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Dysregulated proton and sodium gradients highlight cancer invasion and proliferation

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ABSTRACT

Acidity and salinity of the extracellular fluid, reflecting degrees of acid and sodium contents respectively, regulate essential cellular functions in health and disease, especially cancer. Tumor invasiveness is enhanced by the acidic extracellular milieu as a consequence of upregulated aerobic glycolysis. But recent discoveries also suggest that enhanced proliferative mitosis, which also hallmarks cancer, is impacted by interstitial salinity. Abnormal transmembrane proton/sodium gradients lead to pathophysiological alterations at the cellular level. These novel perspectives mandate pioneering imaging of extracellular acidity and salinity, preferably monitored simultaneously. By dissecting the interplay between dysregulated pH and electrolyte imbalance within the tumor habitat, these biomarkers hold promise for early cancer diagnosis and tracking therapies, from chemotherapy to immunotherapy.

Sodium and pH are vital for numerous processes, from maintaining blood pressure to neuronal firing [1–5]. Various mechanisms located at the cell membrane help regulate sodium and pH to avoid physiological mishaps (Fig. 1). Homeostatic maintenance of extracellular sodium (~150 mM) and intracellular sodium (~15 mM) lends to a strong transmembrane sodium ion gradient, whereas pH values of extracellular (~7.4) and intracellular (~7.2) compartments maintain the transmembrane hydrogen ion (or proton) gradient. Since sodium and pH in blood normally mimic the extracellular space, the weak transendothelial proton/sodium gradients help maintain a healthy blood-brain barrier.

Large transmembrane sodium (and potassium) gradients manifest the cell's hyperpolarized resting membrane potential [1]. During neuronal firing, membrane potential rapidly depolarizes, where the sodium/potassium gradients are transiently reversed but are subsequently reestablished by the sodium-potassium ATP pump (Na^+/K^+-ATPase) (Fig. 1a). Upon neuronal firing, the Na^+/K^+-ATPase consumes one ATP to export three sodium ions from the cell, while at the same time importing two potassium ions into the cell, both against their respective electrochemical gradients. In addition to Na^+/K^+-ATPase, other channels maintain electrolyte levels: voltage-gated sodium channel (VGSC), sodium-calcium exchanger (NCX), sodium-hydrogen exchanger (NHE), sodium-potassium-chloride channel (NKCC), acid sensing ion channel (ASIC), epithelial sodium channel (ENaC), sodium-dependent chloride bicarbonate exchanger (NDCBE), and sodium-bicarbonate channel (NBC) [1–5] **(Fig. 1a)**.

Under normal conditions, salinity of extracellular and intracellular fluids are orders of magnitude different (i.e., ~0.9% vs. ~0.09% respectively). But what happens to compartmental salinity in cancer? Inability to track compartmental sodium in vivo has made it difficult to answer this question. Insights into salinity of each compartment may be crucial for early diagnosis of cancer and tracking new cancer treatments, because salinity impacts mitosis [6] and immune cells sense sodium in the extracellular milieu [7].

Tumors are highly glycolytic even when well-oxygenated [2–5]. This aerobic glycolysis phenotype enables cancer cells to actively metabolize different nutrients while generating acidic by-products that are then extruded by diverse systems **(Fig. 1b)**. While sodium-independent chloride bicarbonate exchanger (NICBE), NBC, ASIC, and ENaC disturb the intracellular pH, the extracellular pH is lowered by action of upregulated proton transporters (e.g., NHE and vacuolar H^+-ATPase) to pump out protons and proton-linked monocarboxylate transporters (MCTs) **(Fig. 1b)**, where lactate/protons are imported by MCT1 and exported by MCT4 [8]. Even carbon dioxide and water from oxidative metabolism contribute to extracellular acidification using an anionic

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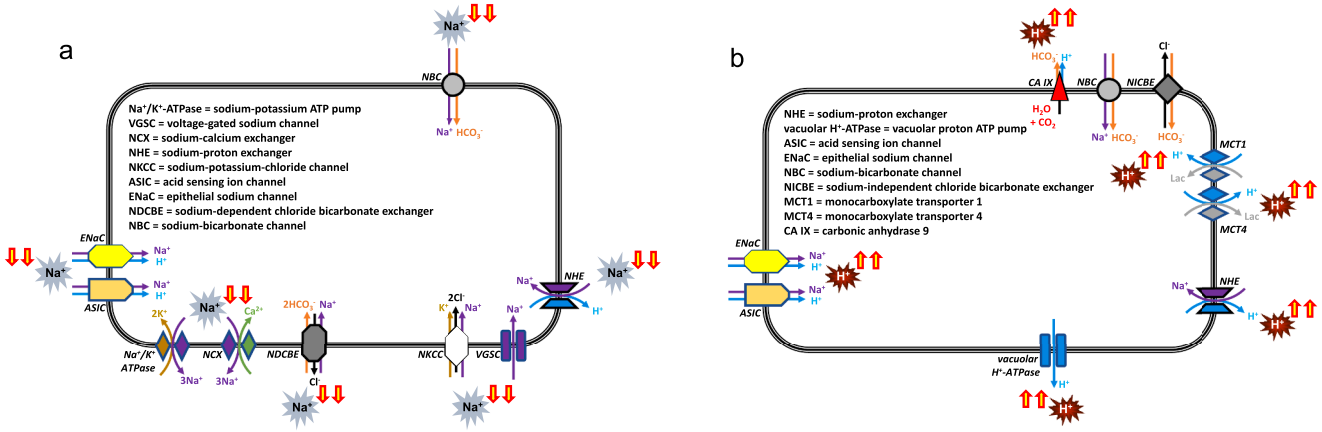

Fig. 1. Mechanisms regulating transmembrane proton/sodium gradients. **(a)** Extracellular sodium is maintained by actions of the sodium-potassium ATP pump (Na^+/K^+-ATPase), voltage-gated sodium channel (VGSC), sodium-calcium exchanger (NCX), sodium-hydrogen exchanger (NHE), sodium-potassium-chloride channel (NKCC), acid sensing ion channel (ASIC), epithelial sodium channel (ENaC), sodium-dependent chloride bicarbonate exchanger (NDCBE), and sodium-bicarbonate channel (NBC). While Na^+/K^+-ATPase is downregulated in cancer cells, upregulation of NHE, VSGC, NKCC as well as an NCX mutant (which moves sodium and calcium in opposite directions) reduce extracellular sodium [1,2]. **(b)** While NHE and vacuolar H^+-ATPase are mainstays in acid extrusion, NBC, ASIC, ENaC and the sodium-independent chloride bicarbonate exchanger (NICBE) also disturb the intracellular pH. But proton-linked monocarboxylate transporters (MCTs), where MCT4 is essential, also contribute to proton (H^+) and lactate extrusion. In fact, anionic exchangers (e.g., carbonic anhydrase 9 or CA IX) can convert carbon dioxide (CO_2) and water (H_2O) generated by oxidative processes to bicarbonate (HCO_3^-) and proton (H^+). The acid-extruding mechanisms are enhanced in cancer cells [3,11].

exchanger (e.g., carbonic anhydrase 9 or CA IX) **(Fig. 1b)**. Tumor acidity also suppresses anticancer immune response [9].

Na^+/K^+-ATPase activity is downregulated in tumors due to the glycolytic shift [2–5], thus impacting compartmental sodium directly **(Fig. 2)**. However, upregulation of ion channels (e.g., NHE, VSGC, and even NCX mutants) and acid transporters (e.g., NHE, vacuolar H^+-ATPase, MCT4, CA IX) together contribute directly to proton/sodium imbalances in the tumor microenvironment [1–5] **(Fig. 2)**. Extracellular acidification within the tumor habitat is vital for invasive properties [2–5] and overwhelming the immune response [9], whereas salinity is important for uncontrolled mitosis [6] and immune cell activity [7]. While extracellular pH imaging has become an invaluable biomarker for early diagnosis and tracking treatments by magnetic resonance imaging (MRI) and spectroscopic imaging (MRSI) [10], imaging compartmental sodium levels is still a desirable goal [11].

Translational MRI/MRSI methods are integral to diagnosing and tracking disease [10]. Clinical MRI is largely based on detection of the ^{1}H nuclei of water molecules in soft tissues, where endogenous ^{1}H-MRI contrasts (based on ^{1}H relaxation) provide decent anatomical contrast. However, paramagnetic lanthanide ions, specifically gadolinium conjugated with a chelating molecule (e.g., Dotarem), can provide superior ^{1}H-MRI contrast (by accelerating ^{1}H relaxation) because the gadolinium agent extravasates through leaky blood vessels to brighten the lesion's appearance. While other nuclei (e.g., ^{23}Na, ^{31}P, or ^{17}O) are rarely considered for clinical applications, imaging these in addition to ^{1}H could offer illuminating physiological insights [10].

^{23}Na-MRI has potential to screen for cancer [12]. Normal ^{23}Na-MRI, however, cannot distinguish between sodium from different compartments because their signals overlap. However, ^{23}Na-MRSI can separate ^{23}Na signals in vivo from intracellular and extracellular compartments [13]. This is achieved by injecting a small amount of a contrast agent, similar to Dotarem but with other paramagnetic lanthanides. Since these agents extravasate into the extracellular space, ^{23}Na-MRSI can readout independent ^{23}Na signals from each compartment with high fidelity. Examination of several glioblastoma models in rodent brain show that tumors (compared to normal tissue) redistribute sodium from the extracellular milieu. Thus, within the tumor habitat, the extracellular salinity drops while the intracellular salinity remains unchanged (or slightly rises) to reduce the transmembrane sodium gradient in tumors. At the same time, sodium redistribution strengthens the transendothelial gradient.

A weak transmembrane sodium gradient implies that cancer cells are depolarized [6], typical of cells in a proliferative state which maintain a less negative membrane potential [1]. Similarly, a strong sodium transendothelial gradient in tumors suggests a weakened blood-brain barrier to support angiogenesis [1]. Since cancer and immune cells can sense sodium [7], compartmental sodium in the tumor niche could be important for cancer treatments tackling different mechanisms of proton/sodium imbalances **(Figs. 1 and 2)**. Given the range of ^{1}H-MRI [14] and ^{1}H-MRSI [11] methods that can measure pH dysregulation in cancer, combining ^{23}Na-MRSI innovations [13,15] can allow pioneering explorations of how transmembrane sodium/proton gradients synchronously change within the tumor habitat, with and without therapy [1–5].

Of the multitude of means by which sodium and pH levels are maintained **(Figs. 1 and 2)**, perturbing NHE function could be a desirable target for cancer therapies [1–5]. Glioblastoma patients exhibiting elevated NHE expression have shorter survival, in part, because greater NHE expression is associated with greater resistance to chemotherapy [16] and tumor recurrence [17]. Since non-selective NHE1 inhibitors (e.g., amiloride) only show marginal effectiveness, selective NHE1 inhibitors (e.g., cariporide) demonstrate preclinical value by limiting sodium influx, and moreover their effectiveness can be enhanced by extracellular acidity [18]. However, activity of NHE inhibitors have so far been explored primarily with acidosis in mind, more so than salinity because of technological challenges [19]. Since extracellular sodium can be easily affected by diet and/or drugs, with sodium levels rarely monitored in situ and/or in vivo, future clinical application of NHE inhibitors will require innovations in merged ^{1}H/^{23}Na imaging with novel MRI/MRSI methods [10–13] to understand the interactions of dysregulated pH and electrolyte imbalances [1–5]. We posit that imaging dysregulated sodium/proton gradients in tumors will also be important for tracking immunotherapy because immune cells can sense both sodium [7] and proton [9] levels in the extracellular milieu.

Declaration of Competing Interest

FH and MHK have no commercial or competing interests.

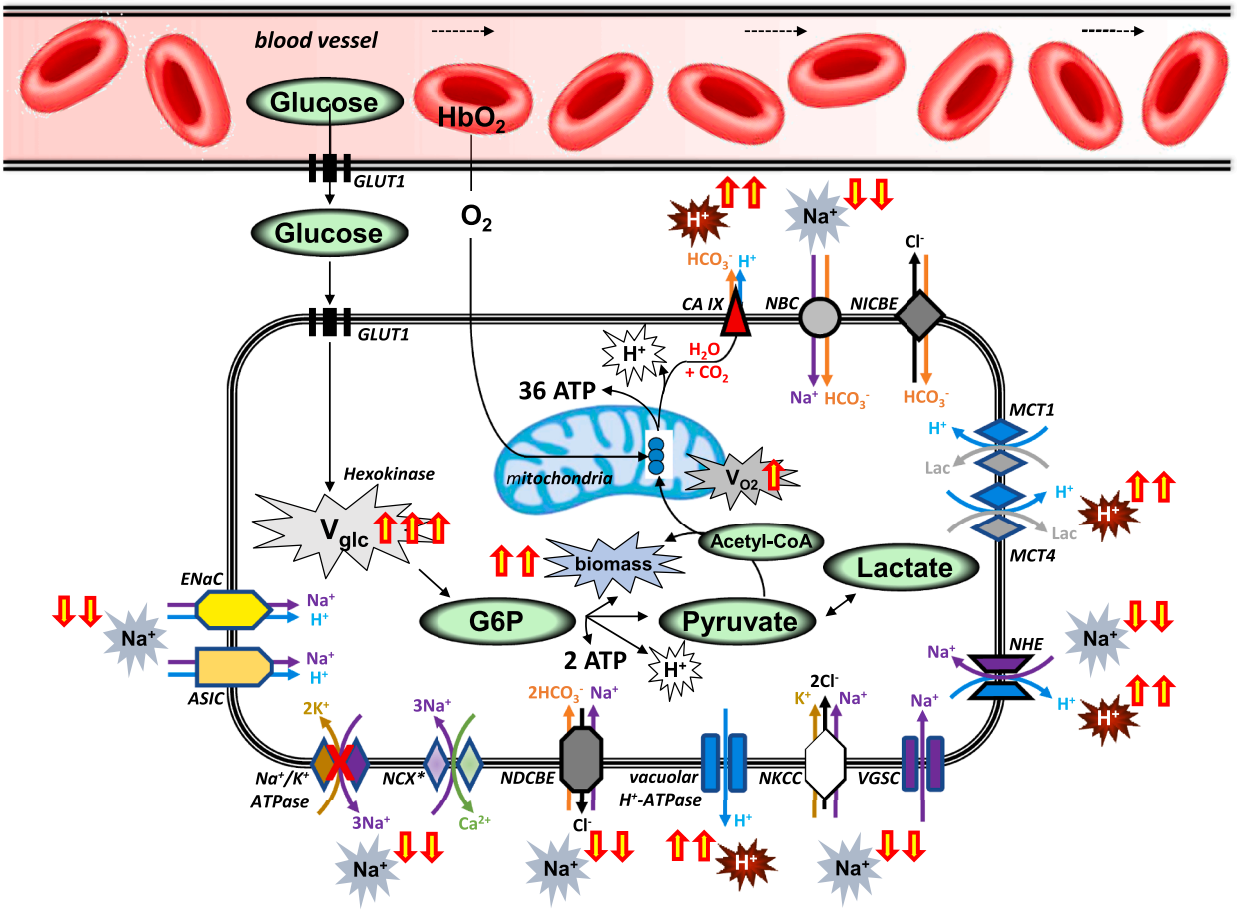

Fig. 2. Metabolic pathways of energy production and biosynthesis in tumors are closely associated with dysregulated transmembrane proton/sodium gradients [1–3, 11]. Glucose is transported across the cell membrane by glucose transporters (e.g., GLUT1), which are upregulated in cancer. Uncoupling between glucose metabolism (V_{glc}) and oxidative metabolism (V_{O2}) generate acidic by-products like lactate and proton (H^+). These are transported out of the cell by MCTs, specifically MCT4, and ion exchangers (e.g., NHE and vacuolar H^+-ATPase). The MCTs, specifically MCT1, also allow transport of other substrates (i.e., β-hydroxybutyrate (BHB) and acetate) [3,11]. Both carbon dioxide (CO_2) and water (H_2O) generated by oxidative processes also produce extracellular acidification using CA IX, generating bicarbonate (HCO_3^-) and proton (H^+). In cancer cells, to maintain intracellular pH homeostasis, both lactate and H^+ are extruded into the extracellular space by upregulation of these mechanisms (**Fig. 1b**). Similarly, there are other mechanisms for maintaining electrolyte balance (**Fig. 1a**). While downregulation of Na^+/K^+-ATPase (red "X") in cancer cells lower extracellular sodium, upregulation of VGSC and NHE and specific mutants of NCX (NCX*) all maintain low extracellular sodium.

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