

## OPINION

## Dysregulated pH: a perfect storm for cancer progression

Bradley A. Webb, Michael Chimenti, Matthew P. Jacobson and Diane L. Barber

**Abstract** | Although cancer is a diverse set of diseases, cancer cells share a number of adaptive hallmarks. Dysregulated pH is emerging as a hallmark of cancer because cancers show a ‘reversed’ pH gradient with a constitutively increased intracellular pH that is higher than the extracellular pH. This gradient enables cancer progression by promoting proliferation, the evasion of apoptosis, metabolic adaptation, migration and invasion. Several new advances, including an increased understanding of pH sensors, have provided insight into the molecular basis for pH-dependent cell behaviours that are relevant to cancer cell biology. We highlight the central role of pH sensors in cancer cell adaptations and suggest how dysregulated pH could be exploited to develop cancer-specific therapeutics.

Invasive tumour cells acquire various adaptive characteristics, including self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, evasion of apoptosis and increased replicative potential<sup>1</sup>. Mutations that underlie these characteristics have been studied extensively. Although less recognized, dysregulated pH is also an adaptive feature of most cancers, regardless of their tissue origin or genetic background. In normal differentiated adult cells, intracellular pH ( $\text{pH}_i$ ) is generally  $\sim 7.2$  and lower than the extracellular pH ( $\text{pH}_e$ ) of  $\sim 7.4$ . However, cancer cells have a higher  $\text{pH}_i$  of  $\geq 7.4$  and a lower  $\text{pH}_e$  of  $\sim 6.7$ – $7.1$  (REFS 2–5). This ‘reversed’ pH gradient creates a perfect storm for metastatic progression (FIG. 1a) and, as suggested by recent evidence, may be permissive for some of the acquired characteristics of cancers. As we describe, an increased  $\text{pH}_i$  is permissive for cell proliferation and the evasion of apoptosis, facilitates metabolic adaptation and is obligatory for efficient directed cell migration. A decreased  $\text{pH}_e$  limits dynamic  $\text{HCO}_3^-$ -dependent buffering, promotes extracellular matrix (ECM) remodelling and stimulates acid-activated proteases to facilitate tumour cell invasion and dissemination.

The increased  $\text{pH}_i$  of cancer cells is paradoxical considering that higher proliferative and glycolytic rates generate metabolic acids. However, changes in the expression and/or activity of plasma membrane ion pumps and transporters that facilitate  $\text{H}^+$  efflux maintain a higher  $\text{pH}_i$  and lower  $\text{pH}_e$  (FIG. 1b). Most notable in cancers is the increased expression and/or activity of:  $\text{H}^+$ -ATPases<sup>6–8</sup>, the  $\text{Na}^+$ - $\text{H}^+$  exchanger NHE1 of the SLC9A family<sup>9–12</sup> and the monocarboxylate- $\text{H}^+$  efflux cotransporters MCT1 and MCT4 of the SLC16A family<sup>13–17</sup>. In normal non-muscle cells, MCTs have a limited role in  $\text{pH}_i$  regulation, but in cancer cells, expression of MCTs is increased. This increased expression confers a selective advantage to cancer cells owing to the high affinity of these transporters for lactate<sup>18</sup> and the ability of cancer cells to promote the conversion of pyruvate to lactate<sup>19</sup>. Additionally, a ‘leading edge’ localization of NHE1 and MCT1 in migrating and cancer cells<sup>20–23</sup> suggests that  $\text{H}^+$  efflux may be spatially organized. The decreased  $\text{pH}_e$  of tumour cells is maintained by a combination of oxygen depletion from limited perfusion, increased intracellular  $\text{H}^+$  efflux (as described above) and high activity of the carbonic anhydrases CAIX and CAXII<sup>24–29</sup>,

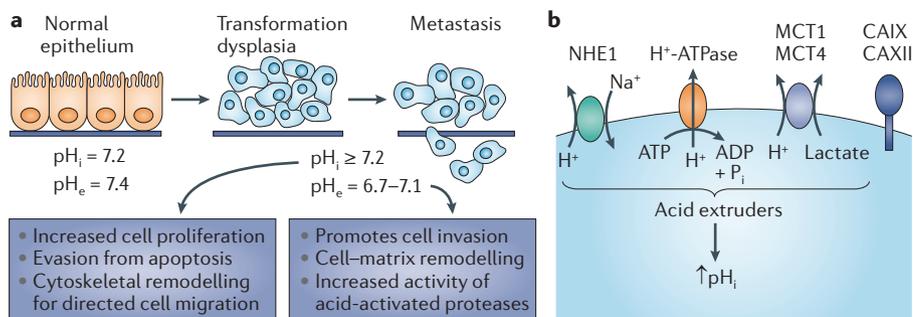
which have extracellular catalytic domains that accelerate the hydration of extracellular  $\text{CO}_2$  to  $\text{HCO}_3^-$  and  $\text{H}^+$ .

Several excellent reviews<sup>30–33</sup> describe cell behaviours that are regulated by pH dynamics in tumours and their environment. In this Opinion article we discuss recent findings on the molecular basis for pH-dependent behaviours relevant to the biology of cancer cells and how dysregulated pH confers adaptive advantages for metastatic progression. We describe how a combination of cellular, molecular and computational approaches can identify and reveal the structural principles of pH sensors, which we previously defined as proteins that have activities or ligand-binding affinities that are regulated by small, physiological changes in  $\text{pH}^{\text{a}}$ . The examples that we present highlight three key aspects of pH as a regulatory signal. First, dynamic changes in  $\text{pH}_i$  can modulate multiple proteins in unison to control complex cell behaviours. Second, although all proteins contain titratable groups with protonation states that can vary with changing pH, only a limited number of proteins are bona fide pH sensors. Thus, physiological changes in pH can specifically regulate selected intracellular and extracellular proteins<sup>34</sup>. Third, changes in pH probably do not act as a binary switch but rather act cooperatively as a modulator or a coincidence regulator with other post-translational protein modifications. Because dysregulated pH is a common feature of cancers, physiological pH sensors hold promise as therapeutic targets to achieve high specificity and broad efficacy.

### pH-dependent cancer cell behaviours

**Proliferation and survival.** Increased  $\text{pH}_i$  is an established permissive signal for cell proliferation<sup>35–38</sup>. Increased  $\text{pH}_i$  also promotes cell survival by limiting apoptosis, which is associated with intracellular acidification<sup>39,40</sup>. Hence, the constitutively high  $\text{pH}_i$  of cancer cells confers the selective advantages of growth-factor independent proliferation and the evasion of apoptosis, two hallmarks of most, if not all, cancers<sup>1</sup>.

Although  $\text{pH}_i$ -dependent cell proliferation is probably mediated by multiple mechanisms, a  $\text{pH}_i$  of  $>7.2$  increases the



**Figure 1 | Dysregulated pH creates a perfect storm for cancer progression.** **a** | Cancer cells have a reversed pH gradient compared with normal differentiated adult cells, including a constitutively higher intracellular pH ( $pH_i$ ) and a lower extracellular pH ( $pH_e$ ), which facilitates the indicated adaptive behaviours. **b** | The increased expression and activity of plasma membrane transporters, particularly acid extruders, and carbonic anhydrases (CAs) maintain the higher  $pH_i$  and lower  $pH_e$  of tumour cells. MCT, monocarboxylate transporter; NHE1, Na<sup>+</sup>-H<sup>+</sup> exchanger 1.

rate at which growth factor-stimulated cells enter S phase<sup>35</sup> and enter and progress through the G2/M phases<sup>41</sup>. A  $pH_i$  of  $\leq 7.2$  delays G2/M entry by limiting the activity of the key mitotic regulatory complex, cyclin-dependent kinase 1 (CDK1)-cyclin B1, in part through the sustained inhibitory phosphorylation of CDK1 on Tyr15 and the decreased expression of cyclin B1 (REF. 41). The regulation of CDK1-cyclin B1 activity by  $pH_i$  seems to be a conserved mechanism for mitotic and meiotic entry. In invertebrate oocytes, a transient increase in  $pH_i$  increases CDK1 activity and is necessary for meiotic re-initiation and maturation, overriding even the repressive action of MAPKs in these cells<sup>42,43</sup>. A higher  $pH_i$  also suppresses the mitotic arrest that is triggered by an activated DNA damage checkpoint<sup>44-46</sup>, and this constitutes a specialized form of mitotic entry. Hence, by conferring the adaptive advantage of bypassing cell cycle checkpoints, the aberrantly increased  $pH_i$  of cancer cells promotes not only unrestricted proliferation<sup>31</sup> but possibly also genetic instability.

The sustained higher  $pH_i$  in cancer cells confers a substantial advantage of resistance to apoptosis. A  $pH_i$  of  $< 7.2$  is a conserved feature of cells undergoing apoptosis, and may be an early and enabling factor<sup>39,40</sup>. Although our understanding of the pH sensors that regulate apoptosis remains rudimentary, pH dynamics probably modulate multiple proteins in unison to control this process. For example, an acidic pH induces conformational changes in the pro-apoptotic protein BAX to facilitate its insertion into the outer mitochondrial membrane to form pores<sup>47</sup>. These pores increase the membrane permeability and the release of cytochrome *c* and other pro-apoptotic proteins into the cytosol. Additionally, the cytochrome *c*-mediated activation of caspases in the cytosol is most

efficient at a  $pH_i$  of  $\sim 6.8$  (REF. 40). There is currently a great interest in enhancing mitochondrial-dependent apoptosis as a therapeutic strategy to limit cancer progression, and identifying the principles of pH sensing by key apoptotic regulators could facilitate the rational design of drugs that target these pH-driven changes.

**Metabolic adaptation.** To maintain their rapid growth and proliferation, cancer cells have a higher need for energy and for the biosynthesis of nucleotides than normal differentiated adult cells. This increased demand is, in part, met by an altered metabolic programme known as the Warburg effect or ‘aerobic’ glycolysis<sup>48-51</sup>. It is a common adaptive feature of cancer cells and other rapidly proliferating cells and is currently receiving a resurgence of interest. The Warburg effect results in ATP being generated by the non-oxidative breakdown of glucose to lactic acid, enables the conversion of metabolites to biosynthetic precursors and occurs even in the presence of oxygen.

An alkaline  $pH_i$  promotes glycolysis and inhibits gluconeogenesis<sup>52-54</sup>, which may depend on the pH-sensitive activity of several glycolytic enzymes. The activity of lactate dehydrogenase (LDH), which converts pyruvate to lactate and regenerates NAD<sup>+</sup> for glycolysis, is maximal at pH 7.5 (REF. 55). In addition, phosphofructokinase 1 (PFK1), the first rate-limiting enzyme in glycolysis, is a recognized pH sensor with a  $> 100$ -fold increase in activity and release from allosteric regulation between pH 7.0 and 7.5 (REFS 56-58). However, the molecular mechanism of pH-dependent PFK1 activity is currently unknown, and our understanding of it is hampered by the lack of a crystal structure of the mammalian tetrameric complex, although the structure of monomeric

rabbit muscle PFK1 was recently reported<sup>59</sup>. Increased  $pH_i$  induces a striking, nonlinear release of PFK1 inhibition by ATP, which suggests that an allosteric mechanism is responsible, analogous to that of haemoglobin, a known pH sensor. We propose that pH probably regulates the activity of mammalian PFK1 by altering the equilibrium between a less active, tense (T) state and a more active, relaxed (R) state, similarly to other metabolites that bind at allosteric sites. A homology model of mouse PFK1 in the T and R states, based on the prokaryotic crystal structure and computationally derived  $pK_a$  values, suggests that a handful of residues might mediate the response to pH changes, including His52 in human muscle PFK1, which is located in the allosteric ATP-binding site (FIG. 2a).

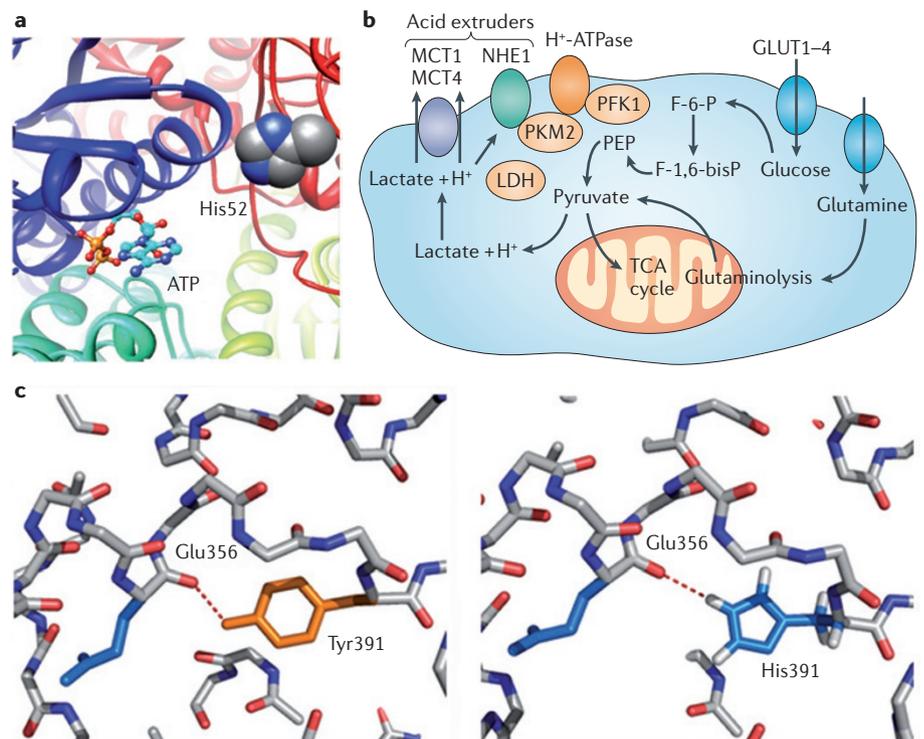
Older studies on pyruvate kinase that was purified from adult, normal tissues suggest that enzyme activity increases with an alkaline shift<sup>60,61</sup>. However, these findings might not be accurate for PKM2, the pyruvate kinase isozyme in cancer cells. Recent findings suggest that the inhibition of PKM2 activity promotes metabolic adaptation, with oncogene- and growth factor signalling-mediated phosphorylation of Tyr105 of PKM2 suppressing its activity<sup>62,63</sup>. Although it is not intuitive that inhibited PKM2 would be permissive for generating pyruvate for lactic acid production, an alternative source of pyruvate in cancer cells is through glutaminolysis (FIG. 2b), which also produces NADPH for biosynthetic pathways<sup>64</sup>. Additionally, recent findings suggest a glycolytic flux mechanism whereby PKM2 activity is inhibited and pyruvate generation is uncoupled from ATP production and relies on a phosphoryl transfer from phosphoenolpyruvate (PEP) to phosphoglycerate mutase<sup>51</sup>, rather than to ADP. If suppressing PKM2 activity facilitates metabolic adaptation, might the higher  $pH_i$  of cancer cells inhibit PKM2? This is suggested by a naturally occurring PKM2-H391Y mutation that abolishes allosteric regulation and is found in patients with Bloom’s syndrome, who are prone to developing cancer. Tyr391 forms a hydrogen bond with the backbone of Glu356 that may restrict the dynamic movement to the R conformation, thus reducing enzyme activity<sup>61</sup>. In the wild-type enzyme, neutral, but not charged, His391 would probably mimic the interactions of Tyr391 (FIG. 2c). If the  $pK_a$  of His391 is upshifted in the physiological range, PKM2 activity could be attenuated at higher  $pH_i$ .

An increased  $pH_i$  can also induce adaptive changes in the expression or localization of glycolytic enzymes. Fibroblasts expressing a mutant, inactive NHE1 have a lower

$\text{pH}_i$  than cells expressing wild-type NHE1 and have a threefold to fourfold decrease in the expression of gluconeogenic enzymes, including phosphorylase kinase, galactokinase and fructose-1,6-bisphosphatase, and a fourfold decreased abundance of LDH<sup>65</sup>, a key glycolytic enzyme for the Warburg effect. Additionally, the lactic acidosis surrounding solid tumours is suggested to enhance metabolic reprogramming through changes in the expression profile of glycolytic enzymes<sup>66</sup>. An important and unresolved question is whether glycolysis is spatially regulated. Recent evidence suggests the intriguing new idea that glycolytic enzymes localize at the plasma membrane — they are enriched in invadopodia<sup>67</sup> (invasive plasma membrane protrusions of cancer cells) and directly bind to several plasma membrane ion transport proteins that control  $\text{pH}_i$ , including the erythrocyte anion exchanger AE1 (REFS 68,69), an epithelial  $\text{H}^+$ -ATPase<sup>12</sup> and NHE1 in fibroblasts (D.L.B., unpublished data). By functioning as a scaffold for a glycolytic metabolon, these ion transport proteins could facilitate glycolytic flux by forcing the proximity of glycolytic enzymes and by generating localized changes in  $\text{pH}_i$ , which would regulate the pH-sensitive activity of glycolytic enzymes, such as PFK1 (FIG. 2b). In metastatic cells, the localized production of ATP by glycolytic flux at the leading edge plasma membrane has been reported<sup>70</sup> and could fuel the cytoskeletal dynamics and enzymatic reactions that are required for membrane protrusion.

**Metastasis and invasion.** The metastatic dissemination of tumour cells, which is the predominant cause of death from cancer, is facilitated by dysregulated pH. An increased  $\text{pH}_i$  is necessary for the directed migration of multiple mammalian cell types, and a decreased  $\text{pH}_e$  promotes degradation of the ECM for cell invasion (reviewed in REF. 33). Recent evidence reveals a molecular basis for  $\text{pH}_i$ -dependent metastasis by showing that pH sensors control distinct stages of cell migration. These findings highlight specificity in pH signalling and the ability of pH to simultaneously coordinate multiple processes that are involved in complex cell behaviours.

The polarity of migrating cells depends on the activity of the low-molecular-weight GTPase CDC42, which requires an increased  $\text{pH}_i$ <sup>71,72</sup>. Although CDC42 is not directly regulated by physiological changes in pH, several guanine nucleotide exchange factors that stimulate CDC42, including DBS, are activated by their release from membrane phospholipids at a  $\text{pH}_i$  of  $>7.2$  (REF. 71).



**Figure 2 | Mechanisms for pH sensing by glycolytic enzymes.** **a** | Molecular modelling can be used to generate hypotheses about the mechanisms of pH sensing, including pH sensing by His52 of phosphofructokinase 1 (PFK1). **b** | Metabolic adaptation includes the increased uptake of extracellular glucose by glucose transporters (GLUT1–4), the increased generation of pyruvate from glycolysis and from metabolism of glutamine (glutaminolysis) and the conversion of pyruvate to lactate and  $\text{H}^+$ . Despite the common view of glycolysis occurring in an ill-defined cytosolic compartment, glycolytic enzymes with predicted pH-dependent activity bind to plasma membrane ion transport proteins that regulate the intracellular pH. **c** | In the pyruvate kinase isozyme M2 (PKM2) H391Y mutant, Tyr391 is predicted to form a hydrogen bond with the backbone of Glu356 (left side of the figure). Similar bonding is predicted in wild-type PKM2 with neutral but not charged His391 (right side of the figure). F-1,6-bisP, fructose-1,6-bisphosphate; F-6-P, fructose-6-phosphate; LDH, lactate dehydrogenase; MCT, monocarboxylate transporter; NHE1,  $\text{Na}^+$ - $\text{H}^+$  exchanger 1; PEP, phosphoenolpyruvate; TCA, tricarboxylic acid.

The *de novo* assembly of actin filaments that drives membrane protrusion in migrating cells also increases with a  $\text{pH}_i$  of  $>7.2$  owing to the reported pH-dependent activities of actin-binding proteins such as cofilin<sup>73,74</sup>, profilin<sup>75</sup>, twinfilin<sup>76</sup>, villin<sup>77</sup> and talin<sup>78–81</sup> (FIG. 3a). For example, newly polymerized actin filaments in metastatic cells require the activity of the actin-severing protein cofilin<sup>82,83</sup>. As is the case for DBS, cofilin activity increases with dissociation from membrane phospholipids at higher  $\text{pH}_i$ <sup>73</sup>. Most proteins bind to phosphoinositides through clusters of lysines and arginines that are generally insensitive to physiological changes in  $\text{pH}_i$ . However, cofilin, the pleckstrin homology domain in DBS<sup>71</sup> and FYVE domains in some proteins<sup>84</sup> contain a histidine at the phosphoinositide-binding pocket that imparts pH-dependent binding affinities. The functional significance of pH-dependent histidine switches for

phosphoinositide binding remains relatively unexplored but could have an important role in pH-regulated metastasis, in which oncogenes drive the synthesis of particular phosphoinositides and the activation of phosphoinositide-binding proteins<sup>85</sup>. As a pH sensor, cofilin also highlights the role of pH dynamics in coincidence regulation, which requires two or more simultaneous inputs or modifications; increased cofilin activity requires both the dephosphorylation of an amino-terminal serine and the deprotonation of a distant histidine (FIG. 3a).

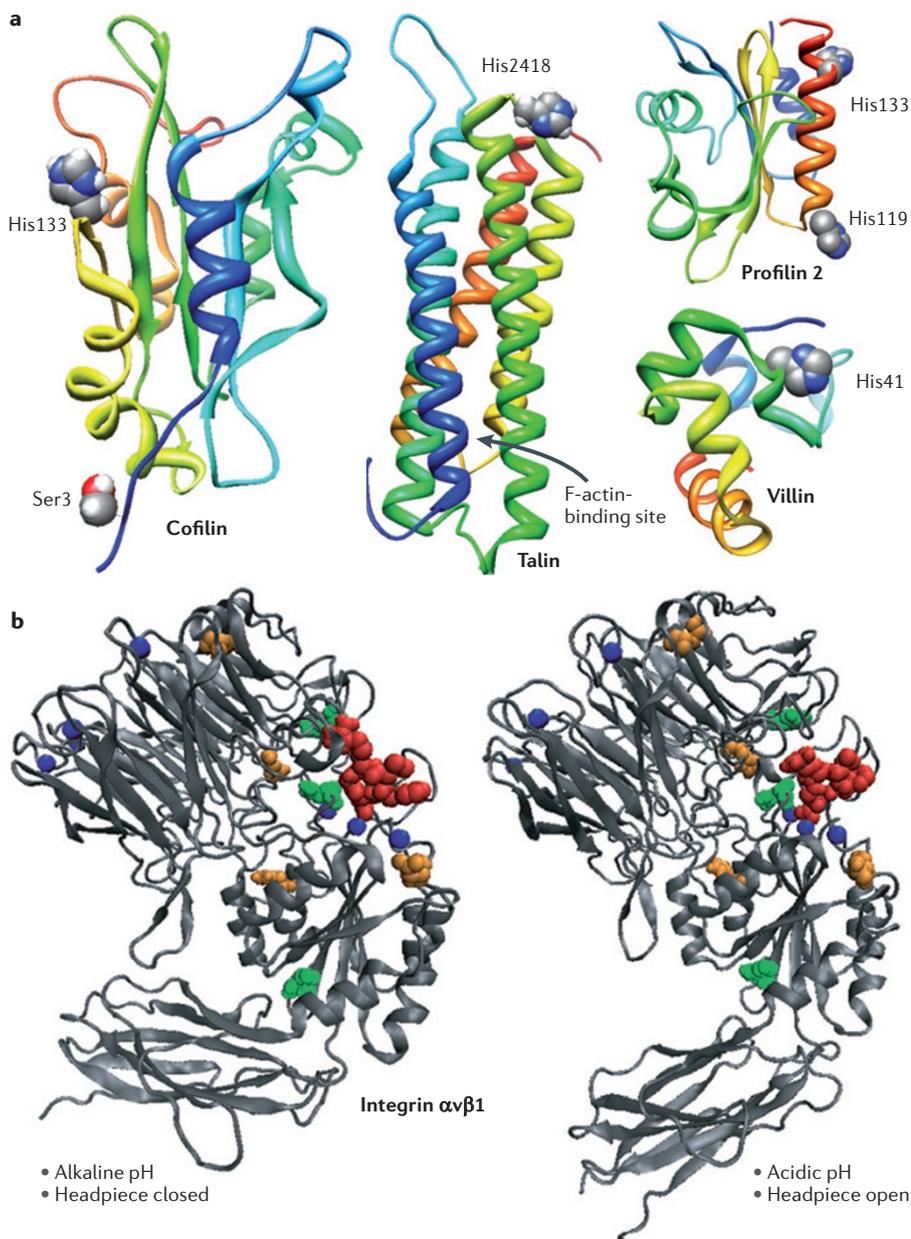
In addition to the *de novo* assembly of actin filaments, metastasis requires the remodelling of cell–substrate adhesions, a process that requires an increased  $\text{pH}_i$ <sup>20,78</sup> and a decreased  $\text{pH}_e$ <sup>86</sup>. The focal adhesion protein talin is a pH sensor that has decreased binding to actin filaments at a pH of  $>7.2$ , and this property permits faster focal adhesion turnover and increased migration<sup>78</sup>. Nuclear

magnetic resonance (NMR) spectroscopy studies of the I/LWEQ actin-binding domain of talin suggest an allosteric mode of regulation, whereby titration of the protonation status of amino acids in a pH-sensor region causes conformational changes at a distant actin-binding site<sup>78</sup> (FIG. 3a). Human

melanoma cells migrate faster at a  $pH_e$  of 7.0 compared with a  $pH_e$  of 7.4, which has been attributed to a lower  $pH_e$  regulating the dynamics of integrin–ECM attachments<sup>87</sup>. Recent findings suggest that pH does not affect the affinity of integrin binding to ECM proteins but rather integrin avidity<sup>88,89</sup>.

Molecular dynamics simulations predict that an acidic  $pH_e$  causes the headpiece of the  $\alpha v \beta 3$  integrin to ‘open’ and to adopt an orientation more amenable to interaction with the ECM, an effect determined by changes in the electrostatic interactions upon protonation of Asp127 of the  $\beta$ -subunit<sup>89</sup> (FIG. 3b). However, Martin and colleagues<sup>90</sup> recently presented a mathematical model which predicts that the speed of tumour invasion does not increase monotonically with decreased  $pH_e$ . By using modelling parameters that include the competitive and cooperative interactions between tumour cells and stromal cells and the processes of cell death and ECM degradation, their data suggest that, although an acidic extracellular environment initially increases invasion, a sustained low  $pH_e$  might kill stromal cells that contribute to continued invasion.

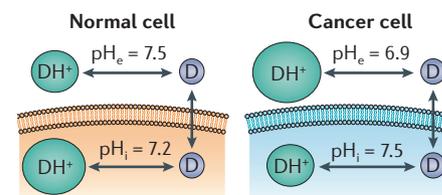
An increased  $pH_i$  and a decreased  $pH_e$  coordinately enhance invasion and metastasis through additional mechanisms. For example, ion fluxes with  $H^+$  extrusion lead to an obligatory influx of water, resulting in osmotic swelling and an accompanying opening of aquaporin channels. These effects increase cell migration<sup>91</sup> and membrane blebbing<sup>92</sup>, an important process in the amoeboid movement of highly metastatic cells. An acidic  $pH_e$  is necessary for the formation and maturation of invadopodia<sup>4</sup> and activates proteases to focally degrade the ECM<sup>4,93</sup>. The activity of matrix metalloproteinase 3 (MMP3; also known as stromelysin 1) is higher at acidic  $pH$ <sup>94,95</sup>, and the expression and secretion of MMP9 increases at lower  $pH_e$  and higher  $pH_i$ <sup>65,96</sup>. Additionally, acid-activated proteases, such as cathepsin B<sup>93,97</sup>, cleave secreted, latent MMPs into active enzymes. These examples of pH-dependent proteins and cellular behaviours reveal potential therapeutic targets and strategies based on using changes in pH to control tumour cell metastasis. The potential of modulating  $pH_e$  to reduce tumour cell metastasis was recently shown by Robey *et al.*<sup>98</sup> by orally administering bicarbonate to mice, which increased both the systemic buffering capacity and the tumour  $pH_e$  and inhibited experimental and spontaneous metastases.



**Figure 3 | Molecular mechanisms of pH sensors in cell migration and invasion.** **a** | Several actin-regulatory proteins have been shown to have pH-dependent activities or ligand-binding affinities. Maximal actin severing by cofilin requires both the dephosphorylation of Ser3 and the deprotonation of His133 (REFS 73,74) (left side of the figure), a coincidence-detection mechanism of regulation. Protonation of His2418 of talin causes conformational changes at a distant site that reduces its binding affinity for actin<sup>77</sup> (middle of the figure). A pH-dependent protein folding has been studied in profilin<sup>76</sup> and the villin headpiece<sup>77</sup> (right side of the figure). **b** | Snapshots of the  $\alpha v \beta 3$  headpiece in its closed (left) and partially open (right) conformations. In molecular dynamics simulations, the partially open  $\alpha v \beta 3$  headpiece is observed more often in acidic extracellular pH conditions than in normal physiological pH conditions<sup>89</sup>. Spheres depict  $Mg^{2+}$  ions (blue), an RGD ligand (red), residues with increased  $pK_a$  that were protonated at both normal and acidic pH (green) or protonated at acidic pH only (orange).

**Implications for therapeutics**

The inverted pH gradient between the inside and the outside of cells that is observed in tumours presents both challenges and opportunities for drug discovery. Dysregulated  $pH_i/pH_e$  has an effect on drug action because most drugs have at least one pH-titratable group. Many commonly used cancer drugs have intracellular targets and are weak bases,



**Figure 4 | Protonation of weakly basic pharmaceuticals reduces cellular uptake.** With a normal pH gradient in normal cells (left side of the figure), weakly basic drugs, exemplified here by doxorubicin with  $pK_a = 8.2$ , accumulate at higher concentrations inside cells than in the extracellular space. When the pH gradient is inverted, such as in cancer cells (right side of the figure), the concentration of the weakly basic drugs can be much higher in the extracellular space than inside cells. The calculations assume that only the neutral form of the drug crosses the membrane. The areas of the circles are proportional to the relative concentrations. D represents the neutral form of the drug, and  $DH^+$  is the protonated (charged) form.  $pH_e$ , extracellular pH;  $pH_i$ , intracellular pH.

possible gain of pH sensitivity that could have adaptive functional significance in the context of the higher  $pH_i$  of cancer cells. Moreover, an *Ingenuity* pathway analysis indicated that nearly 80% of proteins with arginine to histidine substitutions are in signalling modules that regulate cell growth, proliferation or death. An example of a gain in pH sensing is a naturally occurring R337H substitution in the tetramerization domain of the tumour suppressor protein p53 that is associated with an adrenal cortical carcinoma syndrome<sup>111</sup>. This histidine substitution prevents tetramer formation in the presence of an increased  $pH_i$  and hence inhibits DNA binding and p53 function. Although not reported, pH sensitivity might also be acquired with the frequently occurring p53 mutations R175H and R270H in the central DNA-binding domain that are associated with Li–Fraumeni syndrome and multiple types of cancer<sup>112,113</sup>. Despite recent progress on pH-dependent protein–protein and protein–phospholipid interactions, we have limited insight into pH-sensitive protein–DNA binding affinities. Hence, determining whether pH sensing plays an important part in the transcriptional programme conferring tumour formation and survival is an important future direction. Most interactions with the phosphate backbone of DNA are mediated by arginine and lysine residues but, analogous to the phospholipid-binding proteins discussed above, a small number of protein interactions with DNA are mediated by histidines, and these would be candidate pH sensors.

which are protonated (positively charged) at lower pH and neutral at higher pH<sup>99</sup>. The varying ratios of neutral/charged species of a drug can affect its distribution between the extracellular and intracellular spaces, as well as its distribution among subcellular compartments with differing pH<sup>100,101</sup>. This effect occurs because charged species have very low rates of passive permeation through cellular membranes (the role of transporters will be considered below). Weak bases in the acidified extracellular space of a tumour will be preferentially protonated, and less of the drug will be in the neutral form, meaning that less can permeate across the membrane to accumulate in the cell. Conversely, in the more basic cytoplasmic space, the drug will have a higher concentration in the neutral form, which can permeate across the plasma membrane out of the cell, leaving a reduced drug concentration inside the cell. In non-tumour cells, the situation is reversed, and more of the drug accumulates inside the cells, where it can cause toxic effects in healthy tissue.

Using this simple model, the relative concentrations of a weakly basic drug inside ( $C_i$ ) and outside ( $C_e$ ) a cell can be estimated as

$$\frac{C_i}{C_e} = \frac{1 + 10^{(pK_a - pH_i)}}{1 + 10^{(pK_a - pH_e)}}$$

For example, *doxorubicin*, a chemotherapeutic used to treat various cancers, has a  $pK_a$  of 8.2. The above formula predicts that there would be a nearly threefold higher concentration of the drug in the extracellular space than in the cytoplasm, when using  $pH_i = 7.4$  and  $pH_e = 6.9$  (FIG. 4). With a ‘normal’ pH gradient (for example,  $pH_e = 7.4$  and  $pH_i = 7.2$ ), the concentration gradient is inverted, with ~50% higher drug concentration inside the cell. Conversely, the aberrant pH gradient in tumours is a benefit for the cellular accumulation of weak acids. The arguments are simply the inverse of those above, because the weak acid will be preferentially neutral at lower pH. Several studies provide empirical evidence that these pH effects do in fact occur *in vivo* and can affect drug action<sup>99–101</sup>.

Increased  $pH_i$  has also long been recognized as a hallmark of multi-drug resistance (MDR) in tumours, and there has been much discussion, and some confusion, about the possible relationship between  $pH_i$  and MDR, as well as possible effects of  $pH_e$  on MDR, much of which has focused on the role of the ATP-binding cassette transporter ABCB1 (also known as P-glycoprotein or MDR1) in drug efflux<sup>102</sup>. Although it is difficult to make definitive conclusions based on the data presented in the literature, our

view is that the functional connections between  $pH_i$  and ABCB1 activity are likely to be closely related to the discussion of pH-dependent drug distribution above. That is, the rate at which ABCB1 exports a drug probably depends on the protonation state of the drug. It has long been clear that the efflux of negatively charged drugs by ABCB1 is inefficient but many substrates of ABCB1 are weak bases and it is less clear whether ABCB1 preferentially transports positively charged or neutral forms of these compounds. However, there is some direct evidence that ABCB1 preferentially transports the neutral forms of drugs that are weak bases<sup>103</sup>. This is consistent with the widely held view that ABCB1 substrates may enter the transporter binding site through the membrane, which would only be a feasible route for a neutral species. It is also consistent with the highly hydrophobic nature of the binding site revealed in the recent X-ray crystallographic study of mouse ABCB1 (REF. 104). Thus, an altered  $pH_i$  may affect drug distribution not only through modulating passive diffusion but also through modulating active efflux, with the efflux of weak bases being more favourable at higher  $pH_i$ . Finally, we note that pH may also affect other transporters, such as ABCG2 (also known as breast cancer resistance protein), which has been reported to function optimally at an acidic  $pH_e$ <sup>105</sup>.

#### Future directions

Looking forwards, a number of innovative drug delivery strategies have been developed to exploit the low  $pH_e$  in tumours to permit site-specific drug delivery, including nanogels<sup>106</sup>, polymersomes<sup>107</sup>, designed peptides<sup>108</sup> and micelles<sup>109</sup>. Such strategies can help to improve the efficacy of existing drugs. However, a rapidly developing understanding of how an increased  $pH_i$  and a decreased  $pH_e$  facilitate proliferation, survival and invasion may lead to new targets for therapy that might be effective across several classes of cancers. Of course, compounds that selectively decrease  $pH_i$  would also be of great interest, although it may be difficult to achieve this effect only in cancer cells.

Also important is an increased understanding of how dysregulated pH facilitates gain- or loss-of-function mutations in cancer cells. A recent study that identified ~2,000 somatic mutations in cancers revealed that 15% involve histidines<sup>110</sup>, despite the low frequency of histidine residues (~2%) in proteins. Of the mutations involving histidine, 20% are arginine to histidine substitutions, suggesting a

The fundamental question that needs further investigation is whether dysregulated pH is necessary to establish and maintain a malignant phenotype. A xenograft model in immunocompromised mice was used to show an 80% decrease in tumour incidence with oncogene-transformed fibroblasts lacking NHE1 (REF. 114). Tumour incidence is not altered by mutations that limit lactic acid production alone, but is completely abolished by the loss of NHE1 and by mutations that increase lactic acid production. A similar xenograft model of RAS-transformed fibroblasts recently showed that silencing of NHE1 or MCT4 expression reduced the tumour-associated reversal of the pH gradient and limited tumour growth<sup>17</sup>. In other animal models, a number of NHE1 inhibitors reduced tumour size or incidence<sup>115</sup> (reviewed in REF. 116). A notable future need is for alternative syngeneic mouse models and for studies that can assess the effect of pH on the outgrowth of metastatic lesions. These animal studies, combined with an increased understanding of the molecular basis for pH-dependent cell behaviour, should help us to understand how pH dysregulation confers adaptive advantages in cancers and how it might be exploited to limit disease progression.

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#### Competing interests statement

M.P.J. declares competing financial interests; see Web version for details.

#### DATABASES

National Cancer Institute Drug Dictionary: <http://www.cancer.gov/drugdictionary>  
doxorubicin

#### FURTHER INFORMATION

Matthew P. Jacobson's homepage: <http://www.jacobsonlab.org>  
Diane L. Barber's homepage: <http://dbarberlab.ucsf.edu>  
Ingenuity pathway analysis: <http://www.ingenuity.com>

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