Disrupting proton dynamics and energy metabolism for cancer therapy

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Abstract | Intense interest in the 'Warburg effect' has been revived by the discovery that hypoxia-inducible factor 1 (HIF1) reprogrammes pyruvate oxidation to lactic acid conversion; lactic acid is the end product of fermentative glycolysis. The most aggressive and invasive cancers, which are often hypoxic, rely on exacerbated glycolysis to meet the increased demand for ATP and biosynthetic precursors and also rely on robust pH-regulating systems to combat the excessive generation of lactic and carbonic acids. In this Review, we present the key pH-regulating systems and synthesize recent advances in strategies that combine the disruption of pH control with bioenergetic mechanisms. We discuss the possibility of exploiting, in rapidly growing tumours, acute cell death by 'metabolic catastrophe'.

Cytostatic
Pertaining to cytostasis, which is basic cellular function without progression through the cell cycle.

H^+ dynamics
The interaction between extracellular pH and intracellular pH with respect to acid–base movement between the two compartments and their subsequent cellular effects.

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doi:10.1038/nrc3579

Growing solid tumours create hostile environments and thus present tantalizing angles that could be exploited to cause cancer cell-specific 'suicide'. Two physiological processes in particular — intracellular pH (pH_i) regulation and cellular metabolism — have great potential for the development of targeted cancer therapy. This stems from the well-characterized 'Warburg effect' in tumour cells, which describes an altered metabolism by which glycolysis is predominantly used even in the presence of oxygen[1–3]. Compared with normal cells, tumour cells produce increased amounts of H^+ (including lactic and carbonic acids, which are end products of metabolic pathways) owing to enhanced metabolic rates[3,4]. This poses considerable cellular stress, as alterations in pH_i affect the structure and activity of almost every enzyme, which drastically affects cell signalling and metabolic function. Therefore, the resultant increased H^+ production, coupled with incomplete vascularization and poor extracellular clearance, creates a hypoxic and acidic niche that could rapidly become cytostatic and/or lethal to cells[5–7]. Knowledge of H^+ sensing and subsequent cellular signalling pathways has been developing over the past decade following the report of G protein-coupled receptor activation by extracellular acidosis[8]. Numerous proteins have now been implicated in the detection of changes in levels of H^+, HCO_3^-, pH and CO_2 and can induce various cellular signalling events[9,10]. As cell signalling is vital to the control of cell growth, this is an intriguing area of research for tumour biology considering the altered H^+ dynamics that exist in the tumour microenvironment.

Owing to oncogenic transformation and subsequent gene regulation by the hypoxia-inducible factors HIF1 and HIF2 in hypoxic zones[11], cancer cells thrive in their environment and begin to out-compete normal cells according to the principles of Darwinian selection, which allows continued tumour expansion[12]. This selection also allows tumour cells to escape most anticancer therapies[12]. These selection pressures culminate in successful tumour cells adopting enhanced states of both metabolism and pH_i regulation (FIG. 1a,b).

The general model of pH_i regulation in the tumour microenvironment has been extensively reviewed[5,6,10,13–17], and so we focus on key proteins that are implicated in anticancer therapies. However, a framework for the overall pH_i-regulating mechanism is required before expanding on individual proteins in the context of tumour survival. As shown in FIG. 1a, hypoxic zones develop on the basis of their proximity to the vasculature, resulting in increased expression of pH_i-regulating proteins[5,6]. Hypoxia improves cell survival in acidic conditions, which is correlated with an increased ability to correctly regulate pH_i in hypoxic conditions[18–21]. Na^+/H^+ exchanger 1 (NHE1; also known as SLC9A1) is the predominant pH_i-regulating protein; it is found in every cell and is also proposed to have an important role in tumour cells, in which it directly manages free intracellular H^+ when the buffering capacity of intracellular proteins has been exhausted[22,23] (FIG. 1b). In reality, only a few proteins are differentially expressed in tumour cells compared with normal cells in the context of pH_i regulation. These include the hypoxia-regulated,

Key points

- In rapidly growing cancer cells, oncogenes and hypoxia stimulate glycolytic metabolism, which generates increased amounts of lactic and carbonic acids.
- Several pH-regulating systems — Na^+/H^+ exchangers (NHEs), carbonic anhydrases (CAIX and CAXII), HCO_3^- transporters, lactate/H^+ symporters (monocarboxylate transporter 1 (MCT1) and MCT4) and intracellular H^+ pumps — are essential to maintain a permissive intracellular pH (pH_i) to optimize bioenergetic metabolism, cell cycle progression, growth and survival.
- Cells lacking pH-regulating capabilities can enter growth arrest or can be 'killed' by H^+. Targeting pH-regulating proteins in isolation (NHE1, CAs, MCTs and H^+ pumps) impairs tumour progression.
- Targeting the export of lactic acid from tumour cells (by disrupting MCTs) reduces glycolysis and growth rates, thus sensitizing tumour cells to treatment with mitochondrial complex I inhibitors (such as metformin and phenformin).
- We propose the development of an acute 'metabolic knife' treatment that combines targeting of pH control and ATP-driven metabolism to eradicate rapidly growing glycolytic tumours.

extracellular-facing carbonic anhydrases, carbonic anhydrase IX (CAIX) and CAXII, which manage the acidic metabolic production of CO_2 by facilitating H^+ diffusion towards the vasculature. HCO_3^- units can then be recaptured by the tumour cells through HCO_3^- transporters to assist with intracellular buffering of acidic units[5,15–17] (FIG. 1b). The other main difference is in the overexpression of hypoxia-induced monocarboxylate transporter 4 (MCT4), which removes H^+ in conjunction with lactate for the facilitation of continued glycolytic ATP production and thus contributes to pH_i regulation[24–26]. These proteins, coupled with other pH_i modifiers such as H^+ pumps and cytosolic CAII, combine to result in a tumour cell that actively maintains a more alkaline pH_i than a normal cell, despite its acidic surroundings. The way in which individual pH-regulating proteins participate in cell fitness within the tumour microenvironment in the context of both metabolism and acid–base balance is expanded on below.

In addition, this Review describes how interference with H^+ dynamics (both pH_i and extracellular pH (pH_e)) coupled with metabolic disruption could provide a new strategy for anticancer therapeutics[27]. We present the basic features, physiological role and regulation of the most robust pH_i-regulating systems in the context of cancer cell biology (FIG. 1b) and the role of pH dynamics in cell migration and metastasis. We explore the historical development of studies targeting tumour pH and discuss current limitations to this cell-killing strategy. Finally, we provide a perspective on the development of future targeted therapeutic strategies combining disruption of essential elements of pH_i regulation and cellular bioenergetics.

Disrupting pH_i to achieve tumour cell death

Early physiological studies on general pH_i regulation revealed a universal key role for the electroneutral and reversible ion transporter that exchanges one Na^+ ion for one H^+ ion. NHE1 was demonstrated to be involved in cytoplasmic alkalinization of almost all cells during fertilization or on stimulation by growth factors[28]. This amiloride-sensitive growth factor-activatable, ion transporter possesses a H^+-modifier site[29] and was one of the first transporters to be described at the molecular level after the Cl^-/HCO_3^- exchanger anion exchange protein 1 (AE1; also known as SLC4A1)[30,31] (FIG.2 (TIMELINE)). Before the molecular characterization of NHE1, a H^+-suicide technique was developed, and this technique reversed NHE1 ion gradients (FIG. 3A). This led to the isolation of NHE1-defective cell lines to establish the proof of concept that acute reduction in pH_i (6.0 to 5.5) is an effective mechanism to induce cell death in minutes[32]. This technique and NHE1-defective mutant cells enabled the discovery of important interactions among pH_i regulation, cell cycle control and tumour growth[33–35].

pH_i regulation and cell cycle control. The existence of a pH_i threshold (around 7.1–7.2) below which growth factors fail to stimulate G1 progression and cell cycle entry was demonstrated in non-transformed G0- or G1-arrested fibroblasts[36] (FIG. 3B). This pH_i threshold restricted mTOR complex 1 (mTORC1) activation and protein synthesis without affecting activation of either ERK–MAPK or PI3K signalling[33,34]. The block in mTORC1 activation, which is indicated by the lack of ribosomal protein S6 (RPS6) phosphorylation[37], sufficiently accounted for the inhibition of protein synthesis and the G0 or G1 cell cycle arrest. By contrast, imposing the low acidic pH_i threshold at the onset of DNA replication (restriction point (R)) or during S phase did not affect the completion of DNA replication. The down-regulation of mTORC1 signalling during acidification of pH_e and pH_i was recently confirmed in immortalized fibroblasts and breast cancer cells[38].

Disrupting pH_i regulation restricts tumour growth. The effects of genetic disruption of *Nhe1* expression on tumour growth were assessed in three $HRAS^{G12V}$-transformed hamster fibroblast cell lines that differed in their bioenergetic pathways: one cell line was wild type, one was defective in glycolysis and one was defective in oxidative phosphorylation (summarized in REF. 35). The cells defective in glycolysis (which produce 15-fold less lactic acid than the parental cells) were not affected by disruption of *Nhe1*. By contrast, tumour xenografts from the wild-type cell line were severely affected by *Nhe1* disruption: tumours developed in 100% of the mice, but 80% of these tumours had regressed by the end of the experiment (FIG. 3C). Even more drastic was the impact of *Nhe1* disruption on the cell line that produced four-fold more lactic acid than the parental cells owing to a defect in oxidative phosphorylation (FIG. 3C). This cell line, which produced tumours in only 20% of mice, was fully impaired for xenograft tumour formation by *Nhe1* disruption (0% tumour-bearing mice); however, expression of the H^+/lactate symporter MCT4 fully restored tumour formation[25] (discussed further below).

Targeting tumour pH_i-regulating systems

Na^+/H^+ exchangers. Of the nine members of the NHE family, NHE1 has received the most attention in the context of tumour cell-targeted therapy. Although NHE1

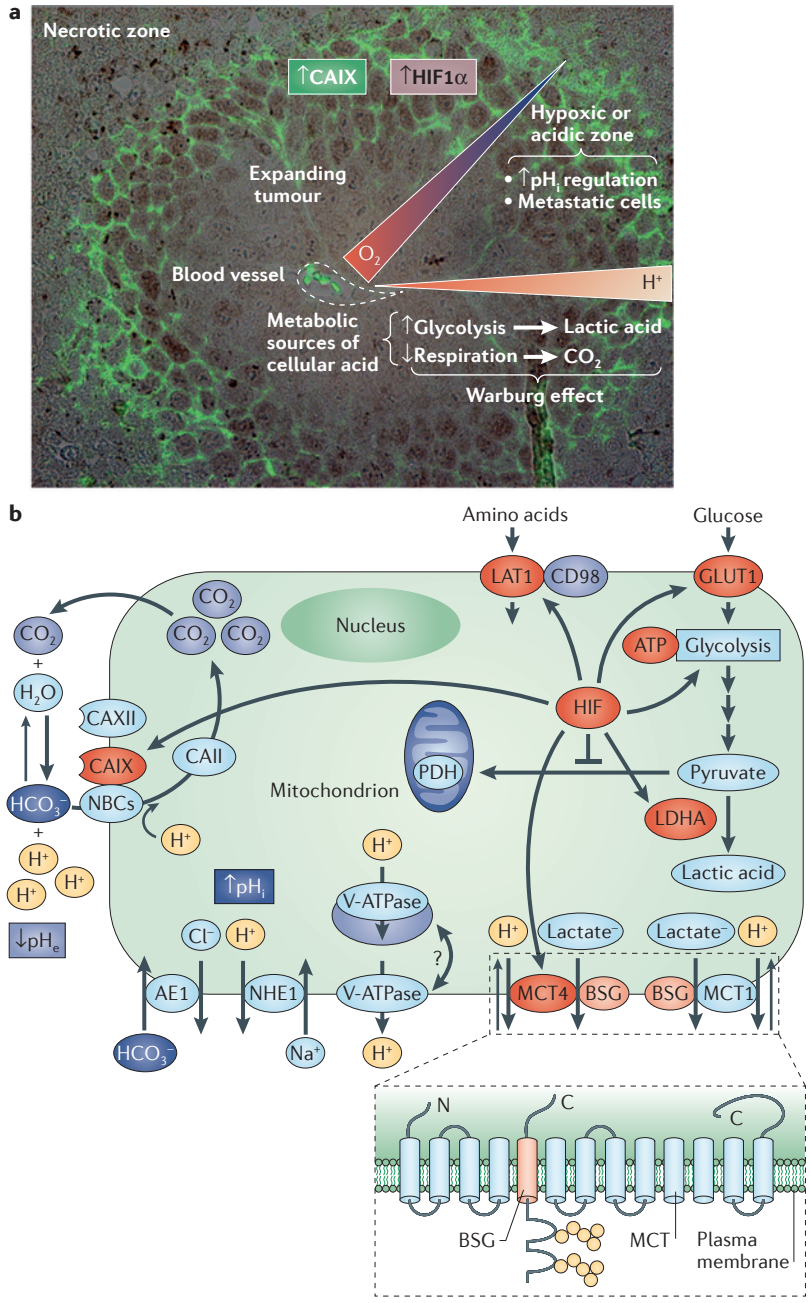


Figure 1 | **The tumour microenvironment presents unique conditions that drive tumour development.** **a** | An image of an LS174 adenocarcinoma cell xenograft tumour with hypoxia-inducible factor 1α (HIF1α; brown) and carbonic anhydrase IX (CAIX; green) immunofluorescent staining, illustrating the altered protein expression that is driven by O_2 and H^+ gradients and sources of metabolic acids found in the tumour microenvironment. **b** | A cellular model of intracellular pH (pH_i) regulation and metabolic interactions in the hypoxic tumour cell. Tumour cells survive their acidic surroundings by coordinating the action of pH_i-regulatory proteins that include Na^+/H^+ exchanger 1 (NHE1), carbonic anhydrases (CAIX, CAXII and CAII), HCO_3^- transporters (Na^+/HCO_3^- co-transporters (NBCs) and anion exchange protein 1 (AE1)), monocarboxylate transporters (MCT1 and MCT4) and potentially vacuolar H^+-ATPases (V-ATPases). Metabolic fuel is provided to the cell by glucose and amino acid transporters (glucose transporter type 1 (GLUT1) and L-type amino acid transporter 1 (LAT1), which is chaperoned by CD98), and hypoxia promotes the expression of certain proteins that are involved in both metabolism and pH_i regulation via the transcriptional activity of HIF. The inset shows MCTs in complex with basigin (BSG; also known as CD147); this complex is essential for proper protein functionality and the maintenance of glycolysis. LDHA, lactate dehydrogenase A; PDH, pyruvate dehydrogenase; pH_e, extracellular pH.

inhibition has been linked to cell death in different tumour cell lines[39–41], dramatic effects on tumour cell survival have not so far been reported for monotherapy with NHE1 inhibitors. This apparent innate resistance is related to the co-expression of other NHE isoforms in epithelial-derived tumour cells[42] that are not affected by NHE1 inhibitors[43]. As NHE1 inhibition formed a promising target in cardiovascular disease owing to its prevention of Na^+/Ca^{2+} exchange following ischaemia, the NHE1 inhibitor cariporide progressed to Phase III clinical trials for the treatment of myocardial infarction[15,44]. Its clinical development has been abandoned, however, owing to poor response and adverse side effects[44,45], which could be attributed to the ubiquitous expression of NHE1 throughout the body[45]. This has dampened the enthusiasm for NHE1-targeted therapies in other clinical settings. However, as the clinical trials involving NHE1 inhibition have only been carried out in patients with cardiac diseases, and the adverse health effects were also cardiac related, it remains to be investigated whether NHE1 inhibition for cancer patients with otherwise healthy cardiovascular systems could be effective. Furthermore, these findings coupled with recent promising *in vitro* use of NHE1 inhibitors in combination with Ca^{2+} exchange inhibitors in glioma cells[46] could warrant further study. NHE1 inhibition is also linked to the improvement of the apoptotic effect of chemotherapeutic agents such as paclitaxel[47], strengthening the potential synergistic value of targeting NHE1 in treatment development.

The expression and activity of NHE1 is induced by hypoxia in pulmonary myocytes[48,49]. These findings generated excitement in the area of H^+ dynamics and cancer because they indicated that NHE1 could be a hypoxic target in tumour cells. However, only limited data for NHE1 upregulation in tumour cells and hypoxic zones[50] have been presented thus far. Recent evidence suggests that NHE1 activity can be upregulated or downregulated depending on cell type and oxygen availability[51,52], indicating a need to further understand the role of NHE1 in the hypoxic microenvironment. Furthermore, a point that is often overlooked in the context of the tumour microenvironment is that extracellular acidosis will in fact decrease NHE1 functionality by altering the H^+ gradient between the cellular space and its environment[53]. Therefore, NHE1 activity is perhaps already limited by the physical parameters that exist in the tumour microenvironment, and these conditions should be considered in future studies.

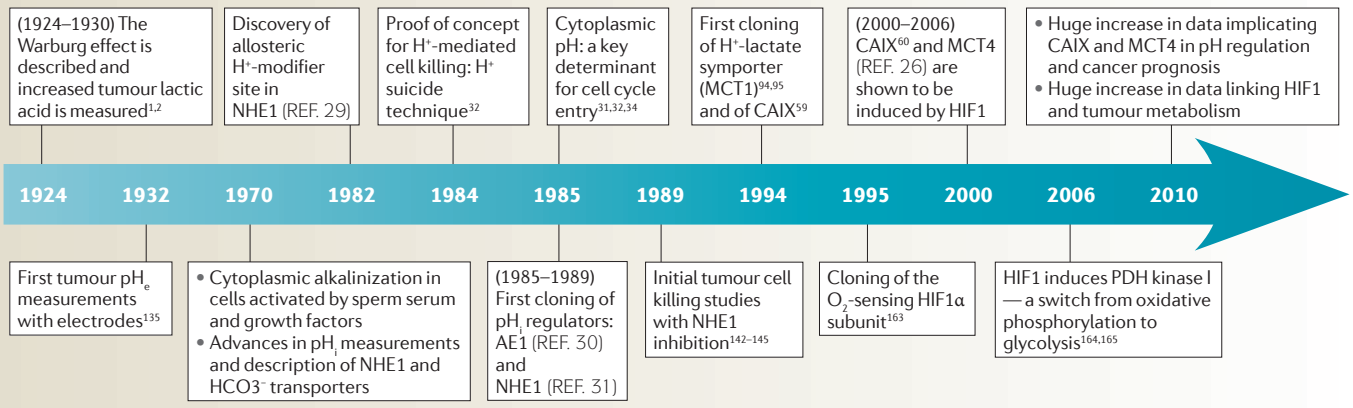
Alkalinization of pH_i by NHE1 is linked to malignant transformation[23], and NHE1 is also strongly implicated in local pH gradients that are associated with fluid-phase endocytosis[54] or macropinocytosis[55]. Interestingly, treatment with the NHE1 inhibitor EIPA was recently demonstrated to compromise the growth of KRAS-transformed tumour xenografts, an effect that was attributed to inhibition of nutrient uptake by macropinocytosis[56]. In addition, pH_i gradients stimulate cell migration and as such NHE1 is proposed to participate in the development of metastases[16,22,57] (discussed below).

Carbonic anhydrases. CAs are a family of 16 proteins distributed throughout the body that catalyse the hydration of CO_2 to generate HCO_3^- (basic) and H^+ (acidic) and have essential roles in gas exchange and pH regulation (reviewed in REF. 58). Following the molecular cloning of CAIX[59], which is normally only expressed in limited areas of the gastrointestinal tract, it was found that CAIX is rapidly induced by HIF[60], leading to its establishment as a marker of poor prognosis in numerous cancer types (for an extensive list, see REFS 13,15). Of the members of the CA family, CAIX is a unique target owing to its nearly exclusive expression by tumour cells at the extracellular surface, which is driven by selection pressures in the tumour microenvironment. Whereas Pastorekova *et al.*[61] showed that CAIX acidifies pH_e, we and others reported that CAIX is a robust pH_i regulator in fibroblasts and hypoxic tumour cells[13,17,18,20,21,62] and as such has a key role in tumour growth together with the other extracellular facing isoform CAXII[18]. Although initial studies revealed that CAIX knockdown (through RNA interference (RNAi)) delayed *in vitro* tumour cell growth[63], effective cell killing was not achieved, which was partly attributed to the concomitant hypoxic induction of CAXII[18]. Our consideration of the magnitude of pH_i changes that are required to affect cell survival could be the reason for reduced efficacy (discussed below), as even combined knockdown of CAIX and CAXII in hypoxic colon adenocarcinoma LS174 cells only reduced pH_i to ~6.9 when pH_e was 6.0 (REF. 16).

More recent work on CAIX disruption has provided encouraging results. Importantly, in the 4T1 mouse model of metastatic breast cancer, knockdown of CAIX (by small hairpin RNA (shRNA)) caused tumours to regress, and consequently 100% of the mice survived with minimal recurrence of the primary tumour (in 20% of the mice)[64]. One of the most interesting findings in this study was that the CAIX inhibitor CAI17 was effective at stalling the progression of mouse 4T1 tumours, but unfortunately it was not as effective on human MDA-MB-231 xenograft tumours[64]. Last, almost no metastases were observed when CAIX was knocked down, which could be a vital finding for the development of metastasis therapies. The importance of CAIX inhibition in the prevention of metastases was indicated by showing that CAIX has a role in the expansion of breast cancer stem cell populations in the hypoxic microenvironment[65].

Neri and Supuran[15] have recently extensively described the development of CAIX inhibition as a clinical target in cancer therapy. Current preclinical studies are combining established clinical applications with CAIX inhibition. In this setting, reduction in CAIX expression has been shown to improve the effect of radiotherapy both *in vitro* and *in vivo* in xenograft experiments[66,67]. Furthermore, CAIX knockdown enhances the effect of the anti-angiogenic therapy bevacizumab in xenografts of HT29 and U87 cells[68]. Recent analysis of human glioblastoma samples indicated high expression of CAIX as an independent marker of prognosis with Kaplan–Meier analysis, revealing a significant decrease in overall survival time (15.2 months with high CAIX versus 34.1 months with low CAIX)[69]. Thus CAIX targeting remains an important cancer-specific treatment strategy, and there are five independent clinical trials currently ongoing using CAIX inhibitors[15]. An interesting note in current clinical trials is that the protein tyrosine kinase inhibitor imatinib also inhibits CAI, CAII, CAIX and CAXII at nanomolar concentrations[70]. Thus, it will be interesting to interpret the positive or negative implications of CA inhibition in imatinib trials. Finally, a monoclonal antibody against CAIX (WX-G250; also known as girentuximab) has recently been shown to increase the median survival time in patients with metastatic renal cell carcinoma when given in combination with interferon-α (IFNα)[71]. This antibody does not inhibit CAIX pH-regulating activity, indicating that other potential roles exist for CAIX in tumour survival.

Timeline | **Historical development of research on H^+ dynamics and metabolic interactions in cancer**



AE1, anion exchange protein 1; CAIX, carbonic anhydrase IX; HIF1, hypoxia-inducible factor 1; MCT, monocarboxylate transporter; NHE1, Na^+/H^+ exchanger 1; PDH, pyruvate dehydrogenase; pH_e, extracellular pH; pH_i, intracellular pH.

Transport metabolon
A group of enzymatic proteins that interact to achieve a more efficient exchange of metabolites.

HCO_3^- transport and the metabolon controversy

A generally accepted feature of tumour cells is that there is a coordination of multiple proteins that provides the efficient pH_i regulation observed in the acidic tumour niche. This is particularly true for CAIX, the high expression of which at the extracellular surface is thought to provide HCO_3^- units that are then rapidly retrieved by the cell by as yet uncharacterized HCO_3^- transporters (FIGS 1b,4a). This proposal of coordinated pH-regulating proteins achieving a common function follows the concept of a membrane transport metabolon, which was originally proposed for the binding of CAII and AE1 (REF. 72). Although there are issues that remain to be resolved with regard to the pH-regulating metabolon (in particular, the specific protein–protein interactions involved have been contested[73–75]), the physiological concept is still very much of interest[76]. A physiological metabolon will remain a topic of interest in tumour cell biology owing to its proposed importance in both pH regulation (CAs and HCO_3^- transporters[10]) and glycolysis (CAs and MCTs[77,78]) (FIG. 4).

Thus far, the definitive molecular identification of the HCO_3^- transporter that is responsible for re-uptake of HCO_3^- has been elusive. HCO_3^- transporters exist in multiple forms with both electroneutral and electrogenic Cl^-/HCO_3^- exchangers (AE1, AE2 (also known as SLC4A2), AE3 (also known as SLC4A3) and the SLC26 proteins) and Na^+/HCO_3^- co-transporters (NBCs) carrying out their respective functions in a tissue-dependent manner[10]. The massive diversity within the HCO_3^- transporting family[79,80] coupled with

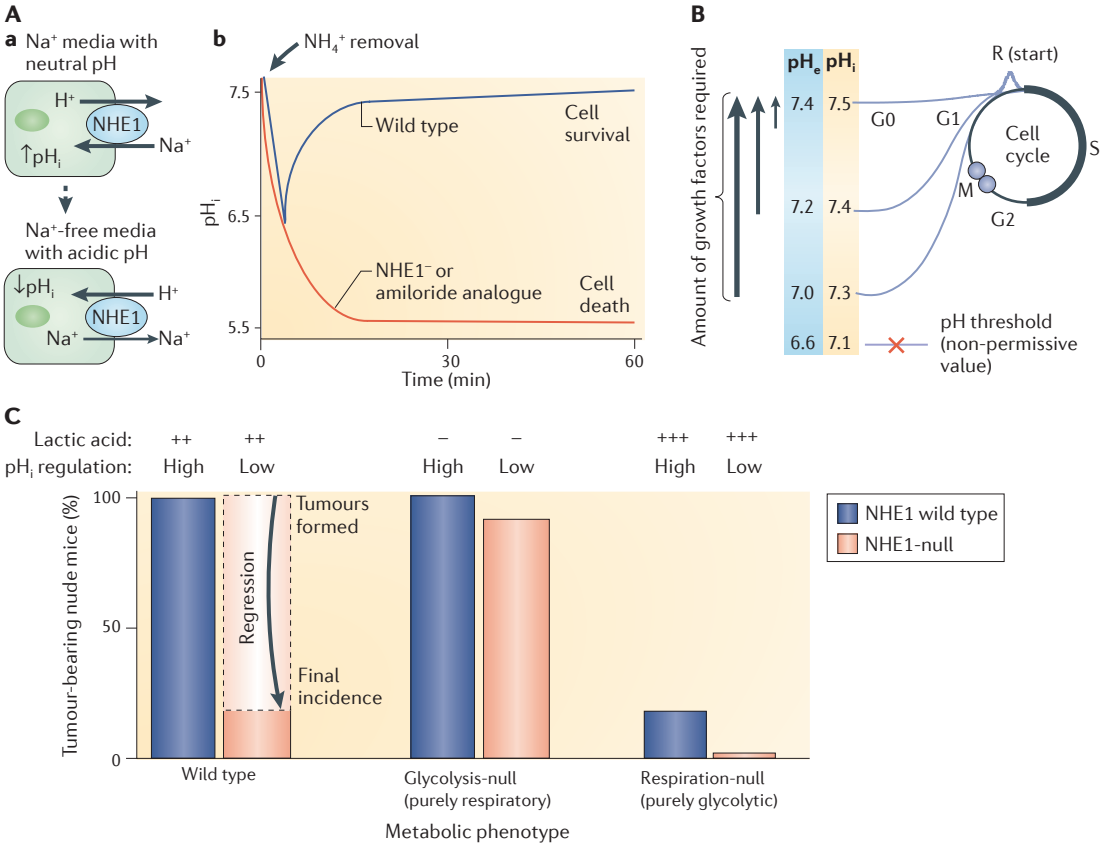


Figure 3 | **Targeting cellular acidification for tumour cell death: proof of concept.** **A** | A summary of the H^+ suicide technique[32,35], which demonstrated that acute intracellular pH (pH_i) acidification can rapidly kill cells. **Aa** | Reversal of Na^+ and H^+ gradients enabled H^+ loading into cells via the reversal of Na^+/H^+ exchanger 1 (NHE1) and led to the selection of NHE1-deficient cells. **Ab** | Strong cellular acid loading using NH_4Cl pre-pulse resulted in mortality for cells deficient in NHE1 activity (owing to NHE1 mutations or pharmacological inhibition with amiloride), which prevented recovery of pH_i. This figure is based on data from REF. 35. **B** | A threshold pH_i value exists to control cell cycle progression. As extracellular pH (pH_e) and consequently pH_i decreases, cells require greater amounts of growth factors to proceed through the cell cycle. Eventually a threshold is reached (below pH_i 7.1) at which point cells will not progress into G1 or S phase even with excessive application of growth factors[33,36]. **C** | Tumorigenicity studies of NHE1 mutants and metabolic mutants established the proof of concept that pH_i regulation is important in tumour development[35]. Wild-type HRAS-transformed fibroblasts that were injected in nude mice always formed tumours. Mutation of NHE1 (NHE1-null) resulted in tumour regression with only 20% of the mice bearing tumours at the end of the study. Tumours derived from cells deficient in glycolysis were mostly unaffected by NHE1 expression. Respiration-deficient cells produce very large amounts of lactic acid and few tumours form with intact pH_i regulation, whereas no tumours formed with NHE1 mutation. Relative lactic acid production and pH_i-regulating capabilities of each tumour type are indicated at the top of the graph.

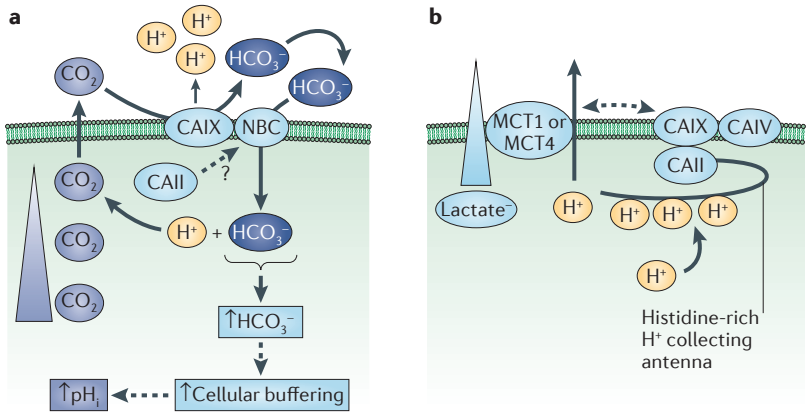


Figure 4 | **Membrane transport metabolons: HCO_3^- and lactate transport. a** | Both extracellular and intracellular carbonic anhydrases (CAs) have been proposed to assist in maintaining HCO_3^- flux across the cell membrane[76] to maintain an alkaline intracellular pH (pH_i) despite acidic extracellular pH (pH_e). Efficient HCO_3^- re-uptake owing to the coordinated action of CAs and Na^+/HCO_3^- co-transporters (NBCs) would then increase cellular buffering capacity to contribute to the maintenance of alkaline pH_i. Additionally, extracellular H^+ diffusion away from the cell surface is assisted by the expression of CAIX[17]. **b** | Extrusion of lactate via monocarboxylate transporters (MCTs) has been shown to be enhanced by the presence of CA isoforms[77,78,107,108]. One of the primary contributing factors to improved lactic acid extrusion is H^+ binding to histidine residues on CAs as opposed to CA catalytic activity. Dashed arrows represent potential interactions.

a lack of clear overexpression in tumours and a lack of specific inhibitors for any HCO_3^- transporters is delaying progress in this area. Initially, a reversal of the normal functioning of a Cl^-/HCO_3^- exchanger was proposed to be a possible player in HCO_3^- re-uptake[5,15]. Indeed, AE1 functions in red blood cells (RBCs) as a bi-directional transporter depending on the gradients that exist at the tissues and the lungs[81]. However, HCO_3^- uptake via a Cl^-/HCO_3^- exchanger has not been described in any other system besides RBCs. An assessment of the tumour microenvironment reveals conditions (low pH_e and non-disrupted Cl^- gradients) that would probably prohibit a Cl^-/HCO_3^- exchanger to function for HCO_3^- uptake, although this remains to be proved.

Expression of the electroneutral NBC (NBCN1; also known as SLC4A7) has been linked to breast cancer susceptibility in genome-wide association studies[82]. Follow-up studies have linked NBCN1 and NHE1 expression with the expression of the truncated form of the ERBB2 receptor and the regulation of pH_i in MCF7 cells[83]. However, it was further shown that NBCN1 inhibition did not alter MCF7 motility, whereas NHE1 inhibition did alter motility[84]. By contrast, a recent report demonstrates the importance of NBCN1 in pH_i regulation in freshly isolated human breast cancer samples, indicating that the overall role of NBCN1 in breast tumour cell biology requires further study[85]. This, combined with the contribution of an electrogenic NBC to tumour pH_i regulation (S.K.P. and J.P., unpublished observations) along with recent data regarding the consistent nature of HCO_3^- transport to tumour pH_i regulation in hypoxia[52], indicates that we may be approaching a better understanding of the overall HCO_3^- uptake mechanism.

Monocarboxylates
Molecules that have one carboxylate group in their structure and require facilitated transport across the plasma membrane; for example, lactate and pyruvate.

Monocarboxylate transporters

Most cancer cells are 'glucose addicts' owing to the predominant use of glycolysis, as initially described by Warburg and revealed by positron emission tomography (PET) imaging of ^{18}[F]-fluorodeoxyglucose (^{18}FDG) tumour uptake in approximately 70% of human cancers[1,3,6]. Despite the fact that the overall ATP yield per glucose molecule is drastically lower through glycolysis than through respiration, it is now well demonstrated that fermentative glycolysis is an efficient pathway to quickly provide energy and carbon building blocks for rapidly growing cells (anapleurosis)[86]. Targeting the strong glucose addiction of cancer cells that is required to maintain glycolysis compared with normal non-dividing cells has consequently developed as a promising therapeutic strategy[25,87–91].

The maintenance of glycolysis requires a continuous expulsion of lactic acid from the cell, which is carried out by MCTs[92]. The MCT family includes 14 different members; however, only four are located at the cell membrane (MCT1, MCT2, MCT3 and MCT4) and have been characterized as carrying out the co-transport of monocarboxylates and H^+ (REF. 93). Importantly for tumour and stromal cells, MCT1 (REFS 94,95) and the hypoxia-induced MCT4 (REF. 26) are specialized for the co-transport of lactate and H^+ (reviewed in REF. 93). MCT1 is implicated in both lactate extrusion and import and has been shown to provide metabolic fuel in the tumour environment through the transfer of metabolites between tumour cells and stromal cells[90]. Although MCT4 has a lower affinity for lactate than MCT1 and MCT2 (K_m of 25 mM versus 4.5 mM for MCT1 and 0.8 mM for MCT2) it has, in contrast to MCT1 and MCT2, an extremely low affinity for pyruvate (K_m of 150 mM) and as such is described as operating almost exclusively as a lactate-exporting protein, which therefore optimizes the reduction of pyruvate into lactate[96,97]. It is thus not surprising that high expression of MCT4 was found in 'glycolytic' tissues, including several hypoxic and rapidly growing tumours[98], such as triple-negative breast cancers[166] and gliomas[99].

Interestingly, MCT1, MCT2, MCT3 and MCT4 require binding with the accessory glycoprotein basigin (also known as CD147 and EMMPRIN) for proper folding in the endoplasmic reticulum, trafficking and insertion into the plasma membrane as functional transporters[25,100] (FIG. 1b). Basigin was first described as an oncogenic protein that induced invasion via the activation of matrix metalloproteinases[101], which resulted in interest in its potential function in glycolysis and tumour cell survival[25]. A combination of knockdown (shRNA of MCT1, MCT4 and basigin), zinc finger nuclease-mediated knockout (of MCT4 and basigin) and pharmacological inhibition (using an MCT1 inhibitor[102]) had antitumoural effects both *in vitro* and *in vivo* in HRAS-transformed fibroblasts and the colon adenocarcinoma LS174 cell line[25]. Similar results were obtained in pancreatic tumour cells[103], indicating that MCT disruption provides an effective avenue for future targeted therapies via the disruption of tumour cell bioenergetics. Moreover, metformin and phenformin

Synthetic lethality
The process of targeting multiple proteins and regulatory systems, by which the combined therapy will induce cell death.

Chronic autophagy
Long-term cellular adaptation towards consumption of cellular components.

Ragulator complex
A multiprotein complex that is responsible for the translocation of mTOR complex 1 to the lysosomal surface.

(which are mitochondrial complex 1 disruptors) sensitize tumour cells to MCT inhibition or knockout, indicating the possibility that synthetic lethality could be achieved through combination therapy[104] (discussed further below). Ultimately, it seems that MCT inhibition in combination with other metabolic or pH_i-regulatory disruption could effectively target aggressive and rapidly growing tumour cells on the basis of their higher metabolic demands. MCTs do not function in the true definition of a pH_i regulator in the sense that they are not activated by a change in pH_i. However, they contribute to the maintenance of cellular alkalinity through the active export of lactic acid, and as such MCTs have a major role in pH_i regulation owing to the high production of intracellular lactic acid in tumour cells. Indeed, the addition of an MCT1 inhibitor in purely glycolytic cells lacking MCT4 induces a rapid decrease in pH_i, leading to growth arrest[25]. MCT inhibitors have also been shown to decrease pH_i in neuroblastoma and melanoma cells[105,106]. Conversely, ectopic expression of MCT4 in $HRAS^{G12V}$-transformed fibroblasts results in a more alkaline tumour pH_i, as monitored *in vivo* by NMR spectroscopy[24]. Similarly, expression of MCT4 in oxidative phosphorylation-deficient tumour cells increased pH_i (REF. 24) and restored full tumorigenic potential[24,25]. These data indicate that MCTs have an important role in pH_i regulation, ensuring an optimal rate of glycolysis and intracellular ATP supply.

Both MCT1 and MCT4 have been proposed to function in a metabolon-like transport model through interactions with either intracellular or extracellular CAs[77,78,107,108] (FIG. 4b). In these models, CAs are hypothesized to enhance H^+ diffusion gradients through their side chain histidine residues, which are proposed to function as an H^+-collecting 'antenna' in the vicinity of MCTs[77,78,107,108]. Interestingly, these enhanced MCT rates are primarily dependent on the histidine residues and not on the catalytic activities of CAs. These data illustrate the important coordination between pH_i-regulating proteins and MCT function in the maintenance of tumour cell energy production via glycolysis. Furthermore, MCT4 expression has implications for metastasis, as indicated by its colocalization with β1 integrins at the leading edge of migrating cells[109,110]. This migratory process is energy demanding, and it is interesting to note that the functional basigin–MCT4 complex could ensure that glycolytic rates are sufficient in this cellular compartment, providing the required metabolic fuel. MCT4 inhibition is thus also of great potential importance for combating tumour cell invasion in hypoxic microenvironments.

H^+ pumps, autophagy and mTOR

Vacuolar (H^+)-ATPases (V-ATPases; commonly termed H^+ pumps) are multisubunit, ATP-driven proteins that have an important role in normal physiological processes, such as receptor-mediated endocytosis, intracellular membrane trafficking and lysosomal acidification, which facilitate the digestion of cellular components[111]. V-ATPases also have a specific role in acid secretion at the plasma membrane in a limited number of cell types, including renal intercalated cells, osteoclasts and epididymal cells (reviewed in REF. 111). H^+ pumps have been proposed to be an integral part of the tumour plasma membrane H^+-extruding system[15,112], the energetic nature of H^+ pumps can assist in moving H^+ out of the cell against an unfavourable gradient (low pH_e).

Convincing evidence for H^+ pumps at the plasma membrane of tumour cells is lacking. A report in the early 1990s created much of the interest related to V-ATPase involvement in cancer cells with a demonstration of functional V-ATPase activity at the plasma membrane based on inhibitor experiments[113]. Following this report, however, the same group searched specifically for V-ATPase localized in the plasma membrane and did not find it in any cell line[114]. This forced them to conclude that "the apparent activity of V-ATPase at the plasma membrane is an epiphenomenon of rapid endomembrane recycling" (REF. 114). However, V-ATPase localization at the plasma membrane of hepatocellular carcinoma (HCC) cells has recently been shown, and V-ATPase expression in these cells was increased compared with normal liver tissue[115]. Mouse orthotopic HCC xenografts treated with the V-ATPase inhibitor bafilomycin effectively reduced tumour growth[115]. Bafilomycin, however, is also known to stabilize HIF1α[116] and inhibit autophagy[117,118] and therefore could have off-target effects outside H^+-pump inhibition.

Owing to the similarity between V-ATPase and the stomach-acidifying (H^++K^+)ATPase, H^+-pump inhibitors — which are commonly exploited as antacids (generically referred to as proton-pump inhibitors (PPIs)) — are at the most advanced stages for clinical use compared with other pH-regulating proteins (reviewed in REFS 15,112). The mechanism of PPIs on V-ATPases remains unclear as they have only been shown to inhibit V-ATPases at very high doses[119,120], and new data indicate that omeprazole specifically does not inhibit the pump function of V-ATPase, despite inducing autophagy markers, showing that PPIs affect the regulatory functions of V-ATPases[121]. PPIs are, however, perfectly suited for a cancer-specific targeted strategy as their activation requires acidity, such as that found in the tumour microenvironment[112]. PPIs have been shown to effectively compromise tumour cell growth by disrupting autophagy and tumour acidosis in both *in vitro* and *in vivo* models[122–125]. Further interest in PPIs relates to the uptake of chemotherapeutic agents (weakly basic or acidic) that can be altered by pH levels[15,126].

We predict that the mechanistic benefit of PPIs for the treatment of cancer is related to a combined effect of inhibiting membrane trafficking, autophagy and mTORC1 signalling. Chronic autophagy, which is observed in the presence of nutrients, has recently been established as a pro-survival cellular adaptation to acidic tumour microenvironment conditions[127]. Furthermore, a link has been made among autophagy, mTORC1 signalling and PPIs (see below)[125]. mTORC1 is the major regulator of cell growth and metabolism through its control over numerous processes, including protein and lipid synthesis (reviewed in REF. 128). PPI administration could disrupt mTORC1 signalling in a number of ways. V-ATPase is a physical component of the Ragulator complex that

Metabolic dormancy
Suppression of cellular metabolism to provide just the minimal energy required to maintain cytostasis.

Ionophores
Molecules that facilitate the movement of ions across the cell membrane, normally by the formation of pores.

is required for mTORC1 function[129] and thus PPIs could directly prevent this interaction. As amino acid levels directly control mTORC1 activity, PPIs could function indirectly on mTORC1 by disrupting the lysosomal pH that V-ATPase maintains at a low permissive level to enable the movement of amino acids in and out of lysosomes[128,130]. Although mTORC1 is known to antagonize autophagy in the short term, it has been demonstrated that mTORC1 is required for the re-formation of lysosomes[131], indicating that it is essential for autophagy in the long term. Therefore, in light of the pro-survival nature of autophagy in advanced tumour cells, particularly in the context of hypoxia[132] and acidosis, PPIs, and also the weak base chloroquine[133], could provide an effective means to target these essential components of tumour cell growth and survival. Exciting potential for future tumour therapy development therefore lies in these interactions among mTORC1, autophagy and altered H^+ dynamics[37,38,129].

Physical limitations of H^+-mediated cell death

Despite the excellent studies regarding tumour acidosis that are discussed here, we still do not have an effective acid-mediated tumour cell death protocol. Thus, we are forced to speculate that an underlying limitation of targeting pH regulation is an inability to decrease pH_i to the level required to cause cell death. We must therefore consider the physical parameters of the tumour microenvironment to critically assess the feasibility of causing appreciable H^+-mediated tumour cell death. For example, an underlying question to consider in the overall discussion of tumour H^+ dynamics is as follows: does the degree of tumour pH_e acidification present an environmental niche that can result in sufficient acidosis to compromise cellular survival if pH_i machinery is disrupted?

Since the description of the Warburg effect, tumour pH_e has been assumed to be acidic owing to the increased production of acidic metabolites coupled with poor clearance owing to incomplete or chaotic vascularization. The first documented measurements of tumour pH_e acidification using electrodes in chicken sarcomas (pH measurements ranging from 6.3 to 6.9) quickly followed Warburg's pioneering studies[134,135] (FIG. 2 (TIMELINE)). Since then, tumour pH_e values of 6.5 have been commonly measured, with some extreme cases reaching below pH_e values of 6.0. Early acidic tumour pH_e measurements recorded using microelectrodes[136–138] have subsequently been confirmed with less invasive MRI and NMR techniques[7,139] (for a summary, see REF. 140). Importantly, the consensus of these observations is that tumour pH_e is consistently lower than normal tissue pH_e, whereas tumour cell pH_i remains higher than normal tissue pH_i (summarized in REFS 5,6,140).

Therefore, an inward H^+ gradient in the range of 0.4–0.7 pH units[136] exists between the extracellular environment and the tumour cell, providing the framework to pose key unanswered questions in H^+ dynamics and cancer: what is the threshold pH_i value of a tumour cell that will allow cell survival? Can this threshold be exploited to cause cell death? How can we ensure acid-mediated tumour cell killing versus induction of metabolic dormancy or cytostasis?

To our knowledge, no empirical studies exist that analyse the degree of pH_i acidification that is required to cause cell death. Studies have demonstrated that a reduction of pH_i by 0.3 units induces a cytostatic growth arrest that can persist over a long period of time and be reversed by an increase of pH_i (REF. 141). Furthermore, the H^+ suicide technique revealed that cell death occurs when pH_i drops below 6.0 (REF. 32). Tannock's group expanded on this concept by demonstrating that NHE1-deficient cells (cells that are unable to maintain pH_i in acidic pH_e) were unable to grow in acidic conditions *in vitro* and did not form tumours in xenograft mouse models[142,143]. A further series of studies attempted to exploit the cytotoxic effect of pH_i acidification in tumour cell lines compared with previous work on fibroblasts. In these studies, cytotoxicity occurred only at more extreme pH_e acidification (<6.5) and in the presence of ionophores (for example, nigericin[144] and carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP)[39,145]), whereas the use of inhibitors of pH_i regulators, such as amiloride and DIDS, increased the pH_e required for cytotoxicity. Analysis of the correlation between pH_i and cell death revealed that, on reaching a pH_i of ≤6.2, cell survival becomes severely compromised[145]. However, as these studies required ionophores, they do not represent an intact pH_i-regulating machinery and thus do not provide direct empirical evidence for the pH_i that is required to kill tumour cells. Furthermore, these early studies were not carried out with the current understanding of the influence of hypoxia on promoting cell survival and pH regulation[18–21,52,61], further clouding our assessment of the degree of disruption to H^+ dynamics that is required to kill cells. Data from fibroblasts have also possibly limited our interpretation of pH_i regulation in cancer cells, as these cells typically are not equipped with the complete range of pH_i-regulating proteins of cancer cells.

Considering the above information, we have to assume that a low pH_i of around 6.0 is required to ensure cell death. Thus, if we could achieve a reduction in pH_i via disruption of pH_i-regulating proteins, movements across membranes by weak acids and bases could maintain cells at a pH_i that permits cytostasis as opposed to cell death (FIG. 5a). Following the clearly illustrated model of weak acid and base movements[146], we observe that the tumour microenvironment presents 'non-physiological' conditions. However, these altered gradients would still favour H^+ entry or leakage into cells, as observed in normal physiological conditions (FIG. 5a). However, if tumour pH_i is substantially reduced, the equilibrium constant for H^+ could become outwardly directed, resulting in passive movement of H^+ from weak acids. This could be particularly important considering that tumour cells are described to have a more positively charged membrane potential than normal cells[147] (that is, –45 mV for normal cells versus –15 mV for cancer cells), which would favour this passive H^+ efflux on pH_i acidification. Thus, as the H^+-mediated tumour cell killing strategy is essentially based on the ability to utilize the movement of weak acids in the tumour microenvironment, we should consider the possibility that these physical

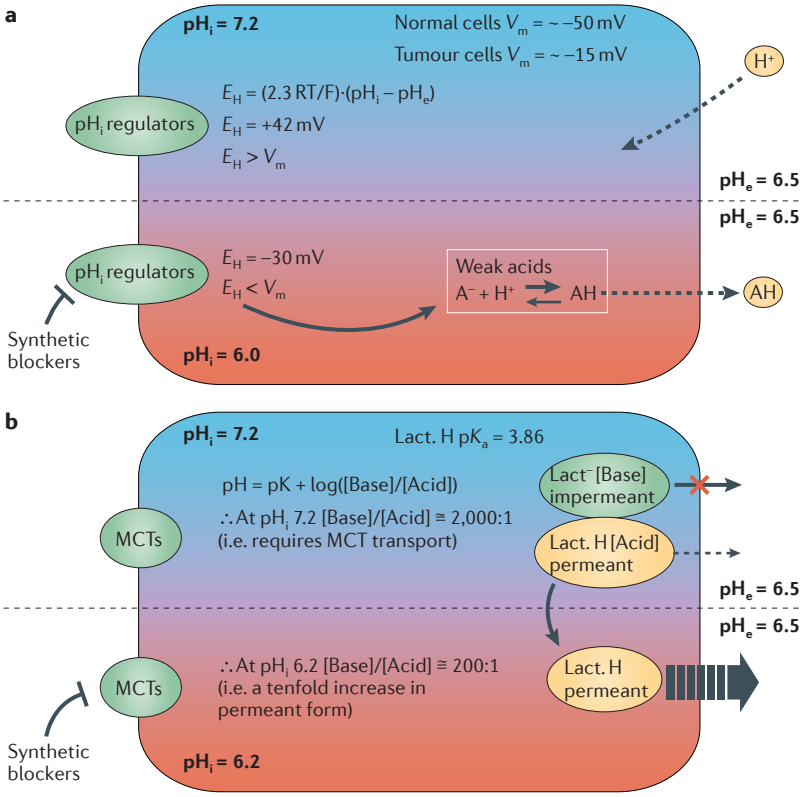


Figure 5 | **Physical limitations for the effective reduction of intracellular pH that would be permissive for cell killing.** **a** | Effective reduction in intracellular pH (pH$_i$; bottom half of the schematic cell) results in an altered Nernst equilibrium potential for H$^+$ (E_H) that would favour H$^+$ efflux from the cell through the movement of permeant weak acids. This movement of weak acids could help to maintain pH$_i$ at a level that does not induce cell killing but that perhaps causes cell cycle arrest. **b** | Lactic acid (Lact. H) can also diffuse across the membrane as a permeant weak acid despite inhibition of all lactate transporting pathways. We illustrate a scenario in which pH$_i$ can drop appreciably owing to the inhibition of lactate-extruding pathways (bottom half of schematic cell), which would result in an increased abundance of the membrane permeant form of lactic acid (based on calculations from the Henderson–Hasselbalch equation) compared with impermeant lactate. This condition could promote a slow but continuous extrusion of lactic acid from the cellular space to allow the maintenance of glycolysis. MCT, monocarboxylate transporter; pH$_e$, extracellular pH. Dashed arrows represent movement across the membrane.

Nernst equilibrium potential
A mathematical formula that describes the equilibrium state of ions between two compartments based on the concentration and electric gradients that exist in the system.

pK_a
The acid dissociation constant that indicates the relative strength of a given acid in solution.

Evolutionary game theory
The application of strategic game theory mathematical modelling to the evolutionary progression of a biological system.

parameters might never reach a permissive level for cell killing. They could, however, reach levels that induce cell cycle arrest.

In our attempts to block lactate efflux from tumour cells, we have come to realize the importance of the capacity for diffusion of membrane-permeant, protonated weak acids. As expected, abrogation of lactate export by facilitated diffusion (through MCT4 knockout and MCT1 inhibition) resulted in a dramatic accumulation of intracellular lactic acid within minutes[25]. However, MCT-impaired tumour cells still passively export a high amount of lactic acid to the culture medium when measured over a 24-hour period (J.P. and I. Marchiq, unpublished observations). This could follow a similar phenomenon to the scenario illustrated in FIG. 5a, with a decreased pH$_i$ favouring the movement of the protonated and permeant lactic acid species across the membrane (FIG. 5b). Thus, considering that the pK_a of lactic acid is 3.86, it is predominantly dissociated into the impermeant form (lactate$^-$ + H$^+$) at a physiological pH$_i$ of 7.2. For example, if the pH$_i$ were to drop to 6.2, although lactic acid would still be strongly dissociated, there would be a tenfold increase in the export diffusion rate of protonated lactic acid associated with an arrest of production of this acid at low pH$_i$ (FIG. 5b). Potential microdomains of pH$_i$ acidification along the cell membrane could therefore facilitate the continued extrusion of lactate despite MCT inhibition. Understanding and overcoming these physical limitations is a major challenge for future studies on pH regulation, metabolic function and advanced therapy.

H$^+$ dynamics and metastasis

Gatenby and Gillies' groups have extensively studied the 'acid-mediated invasion' hypothesis as the mechanism for tumour cell invasion of surrounding tissues and resultant metastases[12,14,148,149]. In this model, the acidic tumour environment induces a breakdown of normal surrounding tissues while the cancer cells that have evolved to withstand their acidic surroundings undergo enhanced migration on exposure to acidosis[150]. In this context the pH$_i$-regulating proteins in cancer cells direct cell migration through the creation of cellular pH gradients[57]. NHE1 and CAIX in particular have been heavily implicated in cell migration owing to their role in mediating intracellular and extracellular H$^+$ dynamics[22,64,151]. Recent supporting evidence for acid-mediated 'niche engineering' in evolutionary game theory was provided by tracking overall tumour growth and the direction of growth with respect to the pH$_e$ using dorsal window mouse tumour xenograft models (using HCT116 and MDA-MB-231 cells)[152]. Indeed, tumour growth is enhanced and directed by the developing acidic pH$_e$, whereas systemic buffering by $NaHCO_3$ treatment prevents this growth[152]. Further evidence showing that NHE1 and glucose transporter type 1 (GLUT1; also known as SLC2A1) are at the leading edge of the tumour and that there is increased extracellular matrix breakdown and vessel formation in acidic zones has strengthened the overall acid-mediated invasion hypothesis.

As a result of these data, a simple approach to prevent metastasis has been proposed via the use of systemic buffers[153,154], and a mouse model of metastatic breast cancer $NaHCO_3$ treatment resulted in increased tumour pH and a dramatic reduction of spontaneous metastases without affecting the primary tumour[153]. These data have been recently extended to a mouse model that develops spontaneous prostate tumours, and studies indicate a time sensitivity of buffering treatment that is dependent on cancer type[155]. HCO_3^- therapy applied before 6 weeks post-birth resulted in almost no tumour development, whereas HCO_3^- treatment started after 6 weeks post-birth resulted in the same tumour incidence as controls[155]. This corresponded to the normal time of tumour formation in this mouse model (between 4 and 8 weeks post-birth), indicating that systemic buffering is perhaps most beneficial as a pH-directed therapy in early development for the treatment of certain tumours[155]. Further experimentation with systemic buffering could provide an exciting and simple way to assist in the combat against fatal metastases.

Unfolded protein response
A stress response within the cell that responds to misfolded proteins and initiates a cascade that leads to apoptotic cell death.

Perspectives

In this Review we emphasize several membrane proteins that are involved in pH_i regulation, a key biological process that ensures tumour growth by exporting metabolism-generated acids. The most rapidly growing tumours are often hypoxic and have resolved the increased nutrient demand by inducing the expression of glucose and amino acid transporters (GLUT1 and L-type amino acid transporter 1 (LAT1; also known as SLC7A5)) through the activity of HIF[156,157] (FIG. 1b). In parallel, tumour cells export acidic by-products through the HIF-mediated induction of CAIX and MCT4 expression, through increased transport efficiency using metabolons (NBCs, CAs and MCTs)[77,78] and through increased affinity for H^+ export (NHE1)[29,43]. Considering the potential killing power of altered H^+ dynamics[32,35], we and others[5,27] envisioned that a combined inhibition of H^+-exporting systems (NHEs, NBCs, CAs and MCTs) would provide a promising new anticancer opportunity. But *in vitro* experiments that combine inhibition of MCTs with NHE1 or inhibition of MCTs with CAs did not improve tumour cell death compared with single inhibition (J.P. and D. Roux, unpublished observations). In both single and combined treatments we obtained tumour growth arrest, but the tumour cells re-grew when the treatment was stopped[25,49]. This acidic pH_i-induced cytostatic effect is due to metabolic dormancy, and the lack of cell killing is due to the failure to decrease pH_i to the lethal level. However, the combination of CA inhibition (targeting hypoxic areas) with either radiotherapy[66,67] or bevacizumab[68] improved the treatment by delaying tumour progression in preclinical studies. Likewise, PPIs or inhibitors of CAs or MCTs are expected to synergize with or be improved by inhibition of the HIF-induced LAT1–CD98 complex[157], which would inhibit mTORC1 and induce apoptosis through the activation of the unfolded protein response (FIG. 6).

However, considering the availability of multiple rationally designed drugs that target key drivers of the major growth signalling pathways (such as receptor tyrosine kinases (RTKs), RAF, MEK, PI3K, AKT and mTOR) and enzymes in metabolic or bioenergetic cascades[158], the question remains as to how we can improve cancer curability. How can we stop the success of accelerated Darwinian selection of tumour cells? The obvious solution is the induction of cell death, and this is why synthetic lethality is so attractive. Recently, a wide range of potential therapies that target metabolism have been proposed to be dependent on altered cellular metabolic phenotypes[4]. Here, we propose that the pursuit of

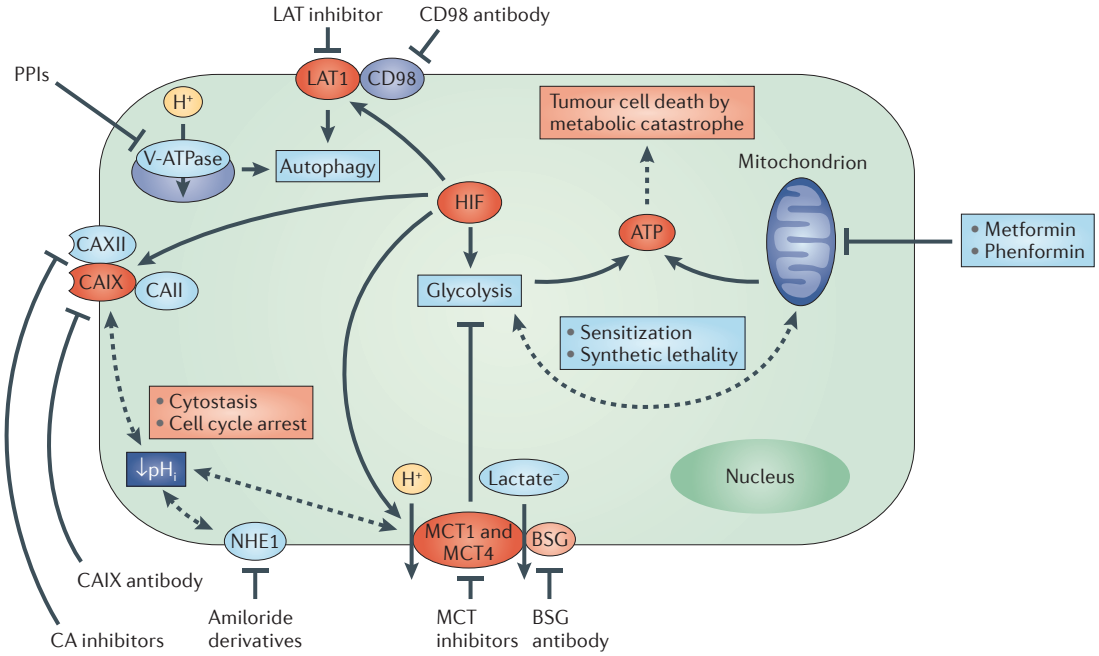


Figure 6 | **Potential strategies to target metabolic-pH cellular interactions to induce 'metabolic catastrophe' and achieve tumour cell killing.** Points to target for inhibition are shown and potential interactions are represented by the dashed arrows. Combined targeting of hypoxia-induced carbonic anhydrase IX (CAIX) and monocarboxylate transporter 4 (MCT4) would contribute to the acidification of intracellular pH (pH_i), which could be further enhanced with inhibition of Na^+/H^+ exchanger 1 (NHE1). Accumulation of cellular lactate owing to MCT inhibition will limit glycolysis, leading to a decrease of cellular ATP levels. This metabolic stress could be enhanced with the application of metformin or phenformin to inhibit mitochondrial ATP production with the intent of conferring synthetic lethality (metabolic catastrophe). Finally, application of proton pump inhibitors (PPIs) could alter lysosomal pH in a way that would disrupt mTOR complex 1 (mTORC1) signalling and thus prevent pro-survival autophagy, which is known to promote tumour progression. Inhibition of the amino acid uptake pathway (by targeting L-type amino acid transporter 1 (LAT1)) would further interfere with mTORC1 signalling. By combining a reduction in pH_i-regulating capacity with a disruption of key metabolic pathways, we predict that highly aggressive cancer cells will enter metabolic catastrophe and subsequently perish. BSG, basigin; HIF, hypoxia-inducible factor.

Metabolic catastrophe
When cellular metabolism is disrupted severely enough to prevent energy (ATP) production and the cell consequently perishes.

ATP crisis
A state in which the cell does not produce enough ATP to meet its energetic demands for survival.

disrupting both pH and bioenergetic regulatory proteins could be an effective means to achieve metabolic catastrophe or ATP crisis in aggressive and rapidly developing tumours. Most cells demonstrate plasticity in their ability to generate ATP from either glycolysis or respiration, whereby blockage of one metabolic pathway rapidly shifts cells to generate ATP from the other. We observed that blocking MCT1 and MCT4 in colon adenocarcinoma cells resulted in reduced rates of glycolysis with a concomitant reactivation of oxidative phosphorylation (I. Marchiq, R. Le Floch, D. Roux and J.P., unpublished observations). These cells then became extremely sensitive to metformin and phenformin. This dual treatment induced a rapid collapse in ATP level and total cell death in 2–3 days[104]. We believe, as was also discussed by Pollak[159], that inhibition of highly glycolytic tumour cells with RTK, RAF or AKT inhibitors will reduce glycolysis and cause a shift to oxidative phosphorylation, which has been shown in imatinib-treated BCR-ABL leukaemic cells[160] or BRAF-inhibited melanoma[161]. These glycolysis-inhibited tumours (achieved by disruption with MCT inhibitors, for example) should then become sensitive to phenformin treatment. This inhibition of glycolysis and mitochondrial complex 1 — a combined treatment that we refer to as the 'metabolic knife' (FIG. 6) — will have to be acute (perhaps lasting 3–5 days depending on rigorous testing) and repeated at intervals to spare normal tissue and resting stem cells and to limit toxicity. The development of this approach to combine acute inhibition of energy-sensing kinases (such as liver kinase B1 (LKB1; also known as STK11) and AMP-activated protein kinase (AMPK))[162] or downstream glycolytic targets (such as lactate dehydrogenase A (LDHA)[88,89] or MCTs[25]) with phenformin are currently ongoing, and time will tell whether real progress will emerge in treating this fatal disease.

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Acknowledgements
Research in the authors' laboratory is financed by European Community (EU7-METOXIA) and French agencies and associations: the Agence Nationale de la Recherche, Ligue Nationale Contre le Cancer (Equipe Labellisée), Institut National du Cancer and grants from the Fondation ARC pour la Recherche sur le Cancer (to S.K.P. and J.C.).

Competing interests statement
The authors declare no competing financial interests.

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