucose uptake, and for many this was the clearest confirmation of Warburg's prediction [19,20]. Although the specificity and sensitivity of FDG–PET to cancerous lesions is near 90% it is acknowledged that FDG uptake is complex and influenced by many factors such as glucose concentration, tissue type and size and inflammatory response given that immune cells also avidly trap FDG [21,22]. Furthermore, being the only alternative to oxidative phosphorylation for ATP synthesis, glycolysis is induced when oxygen becomes limited (hypoxia), a condition frequently observed in solid tumours (see below). FDG–PET does not distinguish between hypoxia-driven and aerobic glycolysis [23]. Thus, the efficacy of FDG–PET depends on the cancer type and stage and this may lead to a significant number of false positive or false negative results. As for the method proving Warburg's hypothesis: the assay only demonstrates increased glucose uptake, at least some of which may be used for nucleotide or lipids synthesis rather than for oxidation into pyruvate and lactate.

Another way to assess an increase in glycolytic flux in cancer is to determine the rate of lactate production. Despite the fact that high lactate concentration can be detected by Magnetic Resonance Spectroscopy techniques, to-date there have been no 'easy' non-invasive methods to study lactate production in tumours but a newly developed non-invasive Magnetic Resonance Imaging technique for analysing tumour's pH may hold some promise for the future [24]. Still, one must be aware of the fact that high levels of lactate may represent other, non-glycolytic, piruvate producing pathways such as alanine transamination or malate conversion by the malic enzyme. The latter could be a side-product of glutaminolysis (Fig. 1).

4. Mitochondrial biochemical defects in cancer

Today, the Warburg effect is regarded as the phenomenon of increased glycolysis in cancer cells even in the presence of oxygen, without a corresponding increase in oxidative phosphorylation. However, the original hypothesis claimed that impaired mitochondrial function caused the glycolytic phenotype and the formation of cancer [4]. This *tout court* statement was immediately criticized by Sydney Weinhouse (for the high pitched debate see Ref. [25]) who demonstrated that mitochondrial function in cancer tissues was normal, and strongly stood against the emerging picture of impaired mitochondria as a cause of cancer. After a methodical analysis of the data available at the time, Weinhouse concluded that information supporting Warburg hypothesis was affected by misinterpretation, mainly of quantitative nature: amongst other flaws, a serious cause of error in the studies of tumour mitochondria was that tumours are typically necrotic and the presence of fatty acid and cellular debris could easily harm mitochondria during their isolation, interfering with their functions [26]. In addition, analysis of the degree of inhibition of glycolysis by oxygen, a phenomenon called the 'Pasteur Effect', showed that aerobiosis reduced glycolysis in cancer as much as it did in normal tissues, indicating a normal oxidative phosphorylation capacity in tumours [27]. Weinhouse concluded that the observed increased

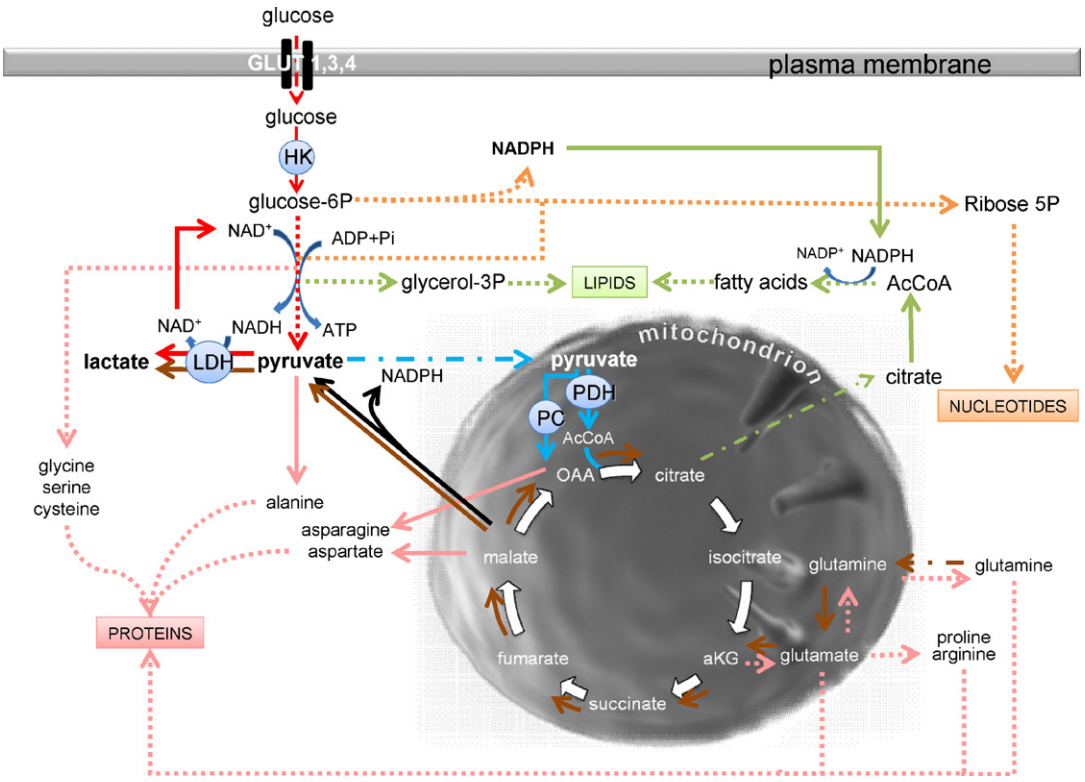

Fig. 1. Mitochondria as a crossroad for catabolic and anabolic pathways in normal and cancer cells. Glucose and glutamine are important carbon sources which are metabolized in cells for the generation of energy and anabolic precursors. The pathways discussed in the text are illustrated and colour coded: red, glycolysis; white, TCA cycle; pink, non-essential amino acids synthesis; orange, pentose phosphate pathway and nucleotide synthesis; green, fatty acid and lipid synthesis; blue, pyruvate oxidation in the mitochondria; brown, glutaminolysis; black, malic enzyme reaction. Solid arrows indicate a single step reaction; dashed-dotted arrows indicate transport across membranes and dotted arrows indicate multi-step reactions. Abbreviations: HK, hexokinase; AcCoA, acetyl co-enzyme A; OAA, oxaloacetate; αKG, α-ketoglutarate.

glycolysis in tumours is not of aerobic, but rather of anaerobic nature.

Several recent studies have confirmed that restricting glycolysis or diverting pyruvate into the mitochondria, can significantly induce respiration in cancer cells [28–30]. These studies confirmed that the fate of pyruvate [either reduction in the cytosol by lactate dehydrogenase (LDH) or oxidation in the mitochondria by pyruvate dehydrogenase (PDH)] determines the direction of tumour metabolism. The inhibition of lactate dehydrogenase or the activation of pyruvate dehydrogenase [via the inhibition of pyruvate dehydrogenase kinase (PDK)], can induce tumour cells to oxidize pyruvate in the TCA cycle and stimulate mitochondrial respiration [28,30]. This indicates that mitochondrial activity is not fundamentally impaired in cancer cells. More recent data showed that for most, but not all, cancer cells glycolysis accounts for about 60% of ATP production [23]. In other words, most cancer cells appear capable of performing respiration, but the rate of oxidative phosphorylation is reduced by a dramatic increase in glycolysis and lactate production.

From a clinical perspective it is important to note that the degree of glycolysis correlates with tumour prognosis. A biochemical analysis of different types of cancer showed that tumour aggressiveness can be defined by a bioenergetic index calculated as the ratio between the activities of the mitochondrial enzyme ATP synthase and the glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase [31]. One can therefore consider that mitochondrial function in cancer cells, even if not damaged, might be inhibited by low oxygen levels, changes in metabolic fluxes and gene expression reprogramming (Fig. 2). The alterations in mitochondrial function in the process of metabolic transformation of cancer cells are discussed below (see also Refs. [32,33]).

4.1. Hypoxia-dependent inhibition of mitochondria activity

Molecular oxygen (O_2) is the ultimate electron acceptor of the mitochondrial electron transfer chain (ETC). It is consumed at complex IV (cytochrome *c* oxidase, COX) where it is reduced to water. The affinity of COX to oxygen is high considering the available oxygen levels in well-oxygenated (normoxic) tissues. The estimated $K_m(O_2)$ of COX is in the range of 1 μM [34] while the concentration of oxygen in slightly hypoxic (yet, physiologic) to normoxic tissues ranges between 6 and 30 μM, respectively (based on measured pO_2 of 4–20 mm Hg which is equivalent to 2.5–10% oxygen) [35,36]. It is rather difficult to measure the concentration of oxygen in solid tumours as it varies spatially, and the further away a particular region of a tumour is from the nearest blood vessel, the lower the pO_2 of that region. However, it is clear that many tumour regions are deeply hypoxic, with oxygen concentrations approaching 0 mm Hg [37]. Thus, it can be argued that in large regions of solid tumours, oxidative phosphorylation is effectively limited leaving glycolysis as the main energy-generating pathway (the Pasteur Effect). It is important to mention that phosphofructokinase-1 (PFK1), a key regulatory enzyme of glycolysis, is negatively regulated by ATP. Therefore, when oxidative phosphorylation is inhibited due to lack of oxygen and ATP levels decrease, glycolysis can be enhanced to compensate for ATP loss. This challenges the 'aerobic glycolysis' hypothesis described above, however, the pathological influence of hypoxia on mitochondrial respiration in solid tumours is still

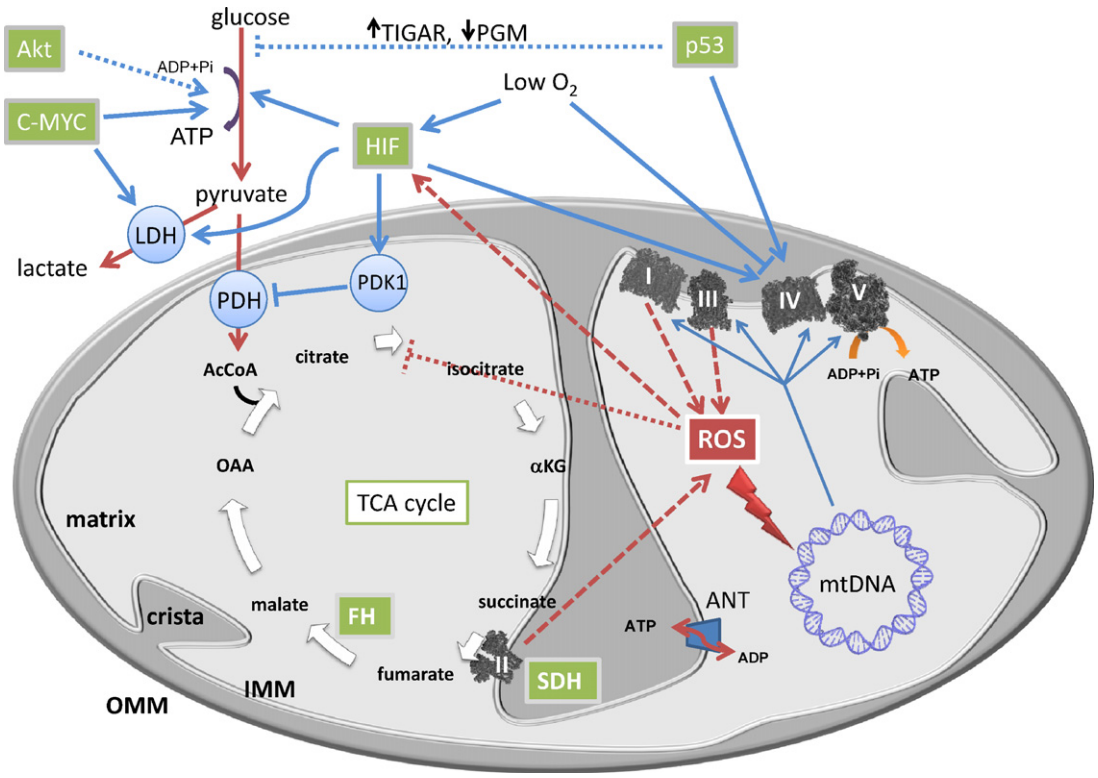

Fig. 2. Mitochondria as a target for multiple metabolic transformation events. Principal metabolic perturbations of cancer cells are induced by genetic reprogramming and environmental changes. The activation of Akt and MYC oncogenes and the loss of p53 tumour suppressor gene are among the most frequent events in cancer. Furthermore, all solid tumours are exposed to oxidative stress and hypoxia hence to HIF activation. These frequent changes in cancer cells trigger a dramatic metabolic shift from oxidative phosphorylation to glycolysis. In addition, direct genetic lesions of mtDNA or of nuclear encoded mitochondrial enzyme (SDH or FH) can directly abrogate oxidative phosphorylation in cancer. 3D structures of the respiratory complexes in the scheme were retrieved from Protein Data Bank (PDB: www.rcsb.org) except for complex I which was retrieved from [87]. PDB codes are as follow: SDH (II), 1 LOV; complex III (III), 1BGY; COX (IV), 1OCC; ATP synthase (V), 1QO1.

unclear and may depend on specific gene expression, activated under these conditions (see below).

4.2. The role of HIF in regulating mitochondrial functions during hypoxia

In response to prolonged hypoxia, cells undergo metabolic adaptation mediated by changes in gene expression. This process is mainly initiated by the Hypoxia Inducible Factor (HIF) family of transcription factors [38]. HIF is a heterodimeric complex composed of α and β subunits. Under normoxia, the protein levels of HIFα are very low owing to its continuous degradation via a sequence of post-translational events (Fig. 3B). The first is HIFα hydroxylation on two prolyl residues, catalysed by the enzymes HIF prolyl hydroxylases (PHD1, 2 and 3), which are oxygen and α-ketoglutarate dependent enzymes [39]. Following hydroxylation, the von Hippel–Lindau gene product (pVHL) mediates HIFα ubiquitylation, leading to its degradation by the proteasome [40]. Under hypoxia, PHD hydroxylation of HIFα is inhibited leaving the stabilized HIFα to interact with HIFβ and activate the hypoxia-mediated response that regulates many biological outcomes among which are angiogenesis and glycolysis.

One consequence of HIF activation is an increase in glucose uptake and phosphorylation due to elevated levels of both the glucose transporter Glut-1 and the glucose phosphorylating enzyme hexokinase (Figs. 1 and 2). This leads to a dramatic increase in the rate of the first two steps of glucose metabolism and is sufficient to explain the substantial FDG trapping (uptake and phosphorylation) seen in tumours by PET imaging [41–43]. Importantly however, recent studies using combined FDG and 18-F-misonidazole, a sensitive hypoxic probe, demonstrated that FDG uptake and hypoxia do not always correlate in vivo [23,44].

As mentioned above, the shift between glycolysis and oxidative phosphorylation is controlled by the relative activities of two enzymes, PDH and LDH that determine the catabolic fate of pyruvate (Figs. 1 and 2). Interestingly, HIF controls this crucial bifurcation point by inducing LDH levels and inhibiting PDH activity by stimulating its inhibitor PDK1 [45–48]. Due to its ability to divert pyruvate metabolism from the mitochondria to the cytosol, HIF is considered a crucial mediator of the bioenergetic switch in cancer cells. It is unclear at the moment whether active inhibition of oxidative phosphorylation by HIF is physiologically advantageous. One option is that it may conserve oxygen under hypoxia in order to sustain other oxygen-dependent activities and to avoid necrotic death [49]. In addition, suppression of pyruvate oxidation in the mitochondria may protect cells from the hypoxia-mediated production of cytotoxic amounts of reactive oxygen species (ROS) [46]. It is of note however that in principle, HIF stabilization may only partially inhibit oxidative phosphorylation since the increased glutaminolysis observed in tumours produces α-ketoglutarate that can feed parts of the TCA cycle and produce reducing equivalents (NADH and $FADH_2$) for the ETC before exiting the mitochondria as malate (Fig. 1) [14,17].

Several tumours display high HIF activity even in the presence of oxygen. Such conditions, known as pseudohypoxia, are mostly evident in tumours with a loss of one of the following tumour suppressors: pVHL, SDH or FH ([50], Fig. 3B). For the reasons described above, pseudohypoxia can be a major driving force for aerobic glycolysis.

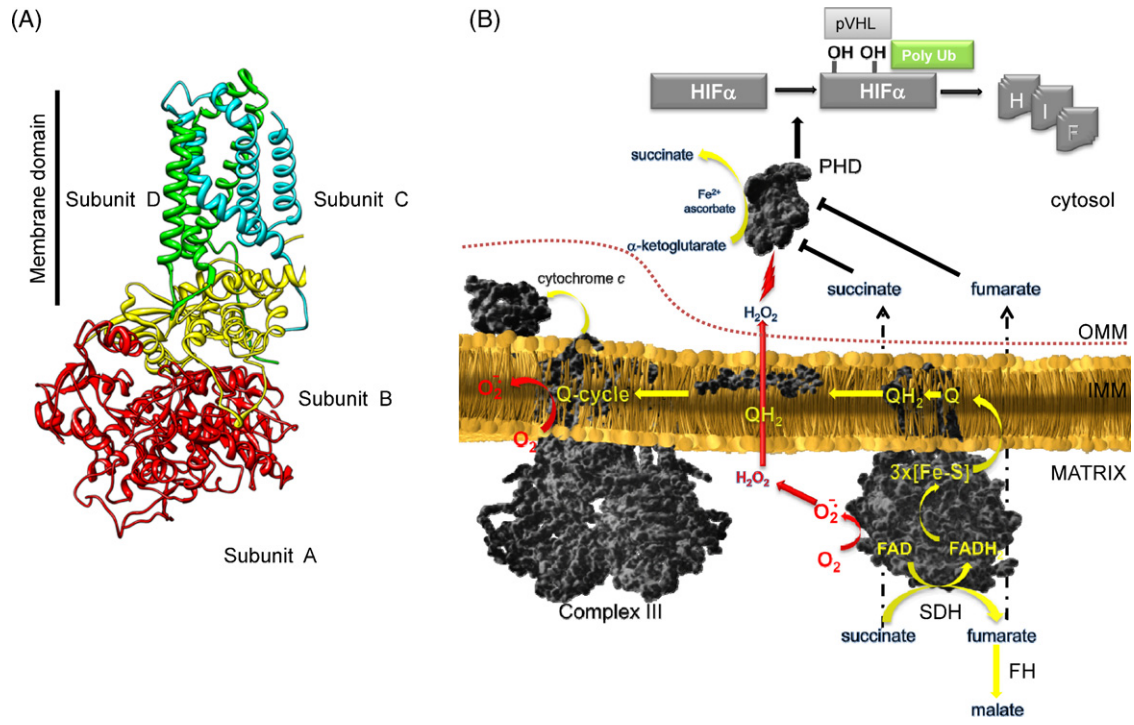

Fig. 3. The physiological roles of SDH in the TCA cycle and the ETC and its potential roles in cancer. (A) Ribbon diagram of SDH structure (PBD code: 1LOV). The catalytic subunits: the flavoprotein (SDHA) and the iron-sulphur protein (SDHB) are depicted in red and yellow, respectively, and the membrane anchors and ubiquinone binding proteins SDHC and SDHD are depicted in cyan and green, respectively. (B) Other than being a TCA enzyme, SDH is an additional entry point to the ETC (most electrons are donated from NADH to complex I—not shown in this diagram). The electron flow in and out of complex II and III is depicted by the yellow arrows. During succinate oxidation to fumarate by SDHA, a two-electron reduction of FAD to FADH2 occurs. Electrons are transferred through the iron–sulphur centres on SDHB to ubiquinone (Q) bound to SDHC and SDHD in the inner mitochondrial membrane (IMM), reducing it to ubiquinol (QH2). Ubiquinol transfers its electrons through complex III, in a mechanism named the Q cycle, to cytochrome *c* (PDB: 1CXA). Electrons then flow from cytochrome *c* to COX where the final four-electron reduction of molecular oxygen to water occurs (not shown in this diagram). Complex III is the best characterized site of ROS production in the ETC, where a single electron reduction of oxygen to superoxide can occur (red arrow). It was proposed that obstructing electron flow within complex II might support a single electron reduction of oxygen at the FAD site (red arrow). Superoxide is dismutated to hydrogen peroxide which can then leave the mitochondria and inhibit PHD in the cytosol, leading to HIFα stabilization. Succinate or fumarate, which accumulate in SDH- or FH-deficient tumours, can also leave the mitochondria and inhibit PHD activity in the cytosol. The red dotted line represents the outer mitochondrial membrane (OMM).

Another link between HIF and the regulation of mitochondrial respiration has been recently proposed. In mammalian cells, the expression of two alternative subunits of COX was shown to be oxygen regulated. HIF simultaneously induces COX4-2 expression and COX4-1 degradation by Lon proteases. This isoform switch leads to optimization of COX activity and overall more effective respiration (with less ROS production) under hypoxia [51].

4.3. Mitochondria activity can be regulated by tumour suppressors and oncogenes: the Crabtree effect revealed?

Back in the 1920s, Herbert Crabtree observed that increased glycolysis in cancer and normal proliferating cells inhibits respiration, an observation is now known as the 'Crabtree effect' [52]. He further suggested that this observation is sufficient to explain the decrease in oxidative phosphorylation-derived ATP in cancer, arguing against Warburg's initial hypothesis that defects in respiration are the cause for increased glycolysis. Years later it was suggested that respiration inhibition by glycolysis was caused by glycolysis competing with oxidative phosphorylation for Pi and ADP [53]. However, the Crabtree effect does not provide an explanation for the actual cause of the observed increased aerobic glycolysis in cancer.

Only recently was it demonstrated that genetic alterations in cancer cells can alter the glycolytic rate. Deregulation of the PI3K/Akt pathway or imbalance in the activity of one of three cancer-related transcription factors, c-MYC, HIF and p53, are sufficient to increase glucose and amino acid metabolism [8,54,55] (Fig. 2). Among a plethora of biological effects, Akt stimulates the expression of the glucose transporter GLUT1, induces the translocation of GLUT4 to the plasma membrane and activates hexokinase 2 (reviewed in Refs. [55,56]). c-MYC increases LDH type A [57] and in synergy with HIF induces PDK1 [58]. p53 is required for efficient oxidative phosphorylation by inducing the expression levels of the nuclear encoded mitochondrial protein SCO_2 which is required for COX assembly [59]. Furthermore p53 controls glycolytic flux by activating TIGAR, a regulator of PFK1, and by downregulating phosphoglycerate mutase (PGM) [60,61]. Activation of PI3K, Akt, c-Myc and HIF and loss of the p53 pathway are amongst the most common alterations observed in human cancer (see 'Subway map of cancer pathways' http://www.nature.com/nrc/journal/v2/n5/weinberg_poster/and [62]). Therefore, one can speculate that these changes are sufficient to provide a causative explanation for the aerobic glycolysis of cancer, and due to the described above Crabtree effect, to the potential decrease in mitochondrial respiration.

5. Genetic mitochondrial defects

5.1. Mitochondrial DNA mutations in cancer

Most mitochondrial proteins are encoded by nuclear DNA and are translated in the cytosol prior to their active transportation into the mitochondria [63]. However, mitochondria contain their own DNA (mtDNA) and ribosomes. mtDNA is a circular molecule of about 16.5 kb encoding all mitochondrial rRNAs and tRNAs and a limited number of ETC proteins [complex I: (ND1–ND6 and ND4L), complex III: (apocytochrome *b* subunit), complex IV: (COXI, COXII and COXIII), and ATP synthase (ATPase6 and ATPase8)]. Unlike nuclear

DNA mtDNA lacks histones, rendering it more susceptible to free radicals and its repair capacity is lower compared to nuclear DNA [64,65]. Somatic mutations in mtDNA occur in many tumours (for reviews see Refs. [66,67]). It is possible that the oxidatively stressed environment of tumours may result in high rates of mtDNA mutations and diminished expression of mtDNA-encoded polypeptides [64] (Fig. 2). Interestingly, mtDNA mutations in tumours are mostly homoplasmic, which means that all cells within a tumour carry the same mtDNA mutation, a phenomenon implicating selective advantage to these mutations. A possible outcome of homoplasmic mtDNA mutations is sub-optimal and even non-functional oxidative phosphorylation that would drives cells to accelerate glycolysis. Indeed, mutations in the mtDNA of oncocytic thyroid carcinomas and renal oncocytomas correlate with low respiratory rate, decreased complex I and III activities, reduced ATP content and high ROS production [68–70]. Still, the pathological relevance of mtDNA mutations in cancer remains controversial. In one study of human renal carcinomas, most mtDNA mutations were silent and no homoplasmy was observed [71]. Furthermore, a critical review of the field suggested recently that the functional consequences of somatic mtDNA mutations might often be limited and of peripheral importance to tumorigenesis. In many cases, the mutations might simply be selected within the mitochondrial pool without having a direct role in tumorigenesis [72,73].

Recently however, it was reported that particular mtDNA mutations can contribute to tumour progression. Cells bearing a mutation in the mtDNA gene encoding NADH dehydrogenase subunit 6 (ND6), have a defective respiratory complex I and subsequently overproduce ROS that increase metastatic potential [74].

5.2. Mutations in nuclear-encoded mitochondrial proteins: SDH and FH as tumour suppressors

An important link between metabolism and cancer was added in the present decade demonstrating that the nuclear encoded TCA cycle enzymes SDH and FH are tumour suppressors [5,6]. Of the two, SDH is also an integral part (complex II) of the ETC (Figs. 2 and 3). Germline mutations in the genes encoding the B, C and D subunits of SDH (Fig. 3A) predispose individuals to hereditary paraganglioma with phaeochromocytoma (HPGL) while germline mutations in *FH* cause hereditary leiomyomatosis and renal cell cancer (HLRCC) (for reviews see Refs. [50,75]). Soon after the discovery of SDH mutations it was proposed that the HIF pathway is activated in the associated tumours [76] but only 4 years later was it demonstrated that impaired HIFα degradation due to the inhibition of its hydroxylation by PHDs is at the heart of the pathology of these tumours [77–79].

Two models that explain anomalous HIFα stabilization under normoxia (pseudohypoxia) due to SDH mutations have been proposed (Fig. 3B). The first suggested that ROS, generated from an impaired complex II, inhibit HIFα hydroxylation by PHDs. However, there are conflicting data as to ROS production in SDH deficient cells [80–82]. This controversy may be attributed to the mechanism used for SDH inactivation (mutations versus siRNAs) or to the methods used for ROS analysis [81]. The second model demonstrated that succinate, which builds up in SDH-deficient mitochondria, can serve as a mitochondria-to-cytosol messenger that inhibits PHD activity [79]. And like succinate, the increased fumarate levels observed in FH-deficient HLRCC tumours also inhibit PHD activity and consequently HIFα degradation [78,83]. The two models, of increased ROS production and of product inhibition are not necessarily mutually exclusive and it is possible that in vivo, both ROS and succinate cooperate to inhibit PHD activity in SDH-deficient tumours [81]. It is important to mention that to date no evidence for an increase in ROS levels in FH-deficient cells has been provided. This accords with the fact that unlike SDH, FH is not part of the ETC and so less likely to generate ROS. As for substrate build up, fumarate accumulation due to FH mutations can also inhibit SDH activity (product-inhibition of SDH—Figs. 2 and 3B). In fact, there is evidence for increased succinate levels in FH-deficient tumours [78].

The crosstalk between succinate and PHDs seems not to be confined to the regulation of HIFα and may be crucial for apoptosis too. It was demonstrated that succinate-mediated inhibition of PHD3 blocks apoptosis of neuronal cells during development [84]. This implies that apoptotic failure, caused by excess succinate due to SDH mutations, plays a role in the pathogenesis of paraganglioma and phaeochromocytoma. A recent study of PHD3$^{-/-}$ mice which demonstrated defects in apoptosis of sympathetic neurons further supports this model [85]. It is yet to be established whether succinate or fumarate inhibit different PHDs thus causing different biological consequences (other than pseudohypoxia). If so, this will explain the differences in the tumour pattern observed in HPGL and HLRCC. Finally, for SDH mutations the role of ROS in PHD inhibition and other downstream effects may also contribute to the differences between these two syndromes.

The genetic reprogramming induced by HIFα stabilization and the change in apoptosis capabilities predispose cells to cancer transformation. Overcoming succinate- and fumarate-related PHD inhibition may have far-reaching consequences for the therapy of tumours with SDH or FH deficiencies. Importantly, it was demonstrated that both succinate and fumarate inhibit PHDs by interfering with the binding of α-ketoglutarate, a required co-substrate of PHD, to the enzyme [86]. An increase in intracellular levels of α-ketoglutarate overcomes the inhibitory effects of succinate and fumarate [86]. This implies that cell permeable α-ketoglutarate derivatives may be developed as therapeutics for HPGL and HLRCC.

6. Conclusions

Warburg's hypothesis was, at the time, a conceptual leap and it resulted in a new understanding of tumour metabolic behaviour. However, almost a century later, the metabolic transformation of cancer is still a riddle. While It is clear today that many, if not most, tumour cells are capable of performing oxidative phosphorylation when forced to, glucose metabolism is increased dramatically in most tumours. Increased glucose metabolism may reflect the need for rapid-production of ATP and/or for anabolic metabolites. Notably, there are examples of tumour cell lines that exhibit inherent decreased mitochondrial functions caused by mutations in either the mtDNA itself or in nuclear-encoded mitochondrial proteins. In other tumours, decreased oxidative phosphorylation could be a consequence of accelerated glycolysis and lactate production due to genetic or environmental alterations. Therefore, owing to the heterogeneity of tumour cells, the oxidative phosphorylation capacity of each particular tumour should be evaluated to determine whether the enhanced glycolysis is indeed a consequence of impaired mitochondrial functions.

This review presented two distinct scenarios, in which mitochondria play a key role in tumorigenesis: mitochondrial dysfunction as the driving cause of tumorigenesis and mitochondrial dysfunction as a 'second hit' in the process of cancer metabolic transformation. In the latter, mitochondria impairment can be the outcome of accelerated glycolysis brought about by the loss of tumour suppressors or the activation of oncogenes. In both cases however, metabolic reprogramming increases the cancer cells' survival and proliferation advantage by increasing ATP production in an oxygen independent manner and providing building blocks for macromolecule biosynthesis, two parameters which are critically required when excessive growth limits nutrient and oxygen supplies. However, it is medically important that regardless of the cause

of metabolic transformation, it may render cancer cells dependent on distinctive metabolic pathways and/or isoenzymes. Identifying and targeting these could take the metabolic transformation process from the realm of intriguing phenomena to the platform of cancer therapeutics.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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