Molecular Imaging of Metabolism in Cancer Metastasis

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Abstract

Cancer progression is characterised by extensive metabolic reprogramming. Renewed enthusiasm in this field has been sparked in part by the realisation that metabolic pathways, oncogenes and tumour suppressors are intimately linked and regulate tumour growth and metastasis through complex reciprocal interactions. The identification of key pathways and enzymes regulating metabolism in cancer cells provides new opportunities for cancer therapy. This has motivated the development of several specific inhibitors targeting metabolic pathways and their therapeutic evaluation in pre-clinical models or in cancer patients. The unravelling of metabolic pathways associated with cancer progression has also highlighted the extensive metabolic heterogeneity that exists between, and within, each cancer type as well as between metastatic sites. The translation of these findings into personalised therapy remains a considerable challenge. To this end, the use of positron emission tomography to non-invasively visualise tumour metabolism is likely to facilitate the implementation of and assessment of new targeted therapies. Here, we briefly review the key metabolic changes associated with cancer progression and discuss recent advances in the field of positron emission tomography for metabolic imaging of cancer and their potential to improve the clinical management of cancer patients.

Extensive metabolic reprogramming — a ‘hallmark of cancer’ — contributes to progression and metastasis.1,2 Cancer cells often display a high rate of glucose consumption, even under oxygen-rich conditions, a process termed aerobic glycolysis or ‘Warburg effect’.3 Warburg postulated that increased energy production by aerobic glycolysis in cancer cells was a consequence of impaired mitochondrial oxidative metabolism.3,4 However, it is now evident that tumours rarely exhibit mitochondrial defects and that most cancer cells still rely on oxidative phosphorylation to produce the majority of their energy, although this varies considerably between cancer types and sites of metastasis.5-8 Moreover, aerobic glycolysis is not exclusive to tumour cells, but is also a common feature of proliferative normal cells.9,11 The current view is that the primary function of elevated aerobic glycolysis is to generate biomass by diverting glycolytic intermediates towards the biosynthesis of macromolecules (nucleotides, lipids, proteins) required for rapidly proliferating tumour cells (figure 1).12 An alternative model of cancer metabolism called the ‘reverse Warburg effect’ has been described more recently. In this model, glycolyticstromal cells under oxidative stress generate lactate, ketone bodies, glutamine and fatty acids that are taken up by metastatic tumour cells to generate energy through the oxidative mitochondrial metabolism.13-15 While this phenomenon may have relevance to the anti-tumour effect of natural antioxidants,16 more work is required to clarify how it can be targeted in metastatic disease.

Increased amino acid consumption resulting from overexpression of cell surface transporters in tumour cells provides an alternative to glucose.17,18 Most notably, glutamine is utilised as a source of nitrogen and carbon to generate biosynthetic components and for mitochondrial metabolism, essential for growth, proliferation and survival (figure 1). This feature has been exploited for tumour imaging by positron emission tomography (PET) as described below.19,20 Alterations in lipid biosynthetic pathways also have been described in cancer.21,22 Unlike most normal cells, tumour cells reactivate de novo lipid synthesis.23,24 Fatty acid synthesis contributes to many aspects of transformation, including survival under oxidative and energy stress, maintenance of high glycolytic rate, growth and proliferation (figure 1).22 Among other alterations, the expression of fatty acid synthase, an enzyme essential for fatty acid synthesis, is increased in several cancers including breast and prostate tumours.25,26 Perhaps the most significant development in recent years is the realisation that metabolic reprogramming is intimately linked to oncogenic signalling.2,27,28 Activation of oncogenic pathways (PI3 kinase/AKT, Ras, Src, B-Raf and Myc), or the loss of tumour suppressors (p53 or PTEN), increases glycolysis by upregulating the expression of glucose transporters and/or several of the glycolytic enzymes.2,29-31 Myc promotes mitochondrial metabolism of glutamine by increasing the expression of ASC2 transporters and glutaminases.12 Induction of the transcription factor HIF-1 under hypoxia leads to increased expression of fatty acid synthase, thereby promoting lipogenesis for...
Figure 1: Schematic of key metabolic pathways in cancer cells. Alterations in glycolysis, through the overexpression of glucose transporters and several glycolytic enzymes, lead to an increased energy production and to the redirection of intermediate metabolites towards the pentose phosphate pathway for the synthesis of nucleotides, proteins and lipids. Metabolic reprogramming also involves alterations in lipid metabolism, mainly due to the increased expression/activity of enzymes responsible for de novo fatty acid synthesis. Enhanced accumulation of amino acids is also essential for cancer cell proliferation. Glutamine transfers its nitrogens to intermediate metabolites of nucleotide and protein synthesis. Its deamination by glutaminases produces glutamate that is further processed to form glutathione, the major cellular antioxidant and α-ketoglutarate to replenish the TCA cycle (anaplerosis) for energy production and fatty acid synthesis. Many of the enzymes (round yellow boxes) and transporters (square blue boxes) regulating metabolism are modulated by oncogenic signalling (e.g. Myc, Ras, p53) and are potential targets for therapy and molecular imaging. PET-probes used in the clinic or still in pre-clinical development are shown in red. Plain arrows denote a one-step reaction while dashed arrows denote a multi-step reaction. HK2 = hexokinase 2; PKM2 = pyruvate kinase M2; LDH = lactate dehydrogenase; PDH = pyruvate dehydrogenase; IDH = isocitrate dehydrogenase; GLS = glutaminase; ACL = ATP-citrate lyase; FASN = fatty acid synthase; CK = choline kinase; GLUT = glucose transporters; MCT = monocarboxylate transporters; LAT1 = system L amino acid transporters; ASC2 = systeme ASC glutamine transporters; X- = system X-; glutamate transporters; 18F-FDG-6-P = 18F-FDG-6-phosphate; Glucose-6-P = glucose-6-phosphate; PPP = pentose phosphate pathway; AcCoA = acetyl CoA; α-KG = α-ketoglutarate; OAA = oxaloacetate; OXPHOS = oxidative phosphorylation; TCA cycle = tricarboxylic acid cycle; Gln = glutamine; 18F-Glu = 18F-labelled glutamate analogues; Glu = glutamate; 18F-Gln = 18F-labelled glutamine analogues; GSH = glutathione; AA = amino acids; 18F-AA = 18F-labelled amino acid analogues; P-choline = phosphocholine.

Metabolic heterogeneity and metastasis

The metabolic activity of tumours is greatly influenced by their microenvironment. This implies not only that tumours from different tissues display different metabolic profiles, but also that the metabolic activity of metastases from the same tumour may differ depending on the site of metastatic lesions. Evidence for this is emerging. Facilitative sugar transporters (GLUTs) that mediate transport of glucose (and other sugars) across the plasma membrane, are often deregulated in cancer and their expression is often correlated with poor prognosis. However, the extent to which specific transporters are upregulated depends on the tumour type and is influenced by various extrinsic factors such as glucose concentration, inflammation and the specific microenvironment of the primary tumour or metastasis. For instance, primary lung cancer is associated with increased GLUT1 expression compared to normal lung tissue, whereas liver metastases show enhanced GLUT3 and GLUT5 expression. A study examining the expression pattern of sugar transporters in over 200 tumour samples by immunohistochemistry revealed a strong upregulation of GLUT1, GLUT2 and GLUT5 in breast cancer compared to normal breast tissues. Increased expression of the high affinity fructose transporter, GLUT5, indicates that some breast tumours may utilise fructose as an energy source in addition to glucose. These findings may have important diagnostic implications as suggested by the authors. In contrast to breast and lung tumours, prostate tumours show lack or decreased expression of most GLUTs, an observation consistent with their greater reliance on fatty acid synthesis and glutaminolysis for growth and the poor imaging sensitivity of 18F-FDG in these tumours.

The link between metabolism and metastatic progression extends beyond elevated sugar transporter expression. Immunohistochemical and differential expression analysis of metabolic genes in metastatic pancreatic cancers revealed increased expression of several genes involved in aerobic glycolysis in primary tumours and site-specific overexpression of GLUT1, pyruvate kinase M2 and hexokinase-2 proteins in metastatic lesions. Similarly, unique alterations in multiple metabolic pathways have been identified in breast cancer brain metastatic variant cells compared to bone metastatic variant cells. Brain metastatic variants were characterised by increased expression of enzymes involved in glycolysis, TCA cycle, and oxidative phosphorylation pathways in addition to the activation of the pentose phosphate pathway and the glutathione system. The authors proposed that these pathways are bi-directional. For example, high glucose concentration or overexpression of FASN is sufficient to trigger the activation of oncogenic pathways and induce a malignant-like phenotype in mammary epithelial cells. The characterisation of cooperative interactions between metabolic and oncogenic has identified several key molecular targets for diagnostic imaging and/or therapy (figure 1) and prompted the development of several metabolic inhibitors, many of which are already under preclinical evaluation.
changes could reflect a predisposition or bioenergetics adaptation of tumour cells to the brain microenvironment that contribute to their survival and growth in brain. It is not clear from this study whether this metabolic reprogramming is characteristic of all or only a subtype of brain-metastatic tumours. Breast cancer is a heterogeneous disease, with at least two molecular subtypes having high affinity for brain, the triple negative and HER2-negative subtypes. Others have reported that HER2-positive tumours are more glutaminolytic, whereas triple negative tumours are more glycolytic or “Warburg-like”.

A similar association between increased lipid metabolism and metastasis has been reported. Lipid accumulation in brain metastatic lesions from melanoma, lung, colorectal and breast tumours was correlated with areas of necrosis. In another study using a pre-clinical model of osteosarcoma metastasis to lung, increased lipid metabolism was correlated with the development of metastatic lesions. Interestingly, ovarian cancers, which have a high propensity for metastasis to the adipocyte-rich omentum, can coerce adipocytes to sustain their bioenergetics requirements and rapid growth in this tissue via direct transfer of lipids from adipocytes to ovarian cancer cells. Similar mechanisms may operate in metastatic endometrial, prostate and breast tumours. Collectively, these studies highlight the metabolic heterogeneity between tumours and metastases, which provides tremendous diagnostic and therapeutic opportunities. However, if the goal is to translate these findings into personalised therapies, a major challenge ahead will be to define the metabolic profiles specific to each tumour type and their site-specific metastases.

**Molecular imaging of tumour metabolism**

Metabolic imaging non-invasively measures the functional state of tumours and metastases, and provides a mean by which to rapidly evaluate tumour response to therapy, resistance to a given treatment or even to predict treatment response. Thus, imaging of metabolism has high potential to improve the clinical management of patients. Several imaging modalities that take advantage of the increased metabolic activity observed in cancer cells have been implemented in the clinic or are in pre-clinical development. These include PET, single photon emission computerised tomography and magnetic resonance spectroscopy. The clinical use of single photon emission computerised tomography and magnetic resonance spectroscopy in cancer patients has been reviewed elsewhere. Here, we focus on the clinical applications and limitations of current radiotracers and recent advances in the field of PET imaging.

**Imaging of glucose metabolism**

The first and most commonly used probe developed for imaging tumour metabolism is the glucose analogue fluorodeoxyglucose radiolabelled with the positron emitter fluor-18 (18F-FDG). The transport of 18F-FDG via glucose transporters directly reflects the cellular use of glucose. However, unlike glucose, phosphorylation of 18F-FDG by hexokinase produces 18F-FDG-6 phosphate, which cannot be further metabolised and therefore is trapped and accumulates in cells. Clinical studies on lymphomas and solid tumours have demonstrated the prognostic value of 18F-FDG for the early assessment of tumour response to conventional therapies. Therapy-induced changes in glycolysis occur as early as a few hours following treatment, and well before any detectable changes in tumour size. Therefore, 18F-FDG uptake informs on the suitability of a chosen therapeutic intervention and allows rapid identification of non-responders who could benefit from alternative interventions. Thus, 18F-FDG is increasingly utilised as a non-invasive marker in clinical studies, validating the efficacy of new therapies for which no reliable biomarkers are currently available (e.g. B-Raf inhibitors for melanoma, c-Kit inhibitors for gastrointestinal stromal tumours and EGFR inhibitors for non-small cell lung cancers). Similarly, 18F-FDG could be a valuable marker of efficacy for new drugs targeting glycolysis or mitochondrial metabolism.

However, 18F-FDG suffers from some limitations. Its sensitivity is low for the detection of micrometastases from breast cancer and melanoma. Moreover, due to the high glycolytic activity in the brain, accumulation of 18F-FDG hinders the detection of gliomas or metastases in this organ. Areas of inflammation are also 18F-FDG avid. Therefore, despite the rapid therapy-induced changes in glucose metabolism, response to chemo or radiotherapy is usually monitored for four to six weeks after the end of treatment to avoid false-positives resulting from therapy-induced inflammation. In addition, 18F-FDG is only weakly taken up and is a poor marker of metabolic activity in tumours that rely more heavily on fatty acid synthesis and glutaminolysis to grow (e.g. prostate carcinoma).

**Imaging of lipid metabolism**

Changes in lipid metabolism provide an alternative for molecular imaging of tumours that are difficult to visualise using 18F-FDG, such as gliomas, prostate and breast cancers. Most commonly used markers of phospholipid biosynthesis are 11C- and 18F-choline and analogues. The specificity of choline-based tracers for cancer cells is the consequence of increased transport and phosphorylation of choline due to an increased expression of choline kinase in transformed cells. Their low urinary excretion makes these tracers more effective than 18F-FDG for identifying patients with recurrent prostate cancer. 11C-acetate was first developed to image oxidative metabolism of the myocardium. 11C-acetate enters cells through monocarboxilic acid transporters before being converted to 11C-acetyl-CoA by acetyl-CoA synthetase. In the myocardium, 11C-acetyl-CoA is used by the TCA cycle and then released from the cells as 11C-O2. In contrast, cancer cells metabolise 11C-acetyl-CoA predominantly into lipids for membrane biosynthesis and cellular energy production. Similar to choline, acetate analogues accumulate more efficiently than 18F-FDG in prostate tumours and are useful tracers for detection of disease recurrences. Recently, 11C-acetate has shown potential as a non-invasive biomarker to monitor therapy response to orlistat, a fatty acid synthase inhibitor.
Imaging of amino acid metabolism

Cancer cells increase their amino acid consumption to accommodate their biosynthetic needs. Hence, amino acid-based tracers are potential alternatives to $^{18}$F-FDG. Amino acids are internalised via plasma membrane transporters, including systems L (leucine-prefering), LAT, A (alanine-prefering), ASCT (alanine-serine-cysteine-prefering) and X_{c} (cytine/glutamate exchange transporter). Increased expression of LAT1, ASCT2 and $X_c$ have been associated with cancer progression and poor prognosis in gliomas, lung, prostate and colon carcinomas, leading to the suggestion that labelled amino acid analogues could be valuable non-invasive prognostic markers for these malignancies.65, 66 Research over the past decades has focused mainly on LAT1 substrates.65,66 These have the advantage of crossing the blood brain barrier and are weakly retained in the normal brain, and thus provide an accurate visualisation of brain lesions.63 However, the clinical use of amino acid-based tracers for imaging extracranial solid tumours is limited by their relatively low specificity compared to $^{18}$F-FDG.

Glutaminolysis metabolism is increasingly recognised as a promising target for cancer therapy.13,18 Surprisingly, glutamine-based tracers are still in their early phase of development.65, 66 This stems in part from the fact that $^{11}$C- and $^{18}$N-labelled glutamine have short half-lives, are rapidly metabolised, and their metabolites are excreted from the cells, making their use as imaging agents difficult. In addition, labelling glutamine/glutamate with the longer-lived isotope $^{18}$F is technically challenging. This has been addressed in part with the development of the glutamine analogue $^{18}$F-$\text{(2S,4R)}$-4-Fluoroglutamine ($^{18}$F-4FGLN) which has excellent tumour targeting properties in preclinical models of glioblastomas and mammary tumours.65,67 While $^{18}$F-4FGLN has yet to be tested in the clinic, preclinical data suggest that it could be useful in patients with tumours that are addicted to glutamine and sensitive to therapies targeting glutamine metabolism, such as tumours carrying N-Myc amplification or HER2+ breast cancer.45,68

Labelled glutamate analogues, including (4S)-4-(2$\text{S,4R}$-fluoropropyl)-L-glutamate ($^{18}$F-FSPG or BAY 94-9392) and $^{18}$F-$\text{(2S,4R)}$-4-Fluoroglutamate ($^{18}$F-4FGLU) have also been evaluated for PET imaging.50,69 $^{18}$F-FSPG was well tolerated in patients and had a similar tumour-to-background ratio to $^{18}$F-FDG in a pre-clinical model of hepatocellular carcinoma, but unlike $^{18}$F-FDG, exhibited low accumulation in inflammatory lesions.60,70 Comparison of the glutamine analogue $^{18}$F-4FGLN and its glutamate counterpart $^{18}$F-4FGLU, showed that both tracers have potential as tumour imaging agents for glioblastomas and prostate cancers.56 However, while glutamine and glutamate belong to the same metabolic pathway, $^{18}$F-4FGLN is taken up by ASCT2 transporters, rapidly converted to various metabolites and a large proportion is incorporated into proteins.66 In contrast, $^{18}$F-4FGLU enters cells via system $X_c$ and is not metabolised nor incorporated into the protein fraction.66 Therefore, $^{18}$F-glutamine analogues could be used to identify tumours that consume glutamine as an energy source and $^{18}$F-glutamate analogues to assess detoxification potential mediated by glutathione, synthesised from glutamate.65,66,69 One possible limitation of $^{18}$F-4FGLN and $^{18}$F-4FGLU is their low penetration through the blood brain barrier.66 Whether this will impede detection of brain lesions remains to be investigated in pre-clinical models of brain tumours and in patients.

Conclusion and perspectives

Metabolic alterations in cancer provide exciting new therapeutic opportunities for this often fatal disease. PET imaging is an integral part of cancer diagnosis and treatment. Advances with the design of new radiotracers will contribute to the clinical translation of anti-metabolic drugs currently in development. However, the apparent metabolic heterogeneity of tumours and metastases poses a significant challenge with regard to the selection of best treatment and imaging modalities for specific tumour type and metastatic site. While $^{18}$F-FDG remains the gold standard for imaging of glycolytic tumours, other radiotracers are required to overcome some of its limitations, particularly in tumours that rely on alternative metabolic pathways for growth. The most promising new tracers currently in development are glutamine and glutamate analogues. Whether these tracers, alone or in combination with $^{18}$F-FDG, could provide more accurate detection of metastatic spread should be further investigated. Currently, most radiotracers only reflect the accumulation of nutrients due to increased transporter expression. Since many metabolic alterations involve changes in the expression or activity of metabolic enzymes, efforts should be put towards developing more specific probes for these enzymes.

References
