Interactions of ion transporters and channels with cancer cell metabolism and the tumour microenvironment

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Major changes in intra- and extracellular pH homoeostasis are shared features of most solid tumours. These changes stem in large part from the metabolic shift of most cancer cells towards glycolytic metabolism and other processes associated with net acid production. In combination with oncogenic signalling and impact from factors in the tumour microenvironment, this upregulates acid-extruding plasma membrane transport proteins which maintain intracellular pH normal or even more alkaline compared with that of normal cells, while in turn acidifying the external microenvironment. Mounting evidence strongly indicates that this contributes significantly to cancer development by favouring e.g. cancer cell migration, invasion and chemotherapy resistance. Finally, while still under-explored, it seems likely that non-cancer cells in the tumour microenvironment also exhibit altered pH regulation and that this may contribute to their malignant properties. Thus, the physical tumour microenvironment and the cancer and stromal cells within it undergo important reciprocal interactions which modulate the tumour pH profile, in turn severely impacting on the course of cancer progression. Here, we summarize recent knowledge of tumour metabolism and the tumour microenvironment, placing it in the context of tumour pH regulation, and discuss how interfering with these properties may be exploited clinically.

1. Introduction

The interest in regulation of cancer cell metabolism has surged over the last decade, from being relatively ignored, to being seen as one of the ‘emerging hallmarks’ of cancer [1]. During this time, it has become apparent that ion transporters and channels play essential roles in the regulation of cancer development [2–6]. As will be described below, the metabolic shift occurring in most cancer cells increases cellular acid production, and many glycolytic enzymes are exquisitely pH sensitive [7–10]. Acid-extruding transporters are, therefore, essential for maintenance of glycolytic metabolism, as well as for allowing normal cell function and survival in cancer cells with this metabolic profile. The extrusion of acid equivalents acidifies the tumour microenvironment, with major functional consequences for the cancer cells and other cells in the tumour microenvironment. One aspect of this which has received relatively little attention in the context of cancer is that the activity of numerous ion channels and transporters is highly sensitive to intra- and/or extracellular pH (pHi, pHe, respectively) [11–13], as well as to hypoxia [14–17]; see [18], and other characteristic properties of the tumour microenvironment. This raises the central question of how cellular metabolism and ion channels/transporters interact reciprocally within the context of the tumour microenvironment. In this review, we summarize recent knowledge of tumour metabolism and the tumour microenvironment—which goes much beyond the well-known ‘Warburg effect’—and the tumour microenvironment, place it into the context of tumour pH regulation, and discuss how interfering with these properties may be exploited clinically.

That cancer cells typically exhibit a metabolic profile markedly different from that of normal cells was first established with Otto Warburg’s discovery.
of the propensity of cancer cells for aerobic glycolysis [19,20]. This phenomenon—the Warburg effect—has been documented in numerous cancers, although the contribution of glycolysis to total ATP production varies widely between cancer types [21]. Importantly, recent evidence has shown that metabolic reprogramming in cancer is far from limited to the Warburg effect. In the following section, we briefly outline the major metabolic changes occurring in cancer cells, with particular focus on their relation to the tumour microenvironment and to changes in cellular pH regulation. For in-depth discussions of the multiple metabolic changes occurring in cancer cells, the reader is referred to several excellent recent reviews [22–30].

2. Overview of the major metabolic changes in cancer cells

Glycolytic metabolism (2 ATP per glucose) is far less efficient in terms of ATP production than complete oxidative phosphorylation (maximally 38 ATP per glucose), and to fill their needs, cancer cells generally consume at least 10 times more glucose than normal cells [31]. It was initially thought that the apparently inefficient glycolytic shift in cancer cells reflected mitochondrial defects precluding or limiting use of oxidative phosphorylation. However, it is now recognized that mitochondrial metabolism is generally fully functional in cancer cells [25,27,29], and the current consensus is that the glycolytic shift is mainly caused by altered regulation of central metabolic enzymes and serves to support cancer cell growth and redox homoeostasis. Thus, rapidly growing cells such as cancer cells not only need ATP, but also building blocks for synthesis of lipids, proteins and amino acids. Furthermore, they need to maintain cellular redox homoeostasis in the face of often greatly increased production of reactive oxygen species. These requirements are closely reflected in their metabolic profile.

After the Warburg effect, the most widely studied metabolic change in cancer cells is a greatly increased use of glutamine as a nutrient [32]. Glutamine enters the cells via e.g. the neutral amino acid transporter SLC1A5 [32]. In the mitochondria, it is converted by phosphate-dependent glutaminase (GLS, upregulated by c-Myc, [25]) to glutamate. Glutamate can in turn be converted to $\alpha$-ketoglutarate (a-KG) and feed into the tricarboxylic acid (TCA) cycle, or it can be used for the production of glutathione (GSH, figure 1), the major thiol-containing endogenous antioxidant and an essential player in tumour cell survival [33]. Several other biosynthesis building blocks are in increased demand in rapidly proliferating cancer cells. Citrate is catabolized by ATP citrate lyase (ACLY) to acetyl-CoA for de novo lipid synthesis [31], and the oxidative branch of the pentose phosphate pathway (PPP) provides ribose-5-phosphate for nucleotide synthesis and reducing power (NADPH) for biosynthesis processes. The citrate required derives from glucose and glutamine, either by condensation of acetyl-CoA and oxaloacetate, or by mitochondrial isocitrate dehydrogenase (IDH2)-mediated conversion of $\alpha$-ketoglutarate [25]. Lipid

![Figure 1: Major metabolic pathways in tumour cells.](http://rstb.royalsocietypublishing.org/)

Figure 1. Major metabolic pathways in tumour cells. The figure illustrates the major metabolic pathways in which changes are known to occur in cancer. See text for details. $\alpha$-KG, $\alpha$-ketoglutarate; ACLY, ATP citrate lyase; GLS, glutaminase; GSH, glutathione; HK, hexokinase; IDH2, isocitrate dehydrogenase; LDH, lactate dehydrogenase; PFK, phosphofructokinase; TCA, tricarboxylic acid; ess AA, essential amino acids; PPP, pentose phosphate pathway; (R)-2HG, (R)-2-hydroxyglutarate.
metabolism is also deregulated in some cancers and can contribute to their proliferative metabolism [29]. Finally, mutations in isocitrate dehydrogenase 1 (IDH1) and IDH2 seem to play important roles especially in gliomas and leukaemias ([34]; see [35]). IDH1 and IDH2 normally catalyse the interconversion between isocitrate and \( \alpha \)-KG (figure 1), but the cancer-associated mutations cause them to instead reduce \( \alpha \)-KG to the structurally similar metabolite (R)-2-hydroxyglutarate ((R)-2HG). 2HG is proposed to act as an ‘oncometabolite’, suggested to at least in part exert its effects by competing with \( \alpha \)-KG for binding to \( \alpha \)-KG-regulated proteins with tumour suppressor roles (reviewed in [35]).

3. Major characteristics of the tumour microenvironment

The tumour microenvironment is a result of the combined effects of poorly organized vasculature, dysregulated growth and metabolism of the cancer cells, and contributions from other cell types—the stromal cells [36,37]. Importantly, the tumour microenvironment is spatio-temporally dynamic, changing substantially as the tumour grows [38]. The genetically unstable and hence very evolutionarily capable cancer cells are selected for overcoming the growth barriers they encounter; in this way, the tumour microenvironment selects for a more aggressive/malignant phenotype [38,39].

(a) The stromal tumour microenvironment

The cancer cells in a growing tumour secrete numerous growth factors, including basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), epidermal growth factor receptor (EGFR) ligands, interleukins, colony-stimulating factor (CSF) and transforming growth factor-\( \beta \) (TGF-\( \beta \)). In addition to stimulating cancer cell growth, these factors also attract and stimulate stromal cells (figure 2), which in turn provide growth- and motility-stimulating signals to the cancer cells. Hence, progression towards more aggressive phenotypes is caused by reciprocal signalling between the cancer- and surrounding stromal cells [1,40]. The exact composition of the stroma varies substantially [41], but generally comprises: (i) cancer-associated fibroblasts (CAFs), normally by far the most abundant stromal cell type [42,43]. In addition to depositing extracellular matrix, CAFs produce a range of tumour-promoting growth factors, such as FGF, EGF, TGF-\( \beta \), VEGF, hepatocyte growth factor (HGF), as well as matrix metalloproteases (MMPs) and other matrix-degrading enzymes [1,40,44,45]; (ii) endothelial
cells forming the tumour-associated vasculature; (iii) pericytes supporting the tumour endothelium [1,44,46,47]; and (iv) tumour-associated macrophages (TAMs) [45], recruited mainly by monocyte chemotactic protein (MCP) (figure 2). TAMs release growth factors (e.g. EGF and VEGF), chemokines, cytokines and matrix-degrading enzymes, thus stimulating angiogenesis, cancer cell growth and invasiveness, and further recruitment of pro-tumorigenic immune cells, while blocking activation of anti-tumorigenic cytotoxic T cells [1,44,46,47]; and (v) cancer stem cells. In addition, tumours can contain tissue-specific cell types, such as pancreatic stellate cells in pancreatic cancers [46,48] and adipocytes in ovarian and breast cancers [49].

Extensive evidence supports the critical role of cancer-stromal cell interactions in cancer progression. For instance, implantation of genetically modified human mammary fibroblasts resembling the activated tumour-associated stroma into the mouse mammary fat pad resulted in malignant lesion outgrowth of subsequently engrafted human mammary epithelial cells [50]. Furthermore, human mammary fibroblasts stimulated MCF-7 breast cancer cell invasion in three-dimensional collagen gels [51] and human CAFs isolated from a prostatic carcinoma stimulated tumour progression by human prostate epithelial cells in an in vivo xenograft mouse model [52].

(b) The chemical/physical tumour microenvironment

The chemical/physical characteristics of the tumour microenvironment are highly cancer-type dependent. However, typical features include (a varying degree of) hypoxia, acidic pH, high lactate levels, and low levels of glucose, ATP and nutrients [3,18,37–39,53].

Hypoxia is one of the most widely characterized properties of the tumour microenvironment. A key mediator of adaptation to hypoxia is the transcription factor hypoxia-inducible factor 1 (HIF-1). In vitro studies show that HIF-1 is activated below 5% oxygen, its activity progressively increasing with decreasing oxygen levels down to 0.2–0.1% [53]. These conditions are frequently present in tumours: hypoxia occurs within 50–100 μm from the nearest blood vessel, progressing to essential anoxia at 100–200 μm from the nearest vessel [3]. Regulation of HIF-1 expression and activity is described in many excellent reviews [39,53–56] and will not be detailed here. Briefly, however, HIF-1 is a heterodimer of HIF-1α and HIF-1β [57]. While HIF-1β is constitutively expressed, HIF-1α is subject to rapid degradation in normoxia in a manner controlled by the oxygen-sensitive prolyl hydroxylase domain-containing proteins and the von Hippel–Lindau (VHL) protein, the substrate recognition unit of an E3 ubiquitin ligase targeting HIF-1α for proteasomal degradation [39,53–55]. When oxygen is limited, prolyl hydroxylation of HIF-1α is inhibited and HIF-1α translocates to the nucleus, heterodimerizes with HIF-1β, and regulates transcriptional activity of its target genes by binding to hypoxia-response elements (HREs) within their promoters [18,39]. Several oncogenic signalling pathways promote HIF-1 induction independently of cellular oxygen levels [58]; see [59], thus, crosstalk between oncogenic and hypoxia-response pathways potentiate HIF-1 activity. HIF-1 contributes to metabolic reprogramming, e.g. by mediating transcriptional upregulation of several glycolytic enzymes and glucose transporter 1 (GLUT1; [55,60]) and repressing mitochondrial function [61,62]. In addition, HIF-1 activity promotes angiogenesis, epithelial–mesenchymal transition, stem-cell maintenance, invasion, metastasis and resistance to chemo and radiation therapy, crucial factors contributing to cancer-related lethality [55]. Acidic pH is another central property of the tumour microenvironment, reflecting the greatly increased cellular extrusion of acid equivalents in conjunction with inadequate perfusion. The pH₅ of the tumour microenvironment has been extensively described [3,37,38,63–66]. The pH₅ in solid tumours can be as low as 6–6.5, while cancer cell pH₅ is generally similar to, or even more alkaline than, that of normal cells [3,37,38,63–66]. Interestingly, acidification of pH₅ was found to be associated with nuclear sequestration of VHL, preventing it from targeting HIF-1α for degradation [67]. This suggests that the acidic tumour microenvironment may contribute to the metabolic shift by favouring maintenance of high HIF-1α levels. In other words, hypoxia and acidic pH₅ engage in a positive feedback loop expected to progressively exacerbate both conditions in the tumour microenvironment.

4. Regulation of cancer cell metabolism: reciprocal interactions with the tumour microenvironment

(a) Cell-autonomous signalling mechanisms regulating cancer cell metabolism

Several cancer-associated signalling pathways are known to impact on cancer cell metabolism (for reviews, see [23,28–30]). In addition to HIF-1α, these include PI3K/Akt signalling, which stimulates hexokinase (HK) and phosphofructokinase (PFK) activity, leading to increased glycolytic flux, and activates ACLY, leading to de novo fatty acid synthesis. Furthermore, Akt activates mTORC1, which in turn stimulates protein synthesis and regulates transcription factors with major roles in metabolic regulation, including c-Myc, sterol-regulatory element-binding protein 1 (SREBP-1) and HIF-1α itself [29,30]. Further, c-Myc stimulates expression of glucose transporters and thus favours ‘glutamine addiction’ [68].

(b) Regulation of cancer cell metabolism by the tumour microenvironment

Cancer cell metabolism interacts reciprocally with the tumour microenvironment in multiple ways, and metabolic reprogramming is at least in part an adaptive response to the changing tumour microenvironment [38,69]. In support of this view, tumours with the same driving mutations exhibit different metabolic profiles depending on their tissue microenvironment [70].

(i) Hypoxia and cancer metabolism

Increased HIF-1 activity upregulates and/or activates a series of glycolysis-stimulating enzymes, including PFK, hexokinase II (HK2), pyruvate kinase M2 (PKM2), and lactate dehydrogenase A (LDH-A), which helps supply NAD⁺ for glycolysis [29,31]. PKM2 is the embryonic form of PK, and the shift from PK to PKM2 causes conversion of phosphoenolpyruvate to pyruvate to be slowed down, favouring preservation of building blocks for biosynthesis over ATP production [31].
Several acid and/or lactate-extruding transporters including monocarboxylate transporter (MCT) isoform 4 and the Na\(^+\)/H\(^+\) exchanger NHE1 are also upregulated by hypoxia through HIF-1α signalling (see §5).

The net result is that lactate and other acid equivalents are extruded by the tumour cells in often greatly increased amounts. Interestingly, metabolic coupling occurs between cancer cells in hypoxic and well-oxygenated tumour regions, and between cancer cells and stromal cells. Specifically, it is proposed that lactate produced by hypoxic tumour cells is taken up via MCT1 in normoxic cancer cells, followed by conversion to pyruvate, sparing the limited supply of glucose for the hypoxic tumour regions [71].

(ii) Cellular pH and cancer cell metabolism

Numerous processes essential to normal cellular metabolism are highly pH sensitive in the physiologically relevant range. These include the activities of LDH [7] and PKF [8,9], which are favoured at alkaline pH\(_i\) and gluconeogenesis, which is inhibited under these conditions [10]. Fatty acid synthesis was shown to be favoured by extracellular acidosis through the upregulation of fatty acid synthase [72]. pH\(_i\), furthermore impacts on cell cycle control [73], ribosome biogenesis [74] and cell death programmes [75,76]; pH\(_i\) must therefore be regulated within a narrow range to maintain normal cellular function. A point frequently considered only superficially is what actually causes the increased acid production in cancer cells. Full oxidation of glucose in fact consumes 38 H\(^+\) while producing a maximum of 38 ATP (2 from glycolysis, 2 from the TCA cycle and up to 34 from the electron transport chain). The H\(^+\) consumed are regenerated in the subsequent degradation of ATP (ATP + H\(_2\)O → ADP + HPO\(_4^{2-}\) + H\(^+\)) in other processes. The CO\(_2\) produced also contributes to net cellular acidification through its hydration to HCO\(_3^-\) and H\(^+\). Glycolysis to the point of pyruvate yields 2 H\(^+\) per 2 ATP produced. In absence of sufficient oxygen (and/or in cells exhibiting a Warburg shift towards glycolytic metabolism), pyruvate is converted to lactate, in turn consuming one H\(^+\) (per pyruvate, i.e. 2 per original glucose) and converting NAD\(^+\) back to NAD\(^-\) to allow glycolysis to go on. Thus, overall, conversion of glucose to lactate has no effect on pH\(_i\), however, again, the subsequent ATP degradation will produce H\(^+\) resulting in cellular acidification. Further, the lactate produced in this manner and cotransported out of the cells with H\(^+\) is an important component of the tumour microenvironment. Moreover, both the GLS-mediated conversion of glutamine to glutamate and the oxidative branch of the PPP produce H\(^+\). Further, conversion of glutamine to glutamate generates NH\(_4^+\), which is secreted at very high rates [32]. The mechanism of NH\(_4^+\) efflux under these conditions is unknown, but transporters, such as NHE1 and NBCn1 (described in the following section), as well as Na\(^+\),K\(^+\)-2Cl\(^-\) cotransport or various K\(^+\) channels, can carry NH\(_4^+\) [77,78] and could contribute.

5. Regulation of acid-extruding transporters by the conditions in solid tumours

To cope with their increased acid production, cancer cells upregulate the expression and/or activity of acid-extruding ion transport proteins and consequently, as described above, generally exhibit a normal or even modestly elevated pH\(_i\). The pH-regulatory proteins involved include the Na\(^+\)/H\(^+\) exchanger NHE1, Na\(^+\),HCO\(_3^-\) cotransporters (NBCs), H\(^+\)-lactate cotransporters (MCTs) and the enzyme family of carbonic anhydrases [2,3,64]. It is a chicken-and-egg question, and to our knowledge still poorly established, whether upregulation of acid-extruding transporters early in transformation is the event that allows the glycolytic shift, or, conversely, the glycolytic shift increases transporter expression through an initial metabolically induced cytoplasmic acidification. It is clear, however, that acidification-induced signalling mechanisms can upregulate the transporters at the transcriptional and/or post-transcriptional level, as shown both for NHE1 and NBCn1 [3,79]. In addition, oncogenic signalling as well as other factors in the tumour microenvironment can regulate the transporters. Notably, hypoxia, through HIF-1α, induces expression of several plasma membrane-located transporters, exchangers, pumps and ecto-enzymes [18]. Most well known is the plasma membrane-bound extracellular facing CAIX. Increased CAIX expression was shown in regions adjacent to necrosis in ducal carcinoma in situ (DCIS) of the breast correlating with hypoxia [80]. Also NHE1 expression was shown to be increased by hypoxia in pulmonary arterial smooth muscle cells [14] and hepatocellular carcinoma HepG2 cells [15]. When various epithelial cancer cell lines were exposed for 48 h to 2% O\(_2\) or simulated hypoxia dimethylxalylglycine, NHE1 protein expression was reduced in some, but not all, cell lines, suggesting that the effect of hypoxia on NHE1 may be cell type specific [17]. In human mammary cancer, increased NHE1 expression was found in DCIS compared with normal tissue, yet with no correlation between the localization of the hypoxia-responsive glucose transporter GLUT1 and NHE1 [81], hence the possible link of NHE1 expression in cancer to hypoxia requires further investigations. MCTs contribute to the maintenance of cancer cell growth by exporting lactate and thus allowing a continuous high level of glycolysis [82]. In addition, MCT activity contributes to acid extrusion by obligatory symport of H\(^+\) [82]. MCT4, but not MCT1, is upregulated by hypoxia in a manner dependent on HIF-1, owing to HREs in the MCT4 promoter [16]. Thus, upregulation of MCT4 appears to be an adaptation to intratumoural hypoxia, contributing to both the acid-resistant and hyper-glycolytic cancer cell phenotype [16]; see [82].

Hormones and growth factors present at high levels in the tumour microenvironment (figure 2) probably contribute to upregulation and/or increased activity of the acid-extruding transporters. For instance, EGF has been shown to increase NHE1 expression in cervical cancer cells [83]; and we recently demonstrated that NBCn1 is transcriptionally upregulated by EGF, neurulegin and ErbB2/HER2 in breast cancer cells, whereas NHE1 is post-translationally regulated by ErbB2/HER2 signalling [84,85]. NHE1 activity is also enhanced by serum deprivation, simulating the poorly perfused tumour microenvironment [86]. Further, inflammatory mediators including IL-1, IL-6 and TNF-α, which exhibit elevated levels in the tumour microenvironment (figure 2), could contribute to upregulation of acid extrusion. Thus, IL-6 has been shown to induce Notch-3-dependent upregulation of CAIX in breast cancer cells [87], and IL-1 and TNF-α have been shown to activate NHE1 in astrocytes [88]. Finally, as NHE1 is stimulated by mechanical stimuli for example cell spreading [89], it may be regulated by the elevated matrix stiffness and interstitial...
pressure in the tumour microenvironment, although this has yet to be directly studied.

A poorly studied issue is how hypoxia, acidification and other factors present in the tumour microenvironment interact in the regulation of acid–base transport. An interesting study compared the effects of hypoxia, lactic acidosis and both conditions simultaneously, on gene expression profiles in human mammary epithelial cells [90]. Some genes were found to be upregulated synergistically by both stimuli, whereas others, including CAIX, were induced by hypoxia and inhibited by lactic acidosis, resulting in no net effect on the expression level when combining hypoxia and lactic acidosis. This pinpoints that results obtained with hypoxia cannot be used to make predictions regarding the tumour microenvironment.

(a) Relative roles of NHE1 and NBCn1 in acid extrusion in the tumour microenvironment

Because NBCn1, similarly to NHE1, exerts the net effect of extruding acid from cells, it could in principle contribute to malignant transformation in the same manner as NHE1. Genome-wide association studies have shown correlation between a single nucleotide polymorphism in the NBCn1 gene and breast cancer susceptibility [91], suggesting that NBCn1 plays a role in breast cancer biology. This is supported by our recent finding that NBCn1 plasma membrane density was increased in primary breast carcinomas and metastases compared with matched normal breast tissue [81]. Notably, NBCn1 was rather uniformly expressed throughout the tumours in contrast to NHE1, which was most highly expressed in peripheral and well-perfused areas [81]. Another recent study showed NHE1 to localize predominantly to peripheral regions of rat brain tumours [92]. The relative roles of NHE1 and NBC activity in cancer cells during hypoxia were studied by Hulikova et al. [17], who found that whereas NHE1-mediated acid extrusion was highly variable between cell lines and inhibited by hypoxia, NBC-mediated acid extrusion was similar between cell lines and hypoxia-insensitive. Further to this, our recent work suggested that in pH regulation in human breast carcinoma tissue, NHE1 activity was only quantitatively important at very low pH levels [81]. Finally, NHE1 has numerous ‘functional interfaces’ with tumour cell metabolism, not restricted to that of global cellular pH-regulation. For instance, NHE1 has been reported to interact directly with PFK1 [93], and to regulate mitochondrial localization of HK2 [94]. Another major role of NHE1 is regulation of cell volume, which impacts on numerous functions relevant to cancer development, including cell migration and proliferation [95]. Cell volume changes are, for instance, thought to underlie the major role of the Na⁺,K⁺2Cl⁻ cotransporter NKCC1 in glioma cell motility [96].

6. Consequences of pH dysregulation for ion channels, transporters and receptors in the tumour microenvironment

Effects of tumour pH and pHe on cell proliferation, death/survival balance and other pivotal cellular functions in cancer has been covered in recent reviews [2,3,97] and elsewhere in this issue. That altered pH and pHe in tumours may modulate cancer development through effects on ion channels, transporters and plasma membrane receptors has received less attention, despite the fact that numerous ion channels are gated by pH and/or pHe. An important example is the family of two-pore K⁺ channels, many of which are highly pH sensitive in the relevant range. The subfamily including TASK-2, TALK-1 and TALK-2 are activated by extracellular alkalization (pK₂ for TASK-2 is 8.0), and hence would be expected to be inhibited in the acidic tumour microenvironment [11]. TASK-2 is additionally gated open by intracellular alkalization, yet this cannot overcome the inhibitory effect of acidic pH [12]. The regulation of TASK channels by tumour pH is of particular interest because TASK channels are, at least in some cell types, required for apoptosis, through their role in apoptotic volume decrease [95,98,99]. This suggests that acidic tumour pH may contribute to death avoidance in cancer cells through inhibition of two-pore K⁺ channels. Complicating the picture, these channels are also implicated in cell cycle progression [98], which would thus be counteracted by their acid-mediated inhibition. Specifically, TASK-3, which is inhibited by extracellular acidification, is overexpressed in ovarian cancer, and TASK-3 blockers caused a significant reduction in cell proliferation and an increase in apoptosis in SKOV-3 and OVCAR-3 ovarian cancer cells [100]. Other potentially important pH sensors are CLC2 and CLC3 Cl⁻ channels [11] (to the extent that they are expressed in the plasma membrane), as well as acid-sensing ion channels (ASICs) and ASIC-related channels, which have been implicated in bone pain in cancer metastasis [101] and in glioma cell motility [13]. Interestingly, Hv1 voltage-gated proton channels [102] were recently assigned a role in metastatic breast cancer [103,104]. These channels are, however, inhibited by acidic pH and activated by acidic pH ensuring that they are only active at outward electrochemical gradients for H⁺ [105]. Hence, they would be predicted to be inhibited in the tumour microenvironment, and further studies should address their regulation in this setting. Notably, Hv1 channels are required for full activity of another family of enzymes implicated in cancer development, the activity of which is highly proton-producing, namely the Nox proteins mediating NADPH oxidase activity [106,107]. Finally, bona fide acid-sensing receptors such as the G-protein-coupled receptors OGR1, GPR4, G2A and TDA8G, which link increases in pH to increases in cellular Ca²⁺, could be important, although this, to our knowledge, has yet to be directly investigated.

7. Exploiting the pH/tumour microenvironment/metabolism inter-relationship triad for clinical purposes

Anti-cancer drug development research in the past decade has largely focused on the paradigm concept of molecular targeted therapies. The idea is seductively simple: if one can identify a key molecular change(s) underlying survival and progression of a given cancer then by specifically targeting that molecular change, one has a therapeutic handle on that cancer [108–111]. Early successes, such as the discoveries of the Bcr-Abl gene fusion in chronic myelogenous leukaemia, targeted by imatinib [112–114], and of the V600E BRAF oncogenic mutation in melanoma, targeted by vemurafenib [115], raised expectations towards molecular targeted drugs. Overall,
however, targeted therapies have shown relatively modest clinical benefit, with only a fraction of the patients expressing the drug target, and thus predicted to benefit, actually doing so, and most responding patients showing only transient responses. This lack of clinical benefit is ascribed to intrinsic resistance of tumours to inhibition of signalling intermediates, essentially owing to redundancy in signalling pathways. A second problem is that of acquired resistance, where tumour cells acquire mutations allowing them to bypass the effects of the targeted drugs [116–120].

The metabolic adaptations of cancer cells, described above, result in changes in the flux along key cellular metabolic pathways. These changes are the endpoints of an intricate interplay between metabolic pathways and other signalling pathways in the cancer cells and in the tumour microenvironment [121–123]. The realization that some of these metabolic alterations seem to be absolutely required for cancer progression suggested the possibility that targeting cancer metabolism is a potentially valuable therapeutic approach [24,124], particularly in synergy with non-metabolic targeting strategies.

Conceptually, one can define two main areas where cancer metabolism could be of clinical value: imaging and therapeutics. The increased glucose metabolism displayed by tumour cells allows their detection with high sensitivity and specificity using metabolic imaging probes, for example the glucose analogue 2-deoxy-2-[18F]fluoro-D-glucose. Multimodality imaging approaches for tumour cell metabolism imaging are increasingly used in routine clinical practice, both for diagnostic purposes and for staging of cancer patients and evaluation and prediction of treatment response [125–128]. From a therapeutic viewpoint, several agents targeting cancer metabolism are currently under development, and preclinical trials for many of these drugs have shown rather encouraging results (reviewed in [124,129]). Here, we have discussed some of the interactions between cancer cell metabolism, ion transport and the microenvironment. It would stand to reason that some of these could be exploited by anti-cancer agents.

Prompted by preclinical studies showing cardioprotective properties, highly specific NHE1 inhibitors were developed. Two of these agents were tested in clinical trials for efficacy in treatment of heart disease, but showed serious cerebrovascular side effects ([130] and references therein). Another potentially important therapeutic target in the context of the tumour microenvironment is HIF-1. Specific blockade of HIF-1 function by small molecule inhibitors has shown promising anti-proliferative and anti-angiogenic effects in preclinical assays [131–134]; one of these, 2-methoxyestradiol (2ME2), is currently being tested in clinical trials [135,136].

Finally, the acidic tumour pH, while contributing to cancer development, provides a therapeutic opportunity: the design of drugs that take advantage of this to modulate bioavailability [137–139]. A related approach is based on the targeting of hypoxia. Hypoxia-activated prodrugs or bioreductive drugs are designed to deliver a cytotoxic agent to hypoxic areas within a tumour [140–143]. Several clinical trials have shown promising results for this class of drugs, e.g. a phase IIb trial of TH-302, a hypoxia-activated prodrug, in combination with gemcitabine in patients with pancreatic cancer [144].

8. Conclusion and perspectives
We have outlined here how metabolic changes in tumour cells reciprocally interact with the tumour microenvironment, and how this involves major changes in pH regulatory ion transport across the plasma membrane. We suggest that both the tumour microenvironment per se and the specific roles of the pH regulatory transporters may be exploited therapeutically. While the understanding of the roles and regulation of these transporters in cancer has increased greatly in recent years, understanding pH regulation in the context of solid tumours is a particular challenge because it requires models that recapitulate the complex, three-dimensional tumour microenvironment. Future studies should focus on addressing the roles of the pH regulatory transporters in such models as well as in vivo, in order to move toward clinical exploitation of the very promising basic and preclinical evidence implicating these proteins in cancer development.

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