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The altered metabolism of tumors: HIF-1 and its role in the Warburg effect

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Introduction

Almost 90 years ago, Warburg (1930) first reported the propensity for cancer cells to convert glucose to lactate even in the presence of oxygen, a phenomenon he had discovered in his work on tissue slices. Since that time the cause of the "Warburg Effect" has been hotly debated, and it is still not well understood. Interest in this phenomenon is at the present time enjoying high profile attention, as evidenced by an article in *The Economist* in 2007 as well as comprehensive reviews in *Science* (Vander Heiden et al., 2009), *Nature Cancer Reviews* (Denko, 2008) and a complete volume of *Seminars in Cancer Biology* (The Warburg Effect, 2009). This is not only because the Warburg effect underlies the clinical success of FDG-PET as a diagnostic tool in oncology, but also because one of the transcription factors that promotes tumor metabolism, growth, and angiogenesis seems to have a significant role in the Warburg effect. HIF-1 is an oxygen-sensing transcription factor that regulates several hundred genes concerned with many aspects of tumor metabolism and progression. Under hypoxic conditions, a common feature of the tumor environment, the HIF-1dimer is formed and (amongst other things) it enhances aerobic glycolysis through co-ordinated up-regulation of glycolytic enzymes and down-regulation of mitochondrial oxidative metabolism, suggesting the possibility of a role in the Warburg effect (Semenza, 2007).

Whatever cause underlies the cancer (e.g. virus, mutation, DNA damage etc), tumor metabolism appears to be similar across a broad range of cancer types (Griffiths and Stubbs, 2005). In this review we discuss HIF-1 targets that relate specifically to tumor metabolism and cellular energy production, factors that must ultimately determine tumor progression. We also consider the conventional assumption that "to generate the energy needed for cellular processes, most cancer cells rely on aerobic glycolysis" (Vander Heiden et al., 2009) and what proportion of total tumor ATP might be made by this pathway.

Warburg's findings

Most living cells depend mainly on oxidative metabolism to obtain the energy necessary for their function and growth, converting glucose to CO_2 and H_2O when oxygen is present but switching (via the

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Pasteur effect) to anaerobic formation of lactic acid in the absence of oxygen. Warburg, in the early part of the last century, discovered that tumor cells metabolised glucose to lactic acid even in the *presence* of oxygen. He hypothesised that cancer cells may have an impaired respiratory capacity resulting in elevated rates of glycolysis, and this dogma became a major focus of cancer research for several decades. Although the capacity for very 'high glycolysis' is a feature of many tumor types, it is not the cause, as Warburg believed (although see Garber, 2006), nor a universal characteristic of tumors.

Much of Warburg's work had been based on ascites cells and tissue slices of tumors. Tissue slices are poor models from a metabolic point of view as they may be too thick for oxygen to diffuse to all the cells, leading to hypoxia in the central region. Another problem was that Warburg studied very poorly differentiated tumors because these were the only ones that were transplantable in the outbred laboratory animal strains available at that time. They had extraordinarily high rates of lactate production and therefore the findings may have been misleading. Later, the Morris (1965) Hepatomas became available, which represented a wide spectrum of growth rate and degree of differentiation. The slow growing well-differentiated hepatomas had very low aerobic and anaerobic glycolysis closely resembling normal liver, whereas the fast-growing poorly differentiated ones had high rates of glycolysis. The phenotype of these tumors included many other major differences in enzymatic activity and isozyme composition that broadly followed their degrees of differentiation and rates of growth (Weber, 1968, 1977; for review see Stubbs et al., 2003). Since then, many tumor models have been developed. The most commonly used are human tumors grown as xenografts in immunocompromised mice, but for metabolic studies these have the disadvantage that they are initiated by implanting thousands or even millions of cancer cells are (usually subcutaneously rather than in the organ where the tumor would normally arise), so the resulting tumor vasculature is very abnormal (Falk, 1980; Field et al., 1991). Another problem is that repeated passage of the most widely used human xenograft tumors has usually led to selection of a rapidly growing and often, hypoxic phenotype.

More recently various transgenic mouse models that develop spontaneous tumors such as MMTV-NEU-NT mammary tumors (Muller et al., 1988) and autochthonous pancreatic tumors (Hingorani et al., 2005) have been introduced. In principle such tumors, which presumably are initiated by transformation of a single cell, would tend to develop a blood supply similar to that of tumors in patients, and should thus be better than transplants for metabolic studies; however, little work of that kind has so far been reported. None of these models truly represent human tumors, although the autochthonous pancreatic tumors come close in that they resemble human pancreatic cancer histologically and respond to treatment in a similar way (Olive et al., 2009).

The HIF-1 transcription factor

Adequate oxygen availability is crucial to most mammalian cells. Stemming from work on erythropoietin and oxygen sensing, Semenza et al. (1994) identified hypoxia inducible factor-1 (HIF-1) as a heterodimeric transcription factor with HIF-1α (and subsequently HIF-2α or HIF-3α) as the oxygen-responsive subunit and HIF-1β as the constitutively expressed subunit (Wang et al., 1995; Gleadle and Ratcliffe, 1998). The α subunit undergoes O_2-dependent hydroxylation, leading to binding of the von-Hippel-Lindau tumor suppressor protein and subsequent ubiquitin-mediated proteasomal degradation. No hydroxylation occurs under hypoxic conditions, so HIF-1α accumulates and binds to HIF-1β, forming the active HIF-1 complex. HIF-1 upregulates a vast network of genes, by binding to hypoxia response elements (HREs) containing the core sequence 5′-RCGTG-3′ in their promoters (Fig. 1). Thus HIF-1α enables the cell to sense when oxygen availability is insufficient and to undergo adaptive changes in gene expression that either enhance oxygen delivery or promote survival in a hypoxic environment. Intratumoral hypoxia (pO_2 values < 10 mm Hg) has been commonly observed in both human and animal tumors, and is associated with increased risk of invasion, metastasis, and patient mortality. Both this hypoxia and also constitutive properties of tumor cells will cause activation of HIF-1, resulting in transcriptional activation of hundreds of genes, including those involved in energy metabolism, angiogenesis and autophagy (Semenza, 2007). Moreover the adaptive change to a more glycolytic phenotype observed under hypoxia is maintained even during subsequent culture under normoxia, suggesting stable genetic changes (Gatenby and Gillies, 2004).

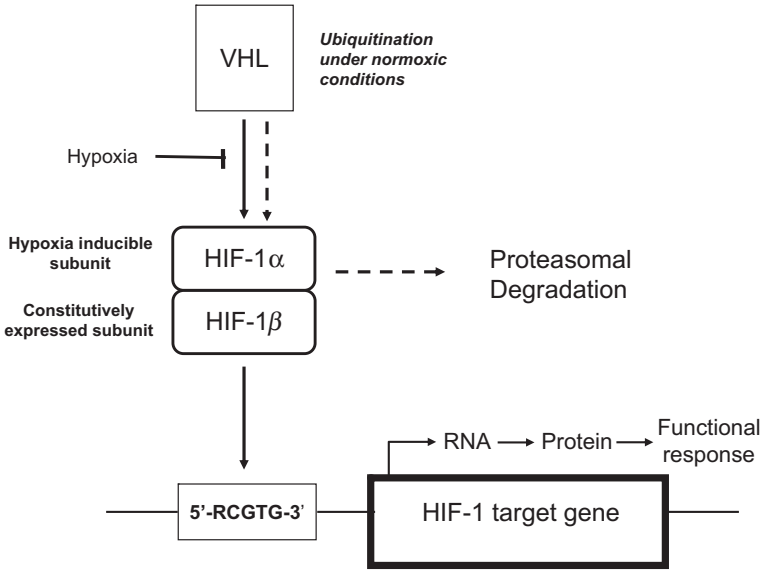

Fig. 1. HIF-1 mechanism. Under well-oxygenated conditions the HIF-1α subunit undergoes an oxygen dependent hydroxylation of conserved prolines and binds to the von-Hippel-Lindau tumor suppressor protein (VHL) targeting it for proteosomal degradation. Under hypoxic conditions HIF-1α binding to VHL is inhibited because prolyl hydroxylation is absent, resulting in its accumulation and dimerisation with HIF-1β (the constitutively expressed subunit) and binding to the core DNA recognition sequence 5′-RCGTG-3′ to increase transcription of many genes including glycolytic enzymes and glucose transporters.

Postulated role of HIF-1 in energy production by tumors

Most cells preferentially metabolise glucose by oxidative respiration when O_2 is available (the Pasteur effect), and the oxidation of one molecule of glucose provides 30–32 ATP molecules (Nelson and Cox, 2005). However, under the partially hypoxic conditions in many solid tumors, or when it is constitutively activated in carcinogenesis, HIF-1 upregulates genes encoding glucose transporters (GLUTs) as well as virtually all of the glycolytic enzymes (see Table 1 and Semenza, 2001; Maxwell et al., 2001). The activation of HIF-1 will therefore increase glucose uptake into the cancer cells. Elevated

Table 1
GENE products concerned with metabolism that are regulated by HIF-1.

Gene product	Metabolic function	Reference
Adenylate kinase 3	Nucleotide metabolism	Semenza (2001)
Aldolase A and C	Glycolysis	Semenza (2001)
Enolase 1	Glycolysis	Semenza (2001)
GLUT 1 and 3	Glucose transporters	Semenza (2001)
Glyceraldehyde-3-P-dehydrogenase	Glycolysis	Semenza (2001)
Hexokinase 1 and 2	Glycolysis	Semenza (2001)
Lactic dehydrogenase A	Glycolysis	Semenza (2001)
Phosphofructokinase L	Glycolysis	Semenza (2001)
Phosphoglycerate kinase 1	Glycolysis	Semenza (2001)
Pyruvate kinase M	Glycolysis	Semenza (2001)
Triosephosphate isomerase	Glycolysis	Semenza (2001)
Pyruvate Dehydrogenase	TCA cycle	Kim et al. (2006)
Pyruvate dehydrogenase kinase 1	TCA cycle	Papandreou et al. (2006)
Cytochrome oxidase	Mitochondrial Respiration	Semenza (2007)
Carbonic anhydrase	pH regulation	Wykoff et al. (2000)
MCT4	Lactate efflux	Ullah et al. (2006)

glucose uptake is the molecular characteristic of tumors that underlies the success of FDG-PET imaging, which can detect and map tumors by detecting the rate of uptake of a radioactive glucose analogue (2-^{18}Fluorodeoxy glucose, see Fig. 2). PET is becoming a very important tool in oncology for assessing the presence and spread of tumors and metastases.

It has been hypothesised (Semenza, 2007) that the increase in glycolytic flux compensates for the reduced efficiency of ATP production (only 2 molecules of ATP per molecule of glucose are produced glycolytically compared with 30–32 from complete oxidation). Modern theories of metabolic regulation suggest that several enzymes in a pathway need to be up-regulated to get any significant changes in pathway flux (Fell, 1996), and the co-ordination by HIF-1 of up-regulation of expression of genes encoding the glucose transporters and glycolytic enzymes suggests that HIF-1 may coordinate such a system. Studies by Gillies' group (Robey et al., 2005) show positive correlations between the cellular level of HIF-1α and rates of lactate production (used as a surrogate for glycolysis) depending on the aggressiveness of the tumor cell type (reflecting Weber's findings in 1968 – see below) and also suggesting a link between HIF-1α and the Warburg effect.

Another metabolic target of HIF-1 is pyruvate dehydrogenase kinase (PDK), which prevents or slows the conversion of pyruvate to acetyl-CoA for entry into the tricarboxylic acid (TCA) cycle by inhibiting the PDH enzyme complex (Kim et al., 2006, Papandreou et al., 2006). This limitation of acetyl-CoA entry into the TCA cycle down-regulates mitochondrial respiration, and causes accumulation of pyruvate, which is then converted into lactate via lactate dehydrogenase (another HIF-1-regulated enzyme), regenerating NAD^+ for continued glycolysis by O_2-limited cells. It has also been hypothesised that this metabolic switch shunts glucose metabolites from the mitochondria to glycolysis in order to maintain ATP production, optimizing the utilisation of the available O_2 and glucose in order to most efficiently generate ATP. The electron transport chain (ETC) activity is down-regulated by altering the subunit composition of cytochome c oxidase (COX), thus minimising reactive oxygen species (ROS) generation (Kim et al., 2006; Semenza, 2007).

In summary, these HIF-controlled metabolic reactions might ultimately influence the balance between oxidative phosphorylation and aerobic glycolysis, and might therefore tend to induce the Warburg effect. However, this raises a question: to what extent does this increase in glycolysis contribute to tumor energy needs?

Energetic "efficiency" in tumors

The efficiency of energy metabolism is often discussed on the unstated assumption that the cell concerned functions entirely alone. It is often said, for instance, that glycolytic metabolism is

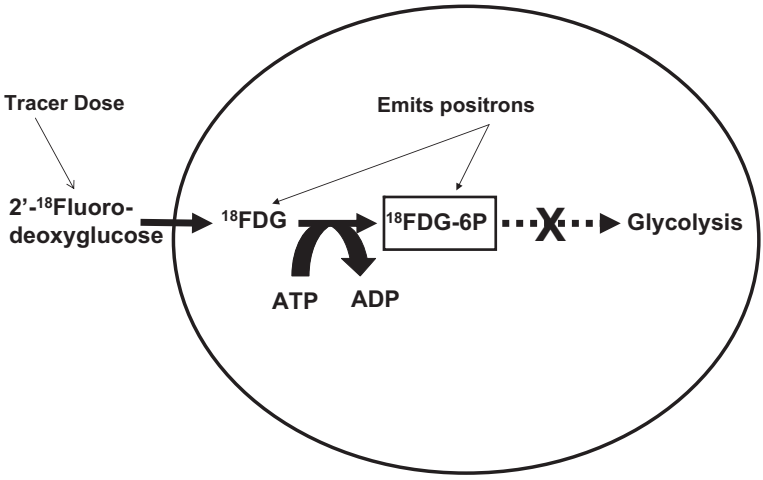

Fig. 2. Conventional FDG-PET. Scheme to describe ^{18}FDG-PET imaging which is used to measure the uptake of ^{18}FDG glucose and used as a tool in Clinical Oncology.

"inefficient" because it produces only two ATP from each glucose molecule instead of 30–32 from complete oxidation, and that this inefficiency would be expected to impair the growth of tumors. However, many tissues in normal organisms function largely (e.g. exercising white muscle) or exclusively (e.g. mammalian red blood cells) by glycolysis, and excrete lactate into the circulation. These glycolytic tissues suffer no impairment because of their "inefficient" ATP synthesis – they simply take in more glucose from the blood stream. Similarly the organism suffers little as the lactate is taken up by other tissues, particularly the liver where most of it is used to make more glucose (i.e. the Cori cycle). There is a metabolic price to be paid in terms of the energy expended on each turn of this cycle, but since the cycle has not been eliminated by natural selection one presumes that the price is worth paying. Tumor cells exploit the same mechanism, taking in as much glucose as they need from the effectively infinite supply in the host's blood stream and excreting lactate for the host to recycle (recently it has been found that parts of some tumors also take up and oxidise lactate (Sonveaux et al., 2008)).

Estimates of the proportion of oxidative vs glycolytic metabolism in tumors

Does the up-regulation of glycolysis and the subsequent generation of 2 ATP (rather than 30–32 from complete oxidation) from every glucose converted to lactate really fill the energy gap when the tissue pO_2 is low? What proportion of the tumor energy (ATP) is provided by glycolytic metabolism to lactate in comparison to oxidative metabolism? These questions have been addressed mainly in cultured cells (for review see Moreno-Sánchez et al., 2007; Zu and Guppy, 2004). From measurements of oxygen and glucose consumption and lactate production the proportion of oxidatively or glycolytically produced ATP can be calculated (Guppy et al., 2002). A meta-analysis of the studies done (again, mainly on cultured cells) over 40 years (Zu and Guppy, 2004) showed that the average contribution of glycolysis to ATP production in a range of 27 tumor types was $17 \pm 18\%$, was not significantly different from the glycolytic ATP contribution of $20 \pm 21\%$ in 16 normal tissues. For instance, in MCF-7 breast cancer cells the ATP production over 5 days of growth was 80% oxidative and 20% glycolytic, with glucose and glutamine contributing 40% to the ATP turnover (Guppy et al., 2002). In contrast, Busk et al. (2008) found that glycolysis accounted for about 60% of ATP production in other types of cultured cells.

Several issues need to be considered in such studies: firstly, do cultured cells represent the biology and growth habits of tumors *in vivo*? One problem is that cell culture is a very artificial environment, and standard lines such as MCF-7 have become adapted to it over numerous generations. Furthermore, cultured tumor cells are usually grown in a very nutritious well-oxygenated medium (although it is possible to make it hypoxic) at a very high medium/cells ratio, whereas *in vivo* tumors are often poorly vascularised and partly hypoxic. This can lead to significantly different growth profiles – for example RCC786-0 cells manipulated to overexpress HIF-1α or HIF-2α have similar growth curves to control (EV) cells when grown in culture (Raval et al., 2005) whereas the growth profiles *in vivo* are very significantly different from each other (see Fig. 3).

Ideally, one would prefer to measure the glycolytic and oxidative flux in solid tumors growing in their original host, but tumors do not have a regular blood supply, so one cannot easily measure their uptake and output of metabolites. However, some specialised solid tumor models do permit direct sampling of metabolites entering and leaving the tumor. In the most comprehensive study of this kind, human tumor xenografts were grown in nude rats on pedicles containing the epigastric artery and vein, and prevented from acquiring other vasculature by polyethylene sheaths (Kallinowski et al., 1988, 1989). This defined blood supply allowed metabolic balance studies in which the arterial and venous flow rates, and their contents of substrates and products were measured; bioenergetic calculations have subsequently been performed from these data (Griffiths et al., 2001; Zu and Guppy, 2004).

A more complete analysis of the published data (see Table 2) has shown that only $\sim$12% of the energy requirement of these tumors came from the phosphorylation of ATP during glycolysis to lactate; the remainder came from oxidative metabolism. It should be noted that in these balance studies across isolated tumors unlike the cultured cell experiments mentioned above, in which glutamine was a major substrate, a net uptake of glutamine was found in only 20% of the xenografts whereas there was a net release in 61% and no change in the others (Kallinowski et al., 1987).

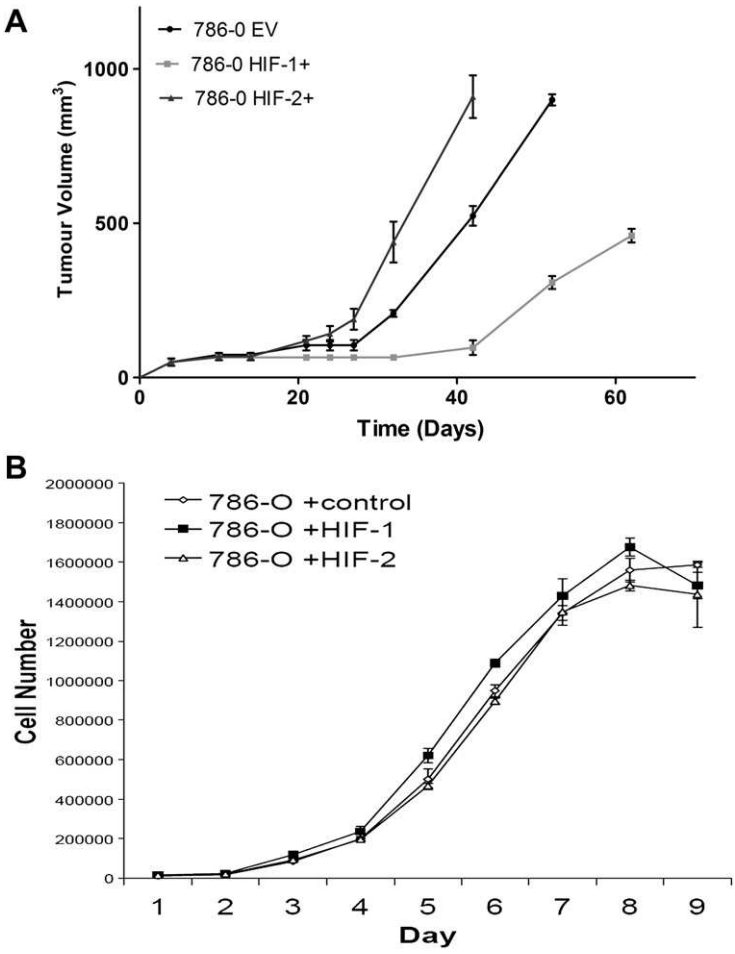

Fig. 3. Different patterns of growth in cultured cells and solid tumors. Differences in growth rates of CCRCC 786-0 cells transfected with HIF-1α (●HIF-1+), or HIF-2α (▲HIF-2+) or empty vectors (■EV) when grown as A) tumors *in vivo* or B) cultured cells *in vitro*.

Even these meticulous studies by Kallinowski et al., (1988, 1989) are open to the criticism that tumors implanted subcutaneously are metabolically different from those that arise spontaneously (Field et al., 1991; Olive et al., 2009), mainly because the blood supply induced by the growth of thousands or millions of implanted cells is even more chaotic than that induced by a tumor growing from a single cell (Falk, 1980). Ideally, therefore, one would like to perform metabolic balance studies on spontaneous tumors, and best of all would be tumors in patients. Holm et al. (1995) performed such a balance study on "real" human tumors *in situ*, by cannulating the mesenteric vessels supplying human colonic carcinomas during operations for bowel resection; in these tumors the energy obtained from glycolysis can be calculated to be 3.1% of the total energy consumption (see also Table 2).

A number of possible confounding factors may skew the calculations. (i) In cell culture, the metabolites measured are uniquely from tumor cells, whereas tumors *in vivo* contain host tissues that also contribute to the measurements. Holm et al. (1995) measured glucose uptake in adjacent peripheral tissues and found that it was less than in the colon tumors by a factor of 30, suggesting that such errors would be negligible. However, one would ideally like to have such data for the tissues of the tumor stroma, and to know the relative numbers of tumor and host cells. (ii) Metabolites other than

Table 2
SUBSTRATE uptake and utilisation data from balance studies on human tumors grown either in nude rats or studied *in situ*.

	Human tumour xenografts in nude rats[a]	Human colon carcinomas *in situ*[b]
Lactic acid output (nmol/g/min)	527	220
Glucose consumption (nmol/g/min)	401	320
O_2 consumption (nmol/g/min)	588	–
Glucose available for oxidation (nmol/g/min)	144[c]	208[d]
CO_2 output (from O_2)(nmol/g/min)	588	–
CO_2 output (from glucose)(nmol/g/min)	850	1296[e]
ATP from glycolysis (nmol/g/min)	527	220
ATP from glucose oxidation (nmol/g/min)	4402	7055
% ATP from glycolysis	**12%**	**3.1%**

[a] From Kallinowski et al. (1988, 1989).
[b] From Holm et al. (1995).
[c] Glucose uptake less half the lactate output with allowances for ketone bodies.
[d] Glucose uptake less half the lactate output with allowances for pyruvate.
[e] With allowances for FFA and ketone bodies.

glucose may be consumed by the tumor. Kallinowski et al. (1988) found that some ketone bodies were consumed by human tumor xenografts. Substituting these data into the data from their 1989 paper, we were able to calculate (Table 2) that ketone bodies would have contributed less than 1% of the carbon uptake. No allowance was made for carbon deposition since Newsholme and Board (1991) showed that the rates of both glycolysis and glutaminolysis are greatly in excess (greater than 400-fold) of the requirements for the biosynthetic processes. The bowel tumors studied by Holm et al. (1995) took up negligible quantities of ketone bodies but they did take up small but significant amounts of essential amino acids and free fatty acids. Because of their high carbon content the fatty acid uptake would lead to a significant amount of ATP oxidative phosphorylation (131 ATP/mole, Atkinson, 1977) so that has also been included in the calculations shown in Table 2. (iii) Recent studies have shown that lactate formed in tumor cells can be taken up again and oxidised by other cells in the same tumor (Sonveaux et al., 2008).

In summary, all these balance studies, both in cultured cells and tumors *in vivo*, suggest that cellular ATP was mainly provided (>80%) by oxidative metabolism. Clearly these results do not support the conventional assumption that tumor cells rely on aerobic glycolysis for their energy needs (e.g. Vander Heiden et al., 2009)

Inhibition studies of HIF-1 metabolic target genes and their outcome

The high profile interest in the Warburg Effect was emphasised when the studies discussed below formed the basis of an article in *The Economist* (2007) concerning dichloroacetate (DCA), a specific PDK-1 inhibitor. Bonnet et al. (2007) had demonstrated that DCA decreased tumor cell proliferation and increased apoptosis in A549 lung cancer cells, suggesting that the tumor glycolytic pathway could be a target for anticancer drug development. Inhibiting PDK caused activation of pyruvate dehydrogenase, providing more pyruvate for the TCA cycle and a more oxidative metabolism; this resulted in the production of ROS which induced tumor cell death by apoptosis. And in a study of another HIF-1 metabolic target gene, LDH-A was knocked down with *i*RNA to slow the conversion of pyruvate to lactate. This resulted in a decreased mitochondrial membrane potential, increased oxidative metabolism and decreased tumor cell growth, whilst increasing the survival rate of animals bearing the tumors (Fantin et al., 2006). Both these studies suggest that decreasing glycolytic and increasing oxidative metabolism in tumors can slow tumor growth (see also Michelakis et al., 2008). The implication of this is that the transcription factor HIF-1, by targeting certain genes associated with energy metabolism, somehow gives the cancer cell an advantage – and that when some of the target genes of HIF-1 are inhibited tumor growth slows. Overall these experiments suggest that if tumor metabolism can be made more oxidative, tumors growth rates may be decreased and thus, by implication, that the Warburg effect may give tumors a growth advantage, albeit by indirect mechanisms.

Other factors that may play a role in the Warburg effect

The following examples suggest that there are other factors that may also play a role in the Warburg effect; i) Hexokinase (HK) may have exclusive access to mitochondrially generated ATP (Pastorino and Hoek, 2003) through interaction with the voltage dependent anion channel (VDAC); ii) most tumors (Mazurek et al., 2005) express a less active isozyme of pyruvate kinase, PKM2, instead of the PKM1 of normal tissues. This results in accumulation of glycolytic intermediates that can be used for anabolism in rapidly growing tumors. Christofk et al. (2008) recently showed that replacement of PKM2 by normal PKM1 abolished the Warburg effect in cancer cells; iii) c-Myc has also been shown to regulate genes encoding glycolytic enzymes (Kim *et al.*, 2004), and through cooperation with HIF-2α, it promotes cell proliferation under conditions in which HIF-1α antagonizes c-Myc (Gordan et al., 2007) or cooperates in a context-dependent manner (Kaelin and Ratcliffe, 2008); iv) akt, a pro-survival signalling protein, has been shown to be able to upregulate glycolysis *independently* of HIF-1 (Arsham et al., 2004).

Alternative hypotheses for the Warburg effect

The rationale behind the hypoxia-induced metabolic switch may not be to maintain ATP production at all, since the proportion of energy derived glycolytically, compared to oxidatively, in tumors is relatively small (3–12%). An alternative rationale has been proposed by Gillies and Gatenby (2007), who hypothesise that the increased glucose uptake and glycolysis observed in tumors are not used for increasing energy production but rather for the production of extracellular acid, which gives cancer cells a competitive advantage and facilitates invasion. The metabolism of glucose to lactic acid in the presence of oxygen, despite its inefficiency in ATP yield, results in increased acid production and regional acidosis. These adaptations confer a significant growth advantage because the cancer cells, through alteration to an acidic environment, is toxic to normal cells, but not to the cancer cells and the acidic extracellular pH leads to increased motility and invasion. Although this hypothesis is usually discussed in the context of the Warburg effect, it does not matter whether the acid is lactate or carbonic acid, both of which have similar pK_a values. However, Table 2 shows that in the studies of Kallinowski et al. (1989) the acid load produced from oxidatively-produced CO_2 and glycolytically-produced lactic acid would have been approximately equivalent (588 or 850 nmol CO2/min/g, vs 527 nmol lactate/min/g). And in the human tumors studied *in situ* by Holm et al. (1995) CO_2 output (1296 nmol/g/min) was more than 5 times higher than lactate + pyruvate output (224 nmol/g/min). This implies that the tumor microenvironment will be acidic, whether the glucose is metabolised oxidatively or glycolytically (see also Swietach et al., 2009).

Another possible mechanism whereby the Warburg effect could allow cancer cells to out-compete more normal cells (in this case both host cells and other cancer cells with more oxidative phenotypes) has been suggested by the work of Pfeiffer et al. (2001). They calculated the growth characteristics of a tissue consisting of purely oxidative cells and cells with both glycolytic and oxidative pathways, competing for the same substrate. Since the cells with an active glycolytic pathway use 15–16 times more glucose than oxidative cells to generate ATP they tended to deplete their surroundings of glucose, to the detriment of the more "frugal" oxidative cells. This phenomenon tends to occur when glucose supply is limited, as could tend to happen in the poorly vascularised central region of a tumor. Both this hypothesis and the acid-secretion hypothesis of Gillies and Gatenby (2007) are compatible with the model in which tumor cells synthesise a substantial amount of their ATP by oxidative metabolism.

Clinical cancers associated with HIF-1 accumulation due to mutations which affect metabolism

In addition to hypoxia, a number of rare cancer mutations are associated with HIF-1 accumulation. If the pVHL tumor suppressor protein is mutated or absent then HIF-1α and HIF-2α, even under oxygenated conditions, are not degraded and many of their targets will be expressed, including up-regulation of glycolysis and suppression of oxidative phosphorylation, leading to a very high risk of renal carcinoma (Kaelin, 2008).

Loss-of-function mutations in fumarate dehydratase and succinate dehydrogenase (associated with rare familial tumor syndromes) increase levels of fumarate or succinate respectively, and inhibit HIF-1α hydroxylation by competitively blocking the binding of 2-oxogluarate, thus activating HIF (Isaacs et al., 2005; Pollard et al., 2005). Similarly in most malignant gliomas (a common form of brain tumor), deficiency of isocitrate dehydrogenase (Yan et al., 2009) results in lower levels of the α-ketoglutarate needed for hydroxylation and proteosomal degradation of HIF-1 (Thompson, 2009). Other metabolites have also been implicated, including pyruvate and oxalacetate. Activation of HIF-1 by these mechanisms will increase the glycolytic flux and thus induce the Warburg Effect in these cancers.

So not only does HIF-1 act as a major regulator of cell metabolism, but changes in cell metabolism (caused in these latter cases by mutations of metabolic enzymes) can have important effects on the HIF response. Recurrent mutations in the genes that regulate metabolism of these types of cancer are the best evidence that alterations in cellular metabolism contribute to the pathology of human cancer. Furthermore, many HIF-1 target genes have been associated with, and correlated to, the aggressiveness of clinical cancers. For example, high levels of lactate (Walenta et al., 2004) and overexpression of carbonic anhydrase (Giatromanolaki et al., 2001) have been linked to poor clinical outcome.

Conclusions

Even though the Warburg effect has been known for almost a century it is still not completely understood. The evidence presented above suggests that the transcription factor HIF-1, by increasing glucose uptake and lactate production, plays an important role in the Warburg effect. Inhibition of this process (with DCA) by diverting the glucose away from glycolysis (and lactate production) into oxidative metabolism inhibits tumor growth. In addition, a number of tumors are now thought to be caused by mutations in TCA cycle enzymes that lead to activation of HIF-1.

The evidence that we have reviewed also suggests that although the Warburg effect causes tumors to have elevated glucose uptake and lactate output, the conventional assumption that 'tumors rely on aerobic glycolysis for their energy needs' is probably wrong. In the few cases where it has been possible to perform metabolic balance studies across tumors *in vivo* it has been found that although the lactate output is greater than that of most normal tissues, the vast majority of ATP synthesis still takes place by oxidative metabolism. The reason for this is that oxidative ATP synthesis uses 15-fold less glucose than glycolysis, so a substantially increased flux through the glycolytic pathway may have only a minor effect on ATP synthesis.

We conclude, therefore, that the Warburg effect in tumors is not primarily aimed at generation of energy, but rather at utilising glucose in a manner suited to proliferation rather than to ATP production (Vander Heiden et al., 2009). Indeed it now seems possible that the apparently wasteful consumption of large quantities of glucose to generate small amounts of ATP could still give cancer cells a selective advantage either by causing hyperacidity of the surrounding host tissues (though even here a substantial proportion of the acid load is from CO_2), or simply by starving adjacent oxidative cells.

Personal memoir – from Warburg to HIF-1 and back

The 50th anniversary of the Advances in Enzyme Regulation meeting is indeed a milestone at which the Warburg effect should be re-visited. I may be one of the few people left who met Otto Warburg (1883–1970), when he visited Professor Sir Hans Krebs, the Director of the Metabolic Research Lab in 1969. Hans Krebs had been Warburg's student in Berlin between 1926 and 1930 and I was Hans Krebs's student. In the late 1960s George Weber was a visiting scientist in the Oxford lab where he would arrive straight from Indianapolis with rats carrying implanted Morris hepatomas! That was my first introduction to studying cancer, and in collaboration with Derek Williamson and Hans Krebs, we published 2 papers in Cancer Research (Williamson et al., 1970; Weber et al., 1971). After Krebs died in 1981, I worked briefly with Peter Ratcliffe, now Nuffield Professor of Medicine in Oxford, who was perfusing kidneys at the time and who, through his interests in renal medicine, went on to make interesting and exciting discoveries about the action of HIF-1 (Jaakkola et al., 2001). Later, in 1984, I joined John Griffiths at St George's Hospital Medical School who worked on Magnetic Resonance (MR) of Cancer. In 1998 we started a collaboration with Adrian Harris, Professor of Medical Oncology in Oxford, studying

in vivo metabolism non-invasively in a model of HIF-1 deficient tumors, using MR. At that time there were <50 publications on HIF-1 in cancer listed on PubMed – now in 2009 there are nearly 3000! We continue this work (with the help of various students), at the CRUK Cambridge Research Institute to where we moved in 2006. Since the 1980s I have been honoured to be invited to the Advances in Enzyme Regulation meetings every year and I am looking forward to the 50th. There cannot be many observations such as Warburg's that continue to intrigue researchers and generate so much debate, including an article in *The Economist*, over nearly a century!

Summary

In recent years it has been suggested that the Warburg Effect (i.e. the propensity for tumors to convert glucose to lactate even in the presence of oxygen) is caused by activation of HIF-1, an oxygen-sensing transcription factor that regulates several hundred genes concerned with many aspects of tumor metabolism and progression. HIF-1 activation occurs both under hypoxic conditions, a common feature of the tumor environment, and also constitutively, at least in some tumor types. Constitutive HIF-1 activation would induce the aerobic glycolysis described by Warburg. HIF-1 enhances aerobic glycolysis through co-ordinated up-regulation of glycolytic enzymes and down-regulation of mitochondrial oxidative metabolism, all phenotypic features consistent with the Warburg Effect. Increased glucose uptake induced by the Warburg effect probably underlies the clinical success of FDG-PET as a diagnostic tool in oncology. However, contrary to the widespread opinion that tumors 'rely on aerobic glycolysis for energy,' balance studies across several human tumor types *in vivo,* show that the ATP generated by glucose metabolism to lactate is still a small proportion (<20%) of the ATP generated by oxidative metabolism. Nevertheless, the increased glucose consumption and lactate output characteristic of the Warburg Effect could give cancer cells a competitive advantage, both by acidifying the surrounding medium and by starving adjacent oxidative cells of glucose. We conclude, therefore, that the role of constitutively activated HIF-1 in increasing the uptake and metabolism of glucose in the presence of oxygen, is not primarily to enhance ATP production, but is a metabolic strategy conducive to proliferation and acidification, which gives tumor cells a selective advantage. A better understanding of the biology underlying the Warburg effect may lead to new anticancer treatments.

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