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The chemistry, physiology and pathology of pH in cancer

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Cell survival is conditional on the maintenance of a favourable acid–base balance (pH). Owing to intensive respiratory CO_2 and lactic acid production, cancer cells are exposed continuously to large acid–base fluxes, which would disturb pH if uncorrected. The large cellular reservoir of H^+-binding sites can buffer pH changes but, on its own, is inadequate to regulate intracellular pH. To stabilize intracellular pH at a favourable level, cells control trans-membrane traffic of H^+-ions (or their chemical equivalents, e.g. HCO_3^-) using specialized transporter proteins sensitive to pH. In poorly perfused tumours, additional diffusion-reaction mechanisms, involving carbonic anhydrase (CA) enzymes, fine-tune control extracellular pH. The ability of H^+-ions to change the ionization state of proteins underlies the exquisite pH sensitivity of cellular behaviour, including key processes in cancer formation and metastasis (proliferation, cell cycle, transformation, migration). Elevated metabolism, weakened cell-to-capillary diffusive coupling, and adaptations involving H^+/H^+-equivalent transporters and extracellular-facing CAs give cancer cells the means to manipulate microenvironmental acidity, a cancer hallmark. Through genetic instability, the cellular apparatus for regulating and sensing pH is able to adapt to extracellular acidity, driving disease progression. The therapeutic potential of disturbing this sequence by targeting H^+/H^+-equivalent transporters, buffering or CAs is being investigated, using monoclonal antibodies and small-molecule inhibitors.

1. Introduction

Hydrogen (H^+) ions (or protons) are the smallest yet arguably the most reactive ions present in living organisms. All biological solutions have a certain concentration of H^+ ions ($[H^+]$) arising from the balance between deprotonation and protonation reactions of water, weak acids and weak bases. Equilibria between H^+ ions and the unprotonated (A) and protonated (HA) forms of molecules is described by an acid-dissociation ($K_a = [H^+] \times [A]/[HA]$) (figure 1). Values of K_a span many orders of magnitude and, consequently, $[H^+]$ can vary greatly between different solutions. For this reason, $[H^+]$ is usually expressed on a logarithmic pH scale [1]. Complex solutes, including many biologically important molecules, are often ascribed with several K_a values, reflecting distinct proton-binding sites. At a given pH, protonatable sites with very high or very low pK_a will be almost completely titrated or unbound, respectively. By contrast, the concentration (in a macroscopic sense) of protonated and unprotonated sites will be balanced if pK_a is near the ambient pH. Such molecules are of major biological importance for two reasons. Firstly, the availability of HA and A protects (buffers) solutions from large pH changes in response to acid–base challenges. Secondly, a sustained change in pH alters the [HA]/[A] ratio, which could have secondary effects if the biological properties of HA and A differ substantially. The sensitivity of proteins to pH has exceptional bearing on cells because proteins act as pH buffers, and their function can change substantially if ionization state is altered by the binding or release of

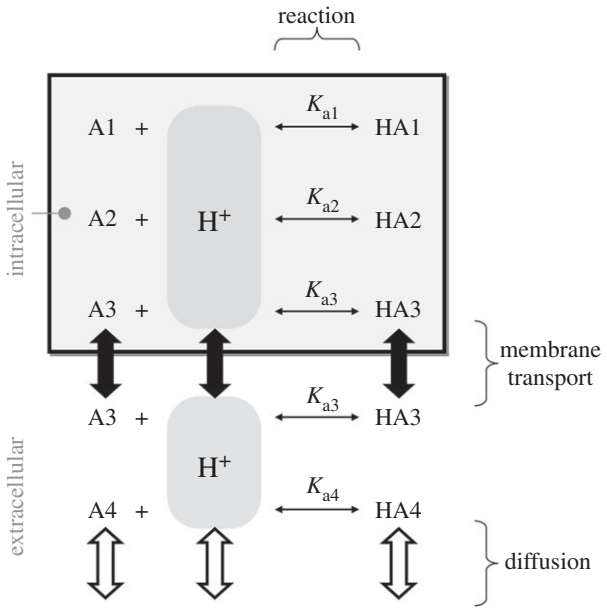

Figure 1. The dynamics of intra- and extracellular pH are determined by reaction, transport and diffusion fluxes. For illustrative purposes, two intracellular buffers (HA1/A1 and HA2/A2), one extracellular freely diffusible buffer (HA4/A4), and a buffer that can cross the cell membrane (HA3/A3) are shown.

H^+ ions (a form of post-translational modification) [2]. It is therefore not surprising that only a narrow range of pH is compatible with eukaryotic function.

Living tissue, unlike a simple salt solution, engages continually in the production or consumption of acids (or bases) through chemical reactions. Because of cellular respiration (yielding CO_2 and lactic acid), most cells are net acid-producers hence intracellular pH (pH_i) has a tendency to fall. A sustained and substantial acid–base challenge cannot be corrected by pH buffers alone because of their finite capacity (i.e. buffering reduces the amplitude of pH-changes but cannot, on its own, eliminate or reverse these). Also in contrast to a simple solution, living tissue is compartmentalized into intra- and extracellular spaces separated by the cell membrane (figure 1). The ability of biological membranes to allow the passage of selected molecules can give rise to pH differences between the compartments. Selective transport of H^+-ions (or molecules that release or take-up H^+ ions such as CO_2 or HCO_3^-: the so-called H^+-equivalents) across membranes is thus an effective means of changing pH_i. As explained later, the usual pH_i-regulatory strategy of cells is to balance the internal production of acid (or base) with an equal 'corrective' efflux of acid (or base) across the cell membrane.

The biological potency and chemical omnipresence of H^+ ions highlight the importance of *regulating* pH (where pH is controlled to suit protein function) and of *adapting* biology to a particular pH level (where gene products are selected or changed on the basis of ambient pH). As will be explained below, these processes are believed to play an important role in cancer disease progression.

2. Low micro-environmental O_2 tension and pH as hallmarks of cancer

Histological studies in the 1950s by Thomlinson and Gray established that human tumours grow around blood vessels and that the outermost cells beyond a distance of approximately 200 µm from blood become necrotic [3]. A gradient of O_2 tension develops across the layer of viable cells, driven by the high metabolic demand of cancer biochemistry and relatively long diffusion distances to the source [4]. O_2 gradients have often been modelled by steady-state diffusion–reaction equations, where D_{O_2} is the O_2 diffusion coefficient and function R describes reactions

$$D_{O_2} \times \nabla^2[O_2] + R([O_2]) = 0. \tag{2.1}$$

The presence of areas with low (<1%) O_2 tension is associated with increased metastasis and poor patient survival [5], giving rise to the notion that hypoxia is a hallmark of malignant cancer. The discovery that hypoxia alters cell biology [6] (e.g. via hypoxia-inducible factor HIF1α [7]) offered a mechanism for adaptive changes, such as the switch-over to glycolytic metabolism (Warburg effect; [8]). Tumour hypoxia has since become a topic of considerable research, achieving promising outcomes with respect to understanding aetiology, improving diagnosis and developing treatments [6,9]. Among other micro-environmental factors specifically identified in tumours, extracellular acidity has emerged as another cancer hallmark [10–12]. Contrary to initial expectations, the intracellular compartment was shown to be alkaline [13] despite low extracellular pH (pH_e). Other than in solid tumours, this trans-membrane $[H^+]$ distribution (acidic extracellularly/alkaline intracellularly) is not commonly observed in tissue. Two questions have emerged in response to these pioneering studies: firstly, how do solid tumours produce low pH_e yet are able to maintain pH_i within favourable limits, and, secondly, how does this trans-membrane pH-distribution affect disease progression?

3. Production and venting of metabolic acids

Cancer cells require a substantial input of energy to support their intensive programme of growth. This explains the high glucose utilization rate, measured to be most typically in the range 0.1–1 µmol (g tissue)$^{-1}$ min^{-1} [14]. Under aerobic conditions, respiration of glucose to CO_2 is coupled to the production of ATP, which consumes an H^+ ion:

$$ADP^{3-} + HPO_4^{2-} \rightarrow ATP^{4-} + OH^-.$$

This acid–base disturbance is then cancelled out by ATP breakdown elsewhere in the cell. As a result, the source of acidity from aerobic metabolism is CO_2, once it hydrates to H^+ and HCO_3^- ions. Under anaerobic conditions, glycolytic ATP production is coupled to the chemical conversion of glucose to anionic lactate [15]:

$$\text{glucose} + 2 \times ADP^{3-} + 2 \times HPO_4^{2-} \rightarrow 2 \times ATP^{4-} + 2 \times \text{lactate}^-.$$

This reaction does not generate (or consume) H^+ ions, indicating that glycolysis *per se* is pH neutral. However, subsequent ATP breakdown releases H^+ ions, explaining how anaerobic metabolism yields acid. Depending on whether respiration is glycolytic or mitochondrial, cancer cells may be producing approximately 1–3 mmol · (l cell)$^{-1}$ min^{-1} of acid (assuming an extracellular/intracellular volume ratio of 1/2; [16]). For a typical intracellular buffering capacity of approximately 30 mmol · (l cell)$^{-1}$ · (pH unit)$^{-1}$ [17], this magnitude of acid-loading would promptly and substantially alter pH_i,

if uncorrected. However, most cells have the capacity to remove respiratory end-products passively across the surface membrane. CO_2 has a high lipid : water partition coefficient, allowing it to cross the lipid bilayer freely. In addition (although not without controversy [18]), specialized gas channels such as aquaporins (AQP) have been demonstrated to increase membrane permeability to CO_2 [19]. Lactic acid, despite a much lower lipid : water partition coefficient, can cross the membrane as H^+-lactate, translocated by H^+-monocarboxylate transporters (MCT), including MCT1 and the hypoxia-inducible MCT4 [20] (according to the SoLute Carrier family naming convention, SLC16A1 and SLC16A3, respectively).

The rate of passive CO_2 and H^+-lactate venting from cells depends on trans-membrane concentration gradients. In well-perfused tissues, outwardly directed trans-membrane gradients are maintained by good diffusive coupling between the cell surface and capillary blood. By contrast, the often inadequate capillary perfusion of tumours gives rise to long diffusion distances and a considerable resistance to solute flux [21]. Extracellular build-up of CO_2 or H^+-lactate will reduce their venting, even across membranes with high permeability. However, CO_2 and H^+-lactate diffusion can be facilitated by biological adaptations that address the rate-limiting steps. Extracellular lactic acid ($pK_a = 3.8$) remains almost completely ionized and the associated H^+ ion is titrated by extracellular buffers. The overall rate of extracellular H^+-lactate diffusion can be rate-limited by the effective mobility of H^+ ions (D_H^{app}). In highly buffered solutions, D_H^{app} depends on the mobility of buffers [22], many of which are large proteins diffusing substantially slower than lactate. However, low molecular weight (mobile) buffers, such as amino acids, phosphates or CO_2/HCO_3^- could facilitate H^+ diffusion and improve H^+-lactate venting (figure 2*a*). Extracellular CO_2 also ionizes ($pK_a = 6.1$) but to a much lesser degree than lactic acid. Although the spontaneous hydration reaction is very slow (time constant 5 s), it can be catalysed by exo-facial carbonic anhydrase (CA) enzymes [23–25], such as the tumour-associated isoforms CAIX and CAXII [26–30]. Catalysed conversion of CO_2 to HCO_3^- and H^+ can facilitate overall CO_2 diffusion by means of a parallel flux of $H^+ + HCO_3^-$, a phenomenon first described *in vitro* by Gros & Moll [31]. Akin to the diffusion of other ionized weak acids, CA-facilitated CO_2 diffusion also requires adequate mobile buffering to carry H^+ ions in parallel to CO_2 and HCO_3^- [32] (figure 2*b*). Net hydration by exofacial CAs will reduce steady-state pH_e and contribute towards micro-environment acidity. Excessive extracellular acidification could become detrimental, but the extent of this may be curtailed by the inhibitory (i.e. negative feedback) effect of H^+ ions on CA activity [33–35].

Mobile buffers usually coexist with fixed buffers, therefore D_H^{app} is typically lower than the diffusivity of CO_2, HCO_3^- or lactate. Consequently, a relatively steep $[H^+]_e$ gradient is needed to drive a diffusive H^+ ion flux to match the flux of CO_2, HCO_3^- or lactate. This may explain why it is important for $[H^+]_e$ at the core of solid tumours to reach levels as high as 250 nM, i.e. $pH_e = 6.6$ [4]. A mechanistic description of tumour pH_e must account for diffusion–reaction processes involving H^+ ions, CO_2, HCO_3^-, lactate and buffers [25], and hence a single equation (such as equation (2.1) used for modelling hypoxia) is inadequate.

Facilitated CO_2 and H^+-lactate diffusion away from the surface of cells is expected to produce a more alkaline pH_i that

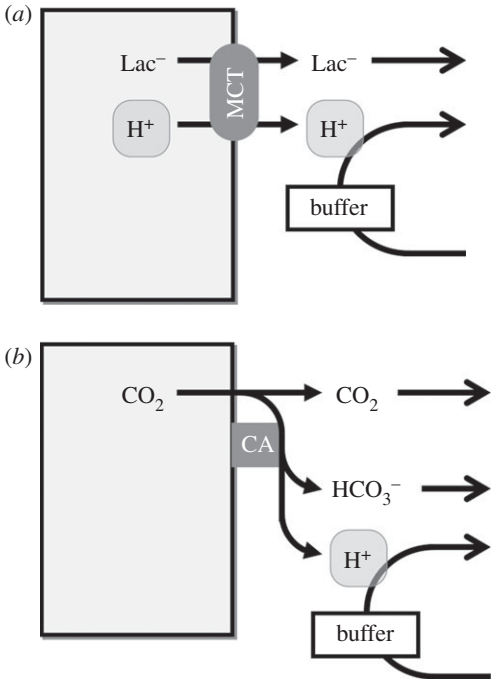

Figure 2. Venting of metabolically produced acids. (*a*) H^+-lactate efflux across the membrane is facilitated by H^+-monocarboxylate transport (MCT). Diffusion of H^+ and lactate away from the cell-surface is necessary for sustained MCT activity. Mobile H^+-buffers can facilitate H^+ diffusion and support H^+-lactate venting. (*b*) CO_2 can permeate the cell membrane through the lipid bilayer or gas channels. Spontaneous CO_2 hydration is slow, but can be accelerated by exofacial carbonic anhydrase (CA) enzymes. Diffusion of the hydration products alongside CO_2 represents a form of facilitated CO_2 diffusion.

better supports cell proliferation. Indeed, facilitated CO_2 venting appears to be a major role for CAIX and CAXII in tumour physiology [25,36], possibly explaining the faster growth rates measured in tumours expressing catalytically active enzyme [35,37]. As demonstrated in skeletal muscle [38], exo-facial CAs also improve H^+-lactate venting by optimizing the ability of CO_2/HCO_3^- to neutralize H^+ ions released by MCT. A similar MCT–CA interaction may be important in tumours.

In the scheme developed so far, H^+-lactate and CO_2 are the principal sources of cellular acid, and their venting is rate-limited by resistances imposed by membranes (permeation) and the tortuous interstitial space (diffusion). Steady-state intracellular $[H^+]$ could be approximated by

$$[H^+]_i = \frac{[H^+]_e \times [\text{lactate}^-]_e}{[\text{lactate}^-]_i} = \frac{[H^+]_e \times [HCO_3^-]_e}{[HCO_3^-]_i}.$$

In some cells, such as erythrocytes [39], steady-state pH_i predicted by this equation is viable because plasma $[H^+]$, $[HCO_3^-]$ and $[\text{lactate}^-]$ are normally well controlled in the body. However, most cells do not have the privilege of direct access to a well-controlled milieu and, consequently, the predicted equilibrium pH_i may not be compatible with biological needs. Due to its stoichiometry, MCT couples the transport of H^+ ions with the transmembrane [lactate] gradient, and any further demand for H^+ transport would have to be met by other means. In addition, many processes, such as cell division, require cells to manipulate pH_i dynamically and independently of pH_e. For these reasons, cells in most tissues, including tumours, have additional mechanisms for regulating pH_i.

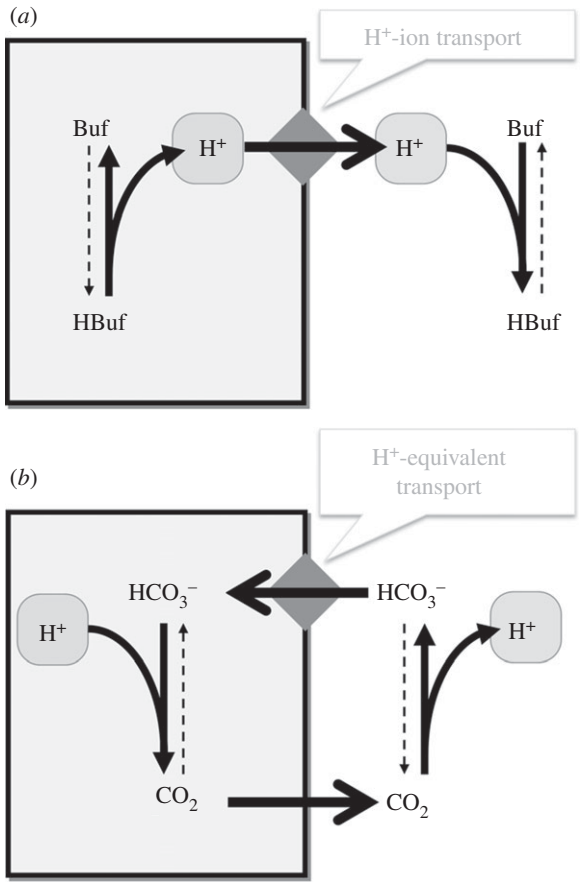

Figure 3. Regulation of intracellular pH by acid-extruding transporter-proteins (diamond symbol). (*a*) Extrusion of H^+ ions alkalinizes the cell. This must also remove buffer-bound H^+ ions to change pH_i substantially. (*b*) Uptake of HCO_3^- (or CO_3^{2-}) ions also alkalinizes the cell because the subsequent reaction with intracellular H^+ ions produces CO_2, the passive venting of which completes the acid-extrusion process. HCO_3^- transport is considered to be H^+-equivalent flux.

4. pH regulation by membrane transport

In a solution where the concentrations and K_a of buffers are held constant, the only means of changing pH is by adding acid (or base). Whereas H^+/H^+-equivalent production (or consumption) by metabolism is not a feasible means of achieving pH_i-homeostasis, cells can control their pH_i by regulating the active transport of H^+ ions or their chemical equivalents (OH^-, HCO_3^- or CO_3^{2-}) across the membrane [40,41]. Experimentally, it is possible to distinguish pH_i-regulating proteins that translocate H^+ (or OH^-) ions from those that translocate HCO_3^- (or CO_3^{2-}) ions by measuring fluxes in the presence and the absence of CO_2/HCO_3^- buffer [42] (figure 3).

For a complete regulatory system, membrane transporters must 'sense' pH_i and respond by producing net H^+/H^+-equivalent efflux or influx when pH_i is either too low or too high, respectively. In practice, most cells express dedicated acid-loading and acid-extruding transporter proteins [40]. By working against each other, acid-extrusion and acid-loading fluxes can correct pH_i disturbances and maintain pH_i around a steady-state point (figure 4*a*). Acid-extruding transporter proteins include Na^+/H^+ exchangers (NHE) belonging to the SLC9 family [43], H^+-ATPase pumps and transporters that produce net HCO_3^- (or CO_3^{2-}) influx, such as electroneutral Na^+–HCO_3^- cotransport (NBCn1/SLC4A7), electrogenic Na^+–$2HCO_3^-$ cotransport

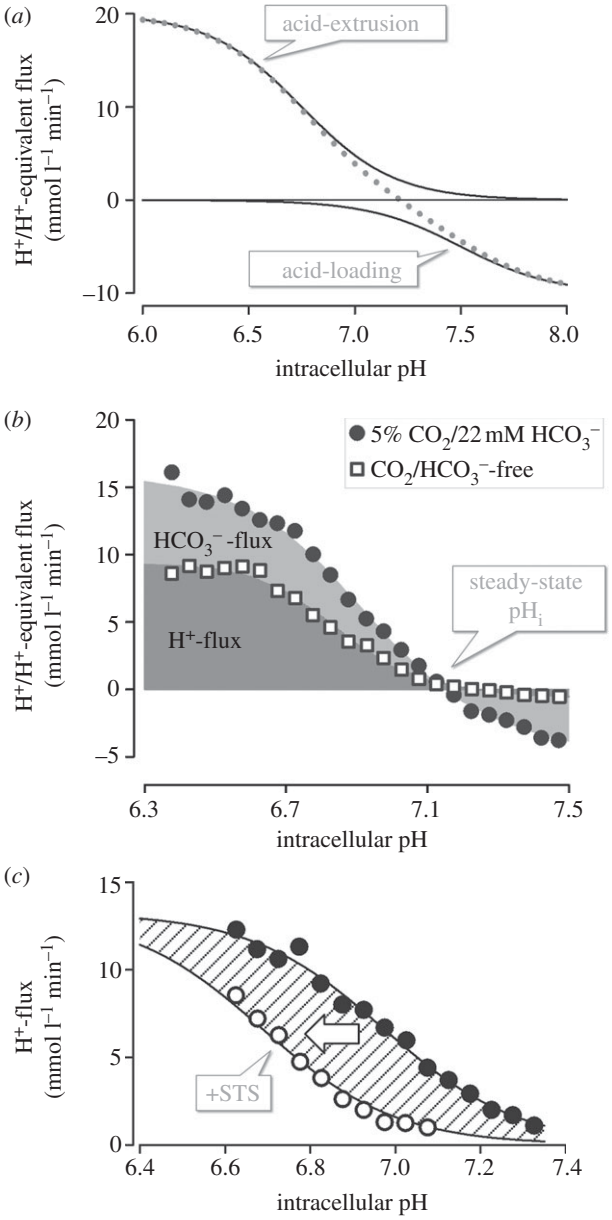

Figure 4. Flux analysis of H^+/H^+-equivalent membrane transport. (*a*) Hypothetical pH_i dependence of flux by acid-extruding and acid-loading transporters: the shape of the pH_i–flux relationship depends on the kinetics of transport, often described mathematically by a Hill equation. Acid extruders and acid loaders work against each other to produce net H^+/H^+-equivalent flux (dotted line). Intracellular pH stabilizes at a point of zero net flux. The angle at which the net flux curve crosses the pH_i axis is a measure of the responsiveness of the pH_i-regulating apparatus to pH_i disturbances. (*b*) Example of flux analysis for colon HCT116 cancer cells cultured under normoxia (see [17]). Fluxes can be dissected into CO_2/HCO_3^--dependent (i.e. involving HCO_3^- transport) and CO_2/HCO_3^--independent (i.e. involving H^+ transport). (*c*) Na^+/H^+ exchange in HCT116 cells is sensitive to kinases: 0.3 pH_i unit acid-shift in pH_i–flux relationship following inhibition of kinases by 20 nM staurosporin (STS) (see [17]).

(NBCe1/SLC4A4) and electroneutral Na^+-dependent Cl^-/HCO_3^- exchange (NDCBE/SLC4A8) [44]. Cl^-/HCO_3^- (or Cl^-/OH^-) exchangers of the SLC4A or SLC26 families are among acid-loading transporter proteins. Many cells use a combination of two or more acid-extruding and acid-loading transporter proteins. It may be speculated that this apparent redundancy is a demonstration of the fundamental importance of pH_i control, particularly in the light of the fact that some nominally pH_i-regulating proteins also service

other regulatory systems (e.g. cell volume [45] or motility [46]), the transport demands of which may—at times—be at odds with pH_i homeostasis. Different types of cancer have been shown to express various combinations of pH_i-regulating transporters [17,47–50] (e.g. figure 4*b*). Despite the heterogeneity of pH_i-regulatory mechanisms, some appear to be constitutive (e.g. HCO_3^--dependent flux, [17,47]), whereas others (e.g. NHE1) emerge as highly dependent on the cell type and conditions.

The energy for actively translocating H^+/H^+-equivalents across the membrane is ultimately financed by ATP consumption. In the case of primary active H^+-ATPase pumps, the transport process is directly coupled to ATP hydrolysis. For the other pH_i-regulating proteins, transport is driven by the energy stored in the electrochemical gradient of coupled ions (i.e. inward $[Na^+]$ or $[Cl^-]$ gradients). The free energy stored in ATP and trans-membrane $[Na^+]$ or $[Cl^-]$ gradients can affect the magnitude of H^+/H^+-equivalent flux produced by pH_i regulators but a more physiologically important modulator of flux is the occupancy of the proteins' pH_i sensor. This can take the form of a binding site involved in the transport-cycle or a dedicated allosteric regulatory site. In the case of Cl^-/HCO_3^- exchanger AE2 (SLC4A2), the allosteric pH_i sensor has been described at the molecular level [51]. By coupling the activity of pH_i-regulating proteins with signalling cascades, cells gain the ability to fine-tune the steady-state pH_i in response to intrinsic (e.g. metabolic status [17,52]) or extrinsic (neural or hormonal [53–55]) influences. NHE1, for example, is sensitive to a wide range of signals [43,54,55] (e.g. figure 4*c*). Factors associated with the tumour milieu, such as extracellular acidity [56], hypoxia [17] and limited HCO_3^- supply [48,56], can also greatly affect H^+/H^+-equivalent flux. These findings highlight the importance of investigating pH_i regulation in the context of the tumour milieu.

In summary, the resting pH_i of a cell can be defined as the steady-state point at which net metabolic acid production is balanced by net membrane H^+/H^+-equivalent transport. These fluxes are likely to show considerable regional variation in solid tumours, resulting in the potential for large pH_i gradients alongside pH_e non-uniformity. This important aspect of tissue pH regulation cannot be investigated by measuring pH in suspensions or two-dimensional monolayers prepared from cultured cells. A more instructive approach to studying pH non-uniformity in tissue is to image cancer-derived multicellular three-dimensional spheroids for pH_i and pH_e (figure 5) [25,36,56].

Active transport of H^+/H^+-equivalents is a means by which cancer cells can maintain an alkaline pH_i, despite the substantial metabolic acid production and low pH_e. However, the capacity of membrane transport to exercise full and autonomous control of pH_i is limited by at least two factors. Firstly, active transport can place a substantial energetic burden on cells. The scale of this can be appreciated from the magnitude of fluxes typically produced by pH_i regulators. To change pH_i, membrane transporters must alter the concentration of free and buffer-bound H^+ ions. In cytoplasm with a typical buffering capacity of 30 mmol. l^{-1} pH^{-1}, for each free H^+ ion there are approximately 10^5 buffer-bound H^+ ions. Consequently, H^+/H^+-equivalent transport must be of the order of several mmol. l^{-1} min^{-1} to change pH_i by a fraction of a unit per minute. Cancer cells are already challenged by a high demand for ATP and restricted respiratory substrate supply, and this may mean that pH_i regulation

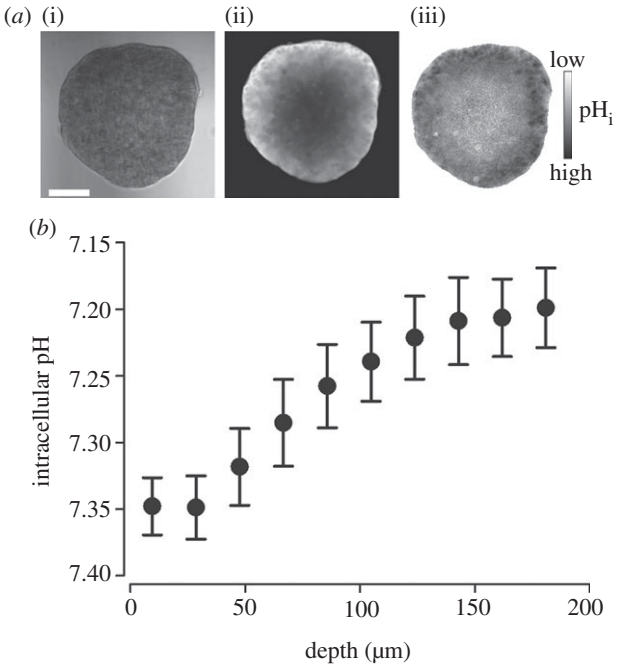

Figure 5. Intracellular pH gradients in multicellular spheroids grown from the ductal breast cancer line T47D. (*a*) (i) Transmission image (scale bar, 100 μm), (ii) fluorescence from intracellular carboxy-SNARF-1 (pH-sensitive reporter-dye), loaded into the intracellular compartment by passive entry of its acetoxymethyl ester; extracellular dye was washed away by perfusion in 5% CO_2/22 mM HCO_3^- buffered normal Tyrode solution. (iii) Ratio of fluorescence at 580 and 640 nm is a measure of intracellular pH. (*b*) Intracellular pH at different depths (average of seven spheroids; mean radius 190 μm). pH-gradient results from restricted diffusion, depth-dependence of metabolic acid loading and H^+/H^+-equivalent membrane transport.

cannot operate at full capacity. Indeed, Na^+/H^+ exchanger activity is reduced at low intracellular [ATP] [52].

A second limiting factor is the effect that pH_i regulators have on extracellular pH. From a tissue point of view, membrane-bound pH_i regulators neither produce nor consume H^+/H^+-equivalents, but change the distribution of H^+ ions between the intracellular and extracellular compartments. During H^+/H^+-equivalent membrane transport, pH_i and pH_e will change in opposite directions. The magnitude of the pH_e change will depend on extracellular buffering and H^+ diffusion. In spheroids, which reproduce many aspects of the tissue microenvironment, the intrinsic extracellular buffering capacity is estimated to be equivalent to 5–10 mmol · (l interstitium)$^{-1}$ · (pH unit)$^{-1}$ [21,56], i.e. lower than in the cytoplasm. As most fixed buffers reside on the surface of membranes, buffering capacity will depend on the degree of cell–cell packing (decreasing in 'looser' regions). Combined with weak diffusional coupling across the tumour interstitium, pH_e changes may be considerable and add to the acidosis imposed by CO_2 and H^+-lactate venting (explaining why glycolysis-deficient tumours still generate low pH_e [57,58]). Displacements of pH_e can slow the transport cycle, either by means of *trans* inhibition or activation of allosteric sites [51,55,59]. For instance, acid extrusion by Na^+/H^+ exchange is inhibited sharply at reduced pH_e [60]. In multi-cellular spheroids, this inhibitory effect can be lessened by increasing extracellular mobile buffering capacity [56]. Since CO_2/HCO_3^- is the principal extracellular mobile buffer, the activity of exofacial CAs can have a substantial impact on the feedback

between pH_e and pH_i-regulating transporters. The sensitivity of at least some pH_i regulators to cellular energetics and to pH_e may protect cancer cells from ATP depletion and excessive extracellular acidification beyond a point that is more damaging than a partially regulated pH_i. In a growing tumour, where diffusion distances and metabolic rate change continuously, these safety checks can be important for the process of somatic evolution which seeks the most viable phenotype.

5. pH sensing, pH-driven selection and clinical perspectives

Cancer cells must be able to detect and respond to micro-environmental factors so that these can then guide somatic evolution. In the case of hypoxia, HIF is a transducer between O_2 tension and cellular effects. The pH sensors involved in cancer disease progression have proved to be more challenging to identify, possibly because of difficulties in distinguishing bona fide pH sensors from the plethora of proteins that bind H^+ ions [2]. Cells sensing an alkaline pH_i have been shown to proliferate [61], enter the cell cycle [62,63], differentiate [64], migrate [65,66], reduce apoptosis [67] and clastogenesis [68], and undergo malignant transformation [69,70]—events that are critical in cancer formation and metastasis. Considering the complexity of these processes, the observed pH_i sensitivity may involve a number of H^+-binding molecular switches. pH sensing is not confined to the cytoplasm: H^+ sensing G-protein-coupled receptors [71], H^+ sensing ion channels (ASICs) [72] and the pH sensitivity of a number of ion channels [73] offer a means by which cells could respond to the pH of the tumour milieu.

Among the titratable sites on proteins, the imidazole group of histidine is an attractive candidate for pH-sensing moieties [74]. Although histidine makes up less than 3% of most proteins, it is commonly found in active or binding sites [75]. The reason for this can be traced to imidazole's pK_a of 6.5, which means that even small changes in pH_i can greatly affect its ionization state and ability to make salt bridges with other amino acids or prosthetic groups. With its unique chemical properties, histidine does not substitute well with any other amino acid [75]. Considering its prominence in active/binding sites, histidine mutations are expected to alter protein function. Among the three most common missense mutations in the tumour suppressor protein p53, two involve substitutions to histidine ($Arg^{175} \rightarrow$ His, $Arg^{275} \rightarrow$ His) [76]. A well-described $Arg^{337} \rightarrow$ His mutation destabilizes p53 tetramerization and hinders its interaction with DNA because a critical salt bridge with Asp^{352} is no longer stable at normal pH_i [77].

Over the long course of cancer disease progression, cells accumulate genetic changes that are retained if selected positively by the micro-environment [78–80]. If extracellular acidity (a complex derivative of diffusion distance, buffering, metabolic rate and membrane transport) were a major selection pressure (as hinted by its prominence as a cancer hallmark) then at least some mutations are likely to relate to genes or gene regulators for H^+/H^+-equivalent transporters, pH sensors or proteins involved in acid-yielding metabolic pathways [79]. Poor prognosis for tumours with low pH_e may indicate that acidity has identified a population of cells with the appropriate pH sensing and regulatory apparatus necessary to thrive and even become resistant to drugs (e.g. weakly basic drugs such as doxorubicin [81,82]). As cancer cells (and possibly stromal cells [83]) have ultimate control over pH_e, the direction and rate of change of this selection pressure can be adapted to optimise disease progression. The higher acid-per-ATP yield of glycolysis, when compared with mitochondrial respiration, may explain the prominence of the Warburg effect in cancer [84]. In summary, the plasticity of pH_i regulation and versatility of protein pH sensitivity offer a mechanism for cancer cells to exploit pH as a selection pressure. By contrast, the genetic stability of normal host cells would hinder their adaptability to the micro-environment.

Growing evidence for the importance of pH in cancer biology has solicited many ambitious ideas for therapy targeting pH-handling proteins with low molecular weight inhibitors or monoclonal antibodies [85–87]. The strategy of blocking acid extrusion from cancer cells and allowing intracellular acid to accumulate to lethal levels may be achieved readily *in vitro*, but efficacy *in vivo* would need to overcome major obstacles. Firstly, acid–base handling proteins are also important for normal cells and inhibition could lead to unacceptable systemic toxicity. It is therefore essential to identify, at the molecular level, acid–base handling mechanisms in cancer that differ substantially from normal cells. For instance, hypoxia-induced MCT4 and CAIX are associated with tumours. Alternatively, drugs could be tailored chemically to become more efficacious in the tumour milieu, e.g. through chemical activation at low pH and/or O_2 tension. A second obstacle is the redundancy in mechanisms for acid extrusion that could compensate for the targeted protein, particularly in cancer cells which have the means to change and adapt dynamically. This raises the question of whether therapy targeted at manipulating pH_i will have the desired clinical efficacy. The hypothesis of acid-driven disease progression has highlighted the importance of *extracellular* pH as a target for therapy. Unlike interventions that alter intracellular pH by targeting one (or more) of many membrane-bound transporter proteins, extracellular pH could be manipulated by altering H^+ diffusivity or buffering capacity. Raising mobile buffering capacity with systemic bicarbonate offers an attractive means of changing the course of acid-driven somatic evolution by ablating the underlying selection pressure [88]. The steady flow of new data in support for the prominent role played by H^+ ions in cancer will keep pH in the spotlight for novel therapeutic approaches for years to come.

6. Concluding remarks

Tissues can regulate and adapt to a particular pH distribution through a two-way interaction between pH and proteins. By continuously evolving superior phenotypes, cancer cells can exploit this interaction to out-compete host cells and metastasize. As the common denominator of a vast array of chemical reactions and transport processes, it is challenging to understand how the concentration of H^+ ions is regulated and sensed. However, the combination of physiological, biochemical, genetic and computational approaches is supplying new ideas on how to exploit the pH/biology interaction in the management of cancer.

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