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REVIEW

Warburg revisited: imaging tumour blood flow and metabolism

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Abstract

In the 1930s, Otto Warburg reported that anaerobic metabolism of glucose is a fundamental property of all tumours, even in the presence of an adequate oxygen supply. He also demonstrated a relationship between the degree of anaerobic metabolism and tumour growth rate. Today, this phenomenon forms the basis of tumour imaging with fluorodeoxyglucose positron emission tomography (FDG-PET). More recently, Folkman has demonstrated that malignant growth and survival are also dependent on tumour vascularity which is increasingly evaluated *in vivo* using techniques such as contrast enhanced computed tomography or magnetic resonance imaging (MRI). Although it is reasonable to hypothesise that the metabolic requirements of tumours are mirrored by alterations in tumour haemodynamics, the relationship between tumour blood flow and metabolism is in fact complex. A well-developed tumour vascular supply is required to ensure a sufficient delivery of glucose and oxygen to support the metabolism essential for tumour growth. However, an inadequate vascularisation of tumour will result in hypoxia, a factor that is known to stimulate anaerobic metabolism of glucose. Thus, the balance between tumour blood flow and metabolism will be an important indicator of the biological status of a tumour and hence the tumour's likely progression and response to treatment. This article reviews the molecular biology of tumour vascularisation and metabolism, relating these processes to currently available imaging techniques while summarising the imaging studies that have compared tumour blood flow and metabolism. The potential for vascular metabolic imaging to assess tumour aggression and sub-classify treatment response is highlighted.

Keywords: *Tumour perfusion; tumour metabolism; angiogenesis; tumour characterisation; response evaluation.*

Introduction

The phenomena known as the 'Warburg Effect' was described by Otto Warburg (1883–1970) during his lifetime of work into cellular metabolism and respiration, for which he was awarded the Nobel Prize in 1931[1,2]. He recognised that glucose can be metabolised either by combination with oxygen, i.e. respiration, or by glycolysis to produce lactate. He also observed that a change from oxidative phosphorylation to the less energy efficient glycolysis, even in the presence of an adequate supply of oxygen, is a fundamental property of the metabolism of cancer cells and that the rate of glycolysis correlated with tumour growth. Today, Warburg's findings underpin the principles of tumour imaging with fluorodeoxyglucose positron emission tomography (FDG-PET).

The Warburg Effect and oxygen delivery

The later identification of hypoxia inducible factor 1 (HIF-1) by Gregg Semenza in 1991 has provided further understanding of the mechanism by which cancer cells exhibit the increased aerobic glycolysis described by Warburg[1]. HIF-1 is a transcription factor which up-regulates a large number of cellular processes that confer a survival advantage to cancer cells. In particularly, HIF-1 increases expression of Glut-1 glucose transporters and hexokinase which are the major determinates of glucose uptake and metabolism. Another important effect of increased HIF-1 activity is increased production of vascular growth factors that stimulate new vessel formation and increased blood flow. Folkman has demonstrated that such new vessel

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formation (angiogenesis) also promotes tumour growth and survival[3].

HIF-1 is frequently expressed constitutively by tumours as a consequence of oncogene mutations including the p53 and Von Hippel Lindau (VHL) genes. Mutations in p53 are commonly found in a variety of tumour types; VHL mutations are particularly associated with renal cancer. The linkage between oncogene mutation, increased expression of HIF and accumulation of FDG has been demonstrated recently by microPET studies showing a two-fold increase in glucose metabolism in VHL knockdown tumour xenografts[4]. Clinical PET studies show that approximately 50–70% of renal cancers demonstrate FDG uptake, consistent with the expected frequency of VHL oncogene mutations in this tumour type[4].

On the basis of constitutive expression of HIF-1 by tumours, it would be reasonable to expect tumour blood flow and metabolism to increase in parallel. Indeed, high levels of angiogenesis and elevated glucose metabolism are both associated with increased metastatic potential and poor patient survival for a range of cancers[5–12]. However, tumour HIF-1 activity can be increased further by tissue hypoxia, which occurs when a tumour outgrows its blood supply. This additional HIF-1 activity ensures adaptation of the tumour to the hypoxic environment by producing an even greater increase in glucose metabolism beyond that secondary to oncogene effects alone, along with other metabolic changes which further increase tumour aggression and resistance to treatment[13].

Therefore, the balance between tumour vascularity and metabolic status offers important information concerning the tumour microenvironment. High glucose metabolism with increased vascularity represents a different biological status within the tumour than high metabolism with poor vascularity, the latter indicating adaptation to hypoxia. Low glucose metabolism with poor vascularity suggests a failure of the adaptive response to hypoxia and/or reduced oncogene effects.

Techniques for imaging tumour blood flow and metabolism

FDG-PET has become an established technique for imaging tumour metabolism in clinical practice and research. Although tumour perfusion imaging is used less frequently in clinical practice, a range of techniques is available including positron emission tomography (PET) with ^{15}O-labelled water, dynamic contrast enhanced magnetic resonance imaging (MRI), contrast-enhanced perfusion computed tomography (CT) and ultrasound. However, there is a growing interest in the use of intravenous contrast media during PET-CT[14]. Extending these applications for contrast media to include perfusion CT would enable anatomical information about tumours to be co-registered with not only metabolic information but also perfusion data in a single examination, without the need for an on-site cyclotron (Fig. 1). The use of CT to assess perfusion as opposed to administration of a second PET tracer such as ^{15}O-labelled water circumvents the need for an on-site cyclotron. Furthermore, because CT depicts perfusion data with higher spatial resolution, some of the limitations of PET perfusion studies can be avoided, including the underestimation of perfusion values in small tumours due to the partial volume effect and the spillover of counts from adjacent structures with high blood flow (e.g. heart, aorta, liver)[15]. CT measurements of perfusion in tumours have also been shown to correlate

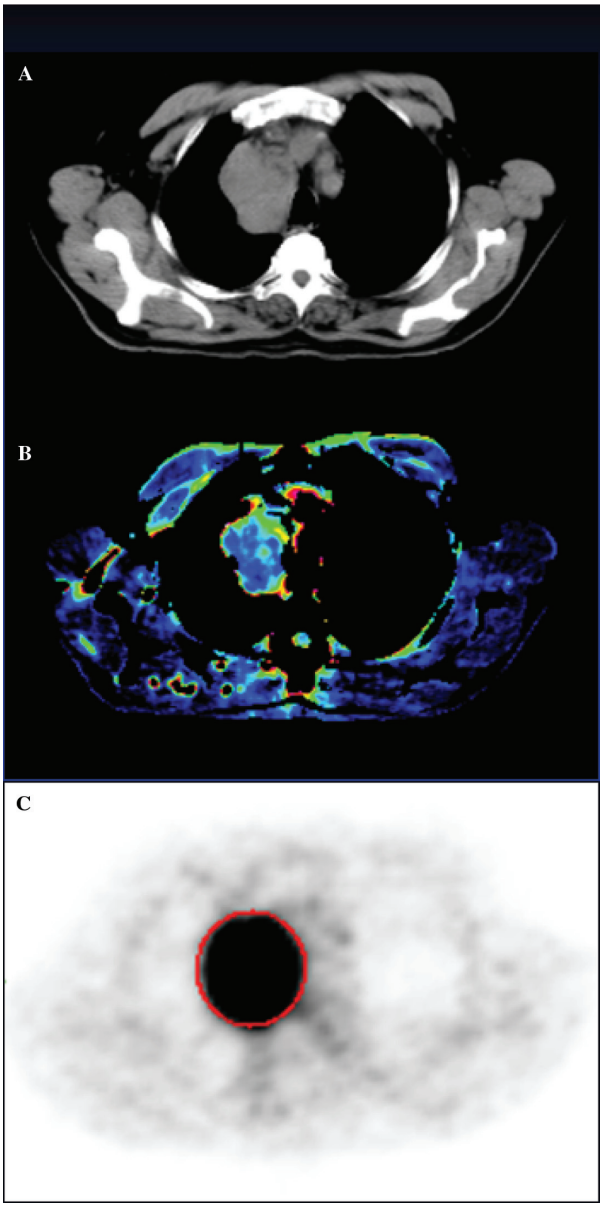

Figure 1 **Conventional CT (A) and images of tumour blood flow (B) and glucose metabolism (C) acquired using perfusion CT and FDG-PET, respectively, from a patient with non-small cell lung cancer.**

with polarographic probe measurements of tumour oxygenation[16].

Imaging studies of tumour blood flow and metabolism

Imaging studies using a range of techniques have shown the relationship between tumour blood flow and glucose metabolism to be highly variable[17–28] (Table 1). Factors influencing this relationship include tumour type, grade and size. Moderate correlations between tumour vascularity and metabolism have been observed in cerebral glioma and breast cancer[17–19]. In non-small cell lung cancer (NSCLC) and cancers of the head and neck, the relationship between tumour circulation and metabolism appears to be dependent on tumour size. Blood flow and metabolism correlate in NSCLCs smaller than 2.5–3.0 cm in diameter, whereas larger tumours exhibit glucose metabolism in excess of blood flow[20–23]. In head and neck cancer, Hirasawa *et al.*[28] observed no correlation between perfusion and metabolism for tumours smaller than 8 cm^2, whereas an inverse correlation was found for larger tumours. Uncoupling of flow and metabolism has also been observed in pulmonary metastases[24]. Studies of liver tumours have shown a negative correlation between blood flow and metabolism, with the ratio of metabolism to blood flow increasing as tumours grow larger[25–27].

An association between mismatched tumour blood flow and metabolism and adverse tumour biology has been illustrated by many of these studies. Aronen *et al.*[17] found that uncoupling of vascularity and metabolism was a feature of high-grade gliomas; Mankoff *et al.*[18] showed that breast cancers with a high ratio of glucose metabolism to perfusion were less likely to respond favourably to treatment. Miles *et al.*[23] found a high metabolic flow difference to be more likely in advanced NSCLC. This adverse impact of high glucose metabolism with low vascularity is also illustrated by separate imaging studies of head and neck cancer that show low perfusion and high FDG uptake to be independent predictors for poor local control following treatment[29,30].

Imaging can also demonstrate regional areas of uncoupling of vascularity and metabolism within tumours (Fig. 2). In their study of locally advanced breast cancer, Mankoff *et al.*[18] illustrated a case in which high metabolism but low blood flow were observed at the centre of the tumour. Following chemotherapy, there was a substantial reduction in tumour size but the patient was left with a core of residual viable tumour, suggesting that regional areas of mismatch may have prognostic implications. Imaging may also depict areas of flow–metabolic mismatch adjacent to a region of frank necrosis (Fig. 2). This finding is in accordance with the results of autoradiographic studies of tumour allografts in which the greatest FDG uptake was found adjacent to areas of necrosis, correlating with local increases in the expression of Glut-1 and hexokinase[31]. Galie *et al.*[32] demonstrated that the epithelial and mesenchymal compartments of syngeneic tumour models exhibit reciprocal patterns of vascularity and metabolism. High vascularity relative to metabolism was found in the stromal capsule and intra-tumoural connectival septa, whereas tumour parenchyma exhibited lower vascularity but greater metabolism.

Table 1 Summary of imaging studies comparing tumour vascularity and metabolism

Study	Tumour type	Techniques	Findings
Aronen *et al.*[17]	Glioma	DC-MRI, FDG-PET	Maximum CBV correlates with maximum FDG ($r = 0.573$, $p = 0.023$)
Mankoff *et al.*[18]	Breast	$H_2{}^{15}O$-PET, FDG-PET	Perfusion and metabolism weekly correlated. High metabolism-flow ratio predicts poor treatment response
Semple *et al.*[19]	Breast	DC-MRI, FDG-PET	Moderate correlation between vascularity and metabolism
Hunter *et al.*[20]	NSCLC	DC-MRI, FDG-PET	Correlation between vascular physiology and glucose metabolism in Stage IIIA ($r = 0.76$, $p < 0.01$)
Tateishi *et al.*[21]	NSCLC	Perf CT, FDG-PET	Vascularity and metabolism correlate in surgically resectable tumours
Hoekstra *et al.*[22]	NSCLC	$H_2{}^{15}O$-PET, FDG-PET	No correlation between perfusion and metabolism in stage IIIA-N2
Miles *et al.*[23]	NSCLC	Perf CT, FDG-PET	Correlation between vascularity and metabolism in small tumours only ($r = 0.85$, $p = 0.03$)
Veronesi *et al.*[24]	Lung metastases	Perf CT, FDG-PET	FDG uptake and angiogenesis independent
Fukuda *et al.*[25]	HCC, CCC and colorectal liver metastases	$H_2{}^{15}O$-PET, FDG-PET	Negative correlation ($r = -0.713$, $p = 0.006$)
Stewart *et al.*[26]	Liver tumours (animal model)	Perf CT, FDG-PET	Glucose metabolism increases and blood flow decreases as tumours grow
Williams *et al.*[27]	Colorectal liver metastases	Perf CT, FDG-PET	Ratio of metabolism to blood flow increases with tumour size
Hirasawa *et al.*[28]	Head and neck cancer	Perf CT, FDG-PET	Negative correlation between perfusion and metabolism for tumours >8 cm^2

DC-MRI, dynamic contrast-enhanced magnetic resonance imaging; CBV, cerebral blood volume; $H_2{}^{15}O$, oxygen-15 labelled water; HCC, hepatocellular carcinoma; CCC, cholangiocarcinoma; Perf CT, perfusion CT.

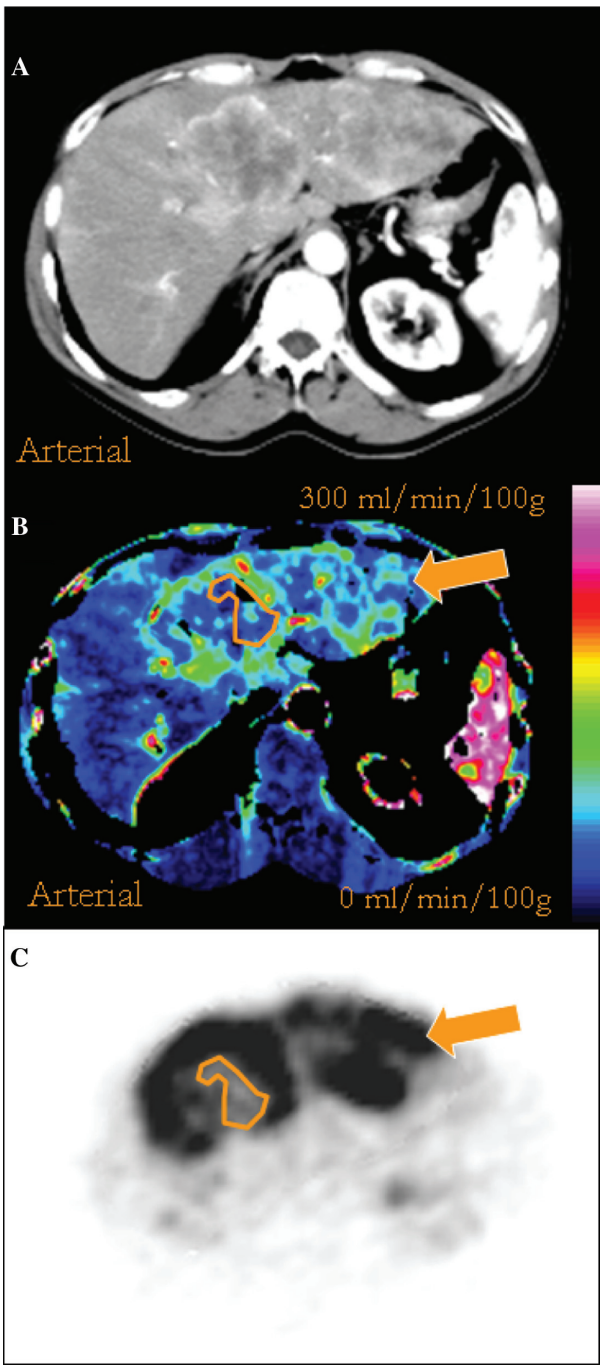

Figure 2 **Conventional contrast-enhanced CT (A), perfusion CT (B) and FDG-PET (C) images of a large hepatic metastasis from colorectal cancer demonstrating regional areas of mismatch between vascularity and metabolism. The orange polygon outlines an area of tumour necrosis with markedly reduced vascularity and metabolism. Regions of reduced vascularity but increased FDG uptake can be seen adjacent to the necrotic zone and in the left lobe of the liver (arrow).**

Changes in tumour blood flow and metabolism following therapy

The application of FDG-PET and tumour perfusion imaging as markers of tumour response is increasing in research and clinical settings as the limitations of current structural imaging approaches are realised. Generally, these functional imaging techniques have been used in isolation. However, there have been a few studies in which both perfusion and FDG uptake have been measured before and after treatment. The findings show that perfusion and glucose metabolism may not change in parallel in response to therapy[33–36] (Fig. 3). Tumour type, drug type and dose, and time since therapy are all factors that may affect the relative magnitude of change in each parameter.

A study of rectal cancer by Willett *et al.*[33] using perfusion CT and FDG-PET reported significant falls in perfusion but no change in glucose metabolism when the vascular endothelial growth factor (VEGF) antibody, bevacizumab, was given alone. A reduction in glucose metabolism was only seen when bevacizumab was given in combination with radiotherapy. A study of locally advanced breast cancer by Mankoff *et al.*[34] found that following neoadjuvant chemotherapy, glucose metabolism tended to fall irrespective of the final pathological response, whereas perfusion increased in tumours failing to respond to treatment but decreased in tumours that subsequently proceeded to partial or complete response. On the other hand, a study of patients with androgen independent prostate cancer treated using thalidomide found that prostate-specific antigen (PSA) response correlated positively with change in glucose metabolism but negatively with change in perfusion[35]. The effect of drug dose is seen in a study by Herbst *et al.*[36] in which the anti-vascular agent endostatin, when given in high doses, resulted in decreased tumour perfusion but increased glucose metabolism. These studies suggest that uncoupling of flow and metabolism appears to be particularly likely following anti-angiogenic therapy, probably reflecting drug-induced hypoxia and secondary stimulation of glucose metabolism.

Based on the results of these studies, it is possible to propose a sub-classification of therapeutic responses into those that are (a) balanced (i.e. a significant reduction in both glucose metabolism and tumour vascularity), (b) predominantly vascular, and (c) predominantly metabolic (Table 2)[37]. A balanced response seems most likely to be associated with a good outcome. It is likely that the predominantly vascular and predominantly metabolic responses will carry different clinical significance. The possibility of modulating tumour responses by adapting therapy for individual patients on the basis of their imaging findings can be envisaged. For example, it may be appropriate to add an anti-vascular drug to a treatment regime producing a predominantly metabolic response. On the other hand, addition of a hypoxia agent may be

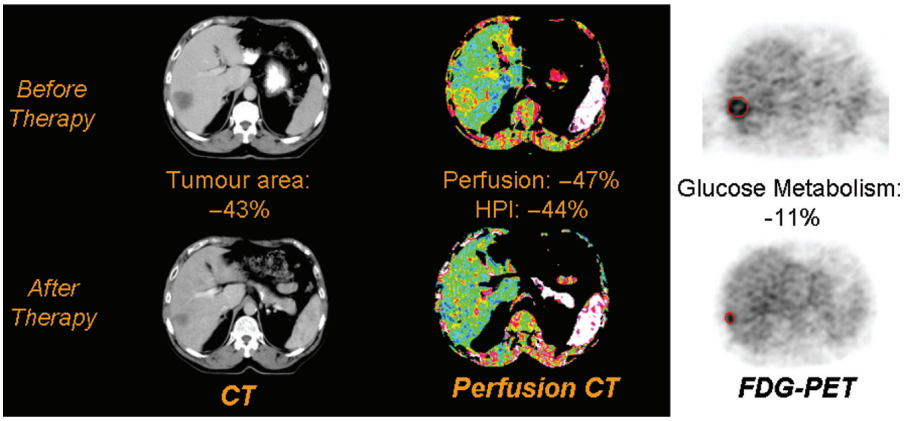

Figure 3 **Changes in tumour size (left), perfusion (centre) and metabolism (right) of a colorectal liver metastases following chemotherapy. There has been a partial morphological response with a predominantly vascular functional response. This combination may indicate adaptation of the tumour to the development of hypoxia during therapy. This response pattern could potentially indicate a need to adapt therapy in order to achieve a full response.**

Table 2 Sub-classification of functional tumour response based upon perfusion and metabolic imaging

	Unchanged or increased perfusion	Reduced perfusion
Unchanged or increased metabolism	No response. *Likely poor outcome*	Predominantly vascular partial response. ?*adapt therapy to target hypoxia*
Reduced metabolism	Predominantly metabolic partial response. ?*adapt therapy to target neovasculature*	Balanced response. *Likely good outcome*

The putative clinical significance of each response class, given in italics, requires confirmation by further clinical trials. Adapted from Miles[37].

appropriate if the response is predominantly vascular. The ultimate goal would be to tailor an individual patient's therapy to the vascular–metabolic response exhibited by their tumour.

Summary

Knowledge of tumour biochemistry and molecular biology dating back to Warburg is fundamental to understanding the application of the techniques currently available for imaging tumour blood flow and metabolism. Tumours exhibit anaerobic metabolism of glucose even in the presence of adequate oxygen. However, glucose metabolism can be further stimulated in the presence of hypoxia associated with poor blood flow. Uncoupling of blood flow and metabolism implying hypoxic stimulation of glucose metabolism is frequently encountered in cancer, particularly in large aggressive tumours and following therapy. Imaging tumour blood flow and metabolism has potential applications for non-invasive characterisation of tumour aggression and may allow novel sub-classification of response with opportunities for personalised cancer care.

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