Sodium homeostasis in the tumour microenvironment

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Abstract

The concentration of sodium ions (Na^+) is raised in solid tumours and can be measured at the cellular, tissue and patient levels. At the cellular level, the Na^+ gradient across the membrane powers the transport of H^+ ions and essential nutrients for normal activity. The maintenance of the Na^+ gradient requires a large proportion of the cell's ATP. Na^+ is a major contributor to the osmolarity of the tumour microenvironment, which affects cell volume and metabolism as well as immune function. Here, we review evidence indicating that Na^+ handling is altered in tumours, explore our current understanding of the mechanisms that may underlie these alterations and consider the potential consequences for cancer progression. Dysregulated Na^+ balance in tumours may open opportunities for new imaging biomarkers and re-purposing of drugs for treatment.

Introduction

The concentration of several key ions, including protons (H^+) (1), potassium (K^+) (2), calcium (Ca^{2+}) (3) and sodium (Na^+) (4) is altered in tumours. This ionic imbalance contributes to several cancer hallmarks, including altered growth signalling, proliferation, angiogenesis, invasion and metastasis (5). Just as intracellular ion concentrations can alter cancer cell behaviour, the extracellular "ionic tumour microenvironment" can determine how cancer, stromal and infiltrating immune cells behave (6). Dysregulation of ion homeostasis within the tumour microenvironment could therefore also contribute to tumour progression. Thus, ion channels and transporters, including those permeant to Na^+, have potential as novel targets for therapeutic intervention.

Control of Na^+ is critical for normal cellular function and homeostatic dysregulation is a key feature of disease states such as acute inflammation (7) and ischaemia (8). Na^+ handling is also altered in cancer: Na^+ is raised in malignant tumours compared to corresponding healthy tissues (9). Tumorigenesis is accompanied by alterations to metabolism, pH regulation, vascularity and cell density that affect the distribution of Na^+ within cancer cells and the extracellular tumour microenvironment. Here, we review the evidence showing that Na^+ handling is altered in tumours, explore the mechanisms that may underlie these alterations and consider the potential consequences for cancer progression. We also highlight potential clinical applications for Na^+ as a diagnostic biomarker and for targeting dysregulated Na^+ within the ionic microenvironment of tumours alongside existing cancer therapeutics.

The extracellular Na^+ concentration ($[Na^+]_e$) is typically an order of magnitude higher (145 mM) than intracellular [Na^+] ($[Na^+]_i$; 12 mM) (10). Therefore, an increase in total tumour tissue [Na^+] could be caused by an increase in the volume of extracellular fluid relative to the volume of intracellular fluid (extracellular volume fraction; Figure 1). The generation of

permeable vasculature in tumours by the angiogenic vascular endothelial growth factor (VEGF) allows leakage of plasma proteins, including glycosaminoglycans and collagen, into the interstitial space (11, 12). Protein leakage into the interstitium will also increase the colloid osmotic pressure, which contributes to the raised interstitial fluid pressure seen in solid tumours (13), potentially expanding the interstitial fluid volume (14, 15). Cell death following successful chemotherapeutic intervention would also be expected to increase the extracellular volume fraction. This would have implications for other ions. For example, K^+ released into the extracellular space following tumour cell death results in an elevated extracellular $[K^+]$ that suppresses the anti-tumour activity of tumour infiltrating lymphocytes (16). Nevertheless, the increase in interstitial fluid volume may underlie the raised total tumour $[Na^+]$. Although it has historically been difficult to measure accurately, there is some evidence that the interstitial fluid compartment is enlarged in some tumour types, including those which induce oedema, such as malignant gliomas and meningiomas (17-19).

Raised total tumour tissue $[Na^+]$ may also be caused by increased $[Na^+]_i$, increased $[Na^+]_e$, or a combination of both. Quantitative measurement of tumour $[Na^+]_e$ is lacking, however, there is evidence that $[Na^+]_i$ is raised in tumours. Early studies using x-ray dispersion microanalysis and flame photometry indicated that the $[Na^+]_i$ of cancer cells from various tumour types was more than double that of cells from adjacent healthy tissues (20, 21). Additional approaches, including measurement of ^{22}Na radioisotope assimilation rate by atomic absorbance spectrophotometry, live cell imaging using the fluorescent Na^+ reporter SBFI-AM, and ^{23}Na magnetic resonance imaging (^{23}Na MRI; Box 1) have broadly confirmed these observations in cultured cells and cancer patients (22-27).

Cellular mechanisms underlying sodium handling in tumours

Numerous plasma membrane channels and transporters facilitate Na^+ flux down the electrochemical gradient from the extracellular space into the cytosol (Table 1; Figure 2).

Altered expression or activity of such mechanisms in cancer cells could account for the raised $[Na^+]_i$ observed in tumours. The ubiquitous Na^+/K^+ ATPase is almost exclusively responsible for the removal of Na^+ from cells and thus maintaining the inward gradient for Na^+. As a result, this pump uses a significant proportion of the total ATP produced by non-excitable cells (28, 29). Altered cellular metabolic activity may thus lead to changes in Na^+/K^+ ATPase activity. For example, when the usage of ATP for cellular proliferation is increased, the provision of ATP to the Na^+/K^+ ATPase may be reduced and, as a consequence, changes in $[Na^+]_i$ and $[Na^+]_e$ would occur (30). Raised $[Na^+]_i$ and possibly $[Na^+]_e$ can both increase Na^+/K^+ ATPase activity to maintain Na^+ homeostasis (31-33). Since this pump is so energetically demanding, increasing Na^+ influx would increase the cellular ATP consumption rate. Evidence suggests that the Na^+/K^+ ATPase is predominantly fuelled by glycolysis in breast cancer cells since this delivers ATP quickly to the site where it is being used (34, 35). However, hypoxia has been shown to inhibit Na^+/K^+ ATPase activity, suggesting that mitochondrial ATP supply is also needed (36, 37).

The inward electrochemical Na^+ gradient set up by the Na^+/K^+ ATPase powers the activity of a number of different Na^+-dependent transport mechanisms. The Na^+/H^+ exchanger (NHE) family is one such mechanism, which uses the inward Na^+ gradient to move H^+ into the extracellular space, thus playing a central role in pH homeostasis (38). The ubiquitously expressed NHE1 is activated by receptor tyrosine kinase signalling, in particular via the Ras-extracellular signal-regulated kinase (ERK) pathway, and by osmotic stress, hormones and growth factors (39). NHE1 is also allosterically activated by an increase in intracellular $[H^+]$, as may be found in glycolytic tumour cells (40, 41). Another major pH regulation mechanism coupled to inward Na^+ transport is the electroneutral Na^+/HCO_3^- cotransporter (NBCn1), which is upregulated in hypoxic tumours (42, 43). The imported HCO_3^- neutralises H^+ generated from high metabolic activity by forming H_2O and CO_2. The Na^+ gradient also powers the import of amino acids into cells via Na^+-dependent amino acid transporters, many of which are overexpressed in cancers (44). Na^+-dependent glucose transporters

(SGLTs), normally responsible for active glucose uptake in the kidney, are also functionally expressed in many cancers (45, 46). In addition, the Na^+-K^+-Cl^- cotransporter (NKCC), a key regulator of osmotic balance and cell volume which facilitates the transport of Na^+, K^+ and $2.Cl^-$ into the cell, is also upregulated in numerous cancers (47, 48). The combined activity of such nutrient and electrolyte transport mechanisms is not only dependent on Na^+ homeostasis within the tumour microenvironment, but is also predicted to raise $[Na^+]_i$, thus potentially contributing to the elevated Na^+ signal observed in tumours.

Na^+ channels expressed on tumour cells also enable Na^+ influx and elevation of $[Na^+]_i$. Voltage-gated Na^+ channels (VGSCs), classically expressed in electrically excitable cells where they initiate action potentials via Na^+ influx, are also expressed in many tumour cell types where they promote cancer cell invasion and metastasis (49, 50). Although the voltage-dependent opening of these channels is transient, they also conduct a 'persistent' inward Na^+ current under resting conditions, thus providing a route for Na^+ to enter the cytosol in non-excitable tumour cells (51-55). The amiloride-sensitive epithelial Na^+ channel (ENaC) and the related acid-sensing ion channels (ASICs) are also Na^+-selective ion channels which permit voltage-independent inward Na^+ current. ENaC and ASICs have been linked to proliferation, migration, invasion and metastasis in various cancers (56, 57). Flow of Na^+ through ENaC and ASICs is regulated by extracellular H^+ (58, 59). Thus, both channels may contribute to elevation of $[Na^+]_i$ in acidic tumours. In addition, N-methyl-D-aspartate (NMDA) receptors may also permit elevated $[Na^+]_i$ in tumours. These ligand-gated, nonselective cation channels are typically expressed in the central nervous system (CNS) and activated by glutamate. NMDA receptors are expressed in numerous tumour types, including non-neuronal tumours such as pancreatic, breast and ovarian cancers, where they regulate invasion and correlate with poor prognosis (60-62). Proteins forming the G protein-coupled receptor-activated Na^+ leak channel (NALCN) have been suggested as potential cancer susceptibility loci (63), and although evidence for its involvement in cancer is limited, NALCN may provide an additional route for Na^+ influx, thus elevating $[Na^+]_i$. Finally, the two-

pore channel (TPC) family of lysosomal and endosomal cation channels can increase cytosolic Ca^{2+} and Na^+ and have been shown to promote lung cancer cell migration (64) and epithelial-mesenchymal transition of breast cancer cells (65).

Pathophysiological consequences of altered tumour Na^+

Dysregulated Na^+ handling in tumours can lead to significant physiological changes at the cellular level, such as altered electrical potential difference across the plasma membrane (membrane potential; V_m), pH, or metabolic activity. These physiological alterations can induce myriad effects on key tumour hallmarks from proliferative ability to invasion into healthy tissue and immune evasion (Figure 3).

Membrane potential depolarisation

Influx of Na^+ into non-excitable cells depolarises the V_m (around 5-10 mV) (22, 66-68). In general, cancer cells exhibit a more depolarised V_m than their normal counterparts (around -5 to -50 mV vs. -50 to -95 mV), which may correlate with their increased proliferative capacity (69). This phenomenon may be due to the changes in V_m that accompany different stages of the cell cycle (70). Indeed, a relatively negative V_m (V_m hyperpolarisation) can prevent DNA synthesis and mitosis (71). Furthermore, stem cell differentiation can only occur if the V_m is hyperpolarised (72). V_m depolarisation leads to reorganisation of charged phospholipids in the inner leaflet of the plasma membrane, which in turn enhances nanoclustering and activation of K-Ras promoting mitogenic signalling (73). V_m depolarisation is also functionally instructive in regulating cytoskeletal reorganisation, morphogenesis, regeneration and tumorigenesis (68, 74-78). In effect, persistent Na^+ entry via ENaCs and VGSCs may increase proliferation, maintain a poorly differentiated phenotype and increase migration via depolarisation of the V_m, all aiding tumour progression. However, V_m depolarisation in tumour cells is likely tightly regulated given that it can also

promote apoptosis and isotonic volume decrease (79, 80) and may thus present an interesting therapeutic target.

Regulation of pH dynamics

The extracellular microenvironment of solid tumours is commonly acidic (pH 6.5–7.2), whereas the intracellular pH of cancer cells is typically neutral or slightly alkaline (81, 82). The acidic tumour microenvironment is a critical contributory factor to many cancer hallmarks such as invasion, altered metabolism, drug resistance and immune evasion (83). The Na^+ gradient across the plasma membrane impacts on pH regulation mechanisms. For example, an altered inward Na^+ gradient will influence the pH-regulating capacity of cancer cells by regulating influx of HCO_3^- via NBCn1 and efflux of H^+ via NHE1. NBCn1 is the predominant means of H^+ extrusion from tumour cells when pH_i is > 6.6, whereas NHE1 is important under more acidic conditions, such as those observed in highly glycolytic cancer cells in a hypoxic tumour (42, 84, 85). Since both transport mechanisms are present in cancer cells (42, 86), they may work in tandem to facilitate Na^+-dependent tumour progression. Increased NHE1 activity leads to intracellular alkalinisation (87) and this may be a critical early event in oncogene-induced malignant transformation (88). Maintenance of a high pH_i by NHE1 activity is permissive for upregulation of both glycolytic activity and protein synthesis required for rapid cell growth and division (1, 89, 90). On the other hand, extracellular acidification promotes invasion and suppresses the immune response (91, 92). Thus, inhibition of the inward Na^+ gradient may provide an effective intervention to manipulate tumour pH for therapeutic benefit.

The acidic pH of tumours may also reciprocally regulate Na^+ conductance. For example, ENaC and ASIC channels are regulated by pH_e (58, 59) and the persistent inward Na^+ current carried by VGSCs is increased under hypoxia or extracellular acidification (93, 94). Similarly, the Na^+/Ca^{2+} exchanger (NCX) is also regulated by pH, with an acidic pH_i inhibiting forward (Ca^{2+} efflux/Na^+ influx) mode action (95). Moreover, given that the sensitivity of NCX

to H^+ requires intracellular Na^+ (96), it is tempting to speculate that the altered pH and Na^+ levels in tumours work in tandem to perturb Ca^{2+} signalling and homeostasis.

Regulation of metabolic activity

Altered tumour [Na^+] leads to changes in glucose metabolism that facilitate cancer progression. In a phenomenon first reported by Warburg in the 1920s (97), cancer cells exhibit upregulated glycolysis with conversion of glucose to lactic acid despite the presence of abundant oxygen ('aerobic glycolysis'). This shift in metabolism towards a more glycolytic phenotype confers numerous survival advantages for cancer cells, including survival within a hypoxic tumour core, and is associated with rapid cell proliferation, acidification of the tumour microenvironment, metastasis and poor patient outcome (98). In addition to directly regulating cancer cell metabolism via the hypoxia sensor HIF-1α, tumour hypoxia may indirectly contribute to a highly glycolytic phenotype via elevation of tumour [Na^+]. Elevations in both tissue and intracellular [Na^+] are observed in ischaemic tissue (99), and hypoxia is known to increase the persistent inward Na^+ current through VGSCs (100, 101). This increase in the persistent Na^+ current would be expected to elevate $[Na^+]_i$ in cancer cells expressing these channels. Moreover, in hypoxic tumours, upregulation of glycolysis and increased extrusion of H^+ by NHE would increase $[Na^+]_i$ (102).

In vitro studies indicate that elevations in $[Na^+]_e$ can drive a highly glycolytic phenotype via the induction of various signalling pathways. For example, early studies revealed that glycolytic lactic acid production of HeLa cells increased as $[Na^+]_e$ increased (103). Elevated $[Na^+]_e$ upregulates the key cancer-associated glycolytic enzymes pyruvate kinase M2, lactate dehydrogenase A and hexokinase II, leading to increased glucose consumption and lactate production (104). Elevated $[Na^+]_i$ may influence cancer cell metabolism due to increased energy demands from Na^+ homeostasis mechanisms. Indeed, Na^+/K^+ ATPase activity can also regulate the expression of glycolytic enzymes, and G-protein GPR35 mutations, which increase Na^+/K^+ ATPase activity, increase the glycolytic rate (105). Conversely, inhibition of

the Na^+/K^+ ATPase reduces expression of the hypoxia sensor HIF-1α, preventing it from upregulating glycolysis via increased expression of the glucose transporter GLUT-1 and hexokinase (106).

Altered $[Na^+]_i$ may also directly affect mitochondrial metabolism by facilitating Ca^{2+} transport between the mitochondria and the cytosol. The mitochondrial Na^+/Ca^{2+} (lithium) exchanger (NCLX) regulates mitochondrial Ca^{2+} content by extruding Ca^{2+} into the cytosol in exchange for Na^+ or Li^+ (107), and is regulated by the cytoplasmic $[Na^+]$. Thus, NCLX uses Na^+ transport to fine-tune the mitochondrial $[Ca^{2+}]$, thereby regulating mitochondrial metabolism, redox homeostasis and ATP production (108, 109). Inhibition of NCLX induces apoptosis in prostate cancer cells (110), suggesting that an elevated $[Na^+]_i$ in cancer cells might promote apoptosis resistance via NCLX. Taken together, these data suggest that elevated tumour $[Na^+]$ and Na^+/K^+ ATPase activity contribute to a highly glycolytic cancer cell phenotype, which would be expected to promote proliferation, tumour acidification and resistance to apoptosis. However, the underlying mechanisms linking tumour $[Na^+]$ to cancer metabolism remain poorly characterised and require further research.

Nutrient transport

Amino acids regulate cancer cell signalling and metabolism (111), raising the possibility that altered amino acid uptake through Na^+-dependent systems might influence cancer progression following changes to the transmembrane Na^+ gradient. For example, the Na^+-dependent SGLT glucose transporters facilitate glucose uptake into cancer cells, and specific blockade of SGLT2 reduces mitochondrial ATP production and cellular proliferation and increases tumour necrosis (45, 112). Moreover, the Na^+-dependent amino acid transporter SLC1A5, which is highly expressed in cancers and is driven by myc expression, imports glutamine (among other amino acids), and activates mammalian target of rapamycin complex 1 (mTORC1) to facilitate proliferation (113, 114). Many cancers, including triple-

negative breast cancer, have a “glutamine addiction” since this amino acid is a key carbon source for fatty acid production and mitochondrial ATP production (115, 116). Changes to the Na^+ gradient may therefore regulate nutrient uptake in cancer cells and these observations raise the interesting possibility that pharmacologically reducing the inward Na^+ gradient may impair the ability of cancer cells to import nutrients.

Cell volume regulation

Na^+ salts are the main contributors to the osmolarity of extracellular fluid, and the osmolarity of intracellular and extracellular fluids must be balanced to prevent cell shrinkage or swelling. The $Na^+/K^+/Cl^-$ cotransporter NKCC1 activity is driven by the inward Na^+ gradient and acts to regulate cell volume by facilitating the accumulation of intracellular Cl^- (117). Solid tumours exhibit a high interstitial colloid osmotic pressure (COP) and hydrostatic pressure in the interstitial fluid (13) which would be expected to hinder cell expansion. In vitro evidence shows that breast cancer cells in spheroids under compression actively extrude Na^+ through NHE1 to reduce intracellular tonicity, leading to osmosis into the cell to resist compressive forces (118). In this circumstance, NHE1 functions in the reverse mode, importing H^+ leading to intracellular acidification. Thus, by regulating Na^+ as well as H^+, NHE1 activity must balance the cell’s pH and volume regulation needs. In hypotonic conditions, cancer cells regulate $[Na^+]_i$ to protect against volume increase (119). Therefore, tight regulation of Na^+ transport is critical for maintaining cell volume in response to changes in COP and hydrostatic pressure in the tumour microenvironment.

Effects of Na^+ on tumour progression

As a result of its impact on physiological behaviour of cancer and stromal cells, substantial experimental evidence supports the role of raised $[Na^+]$ in promoting key aspects of tumour progression, including proliferation, migration, invasion and inflammation (22, 120-122) (Figure 4).

Proliferation

High osmolarity in the tumour microenvironment promotes proliferation via modulation of $[Na^+]_i$. Because the tumour interstitial fluid COP is higher than in healthy tissues (123, 124), and this contributes to a higher osmolarity, inward Na^+ current is enhanced in cancer cells. For example, high osmolarity increases inward Na^+ current through ENaC, which elevates $[Na^+]_i$, promotes tumour cell proliferation and inhibits apoptosis (121, 125, 126). This hyperosmolarity-induced inward current may promote proliferation by triggering brx-dependent activation of the small GTPase Rac1 thus stimulating the mitogen-activated protein kinase (MAPK)/ERK1/2 cascade (127, 128). High $[Na^+]_e$ has also been shown to increase phosphorylation of the salt-inducible serine/threonine kinase SIK3 in breast cancer cells, which promotes proliferation via release from G1/S-phase arrest (129). Moreover, NKCC1 expression, cell shrinkage and Na^+-dependent Cl- accumulation have been established as important regulators of the cell cycle and proliferation in cancer cells (48, 117, 130). On the other hand, moderate hypertonicity has been shown to lead to dormancy (131), suggesting that the level of osmotic pressure within tumours, and the cellular response to this, may be critical for determining fate. High $[Na^+]_e$ has also been shown to induce DNA breaks and temporary cell cycle arrest (132). However, unlike most cases of DNA damage-induced cell cycle arrest, these DNA breaks are not repaired during this period, and the DNA damage persists when cells adapt to high $[NaCl]_e$ and start to proliferate, with implications for oncogenesis (133-135).

Migration

Several Na^+ transport systems have been shown to control tumour cell migration. For example, NHE1 has been shown to work in concert with aquaporins to allow cancer cells to move through confined spaces by taking in water and ions at the leading edge and expelling water from the trailing edge. This is achieved partly by concentrating NHE1 and aquaporin AQP5 at the leading edge of cells (136). Similarly, ENaC expression is increased at the

leading edge of migrating choriocarcinoma cells, promoting their motility (137). In addition, a glioma-specific Na^+ channel made up of ENaC and ASIC subunits promotes migration and cell cycle progression (138). VGSC activity has also been shown to promote acquisition of a mesenchymal-like elongate morphology and thus increase migration of cells from a range of different types of cancer (139-144).

The Na^+ gradient across the plasma membrane is tightly linked to Ca^{2+} transport by the Na^+/Ca^{2+} exchanger (NCX), which is also upregulated in tumour cells (145, 146). NCX classically acts to extrude cytosolic Ca^{2+} following large increases in $[Ca^{2+}]_i$, thereby importing Na^+ (147). Importantly, small changes to the Na^+ gradient across the plasma membrane can alter the equilibrium potential for NCX and thereby lead to its operating in reverse (Ca^{2+} entry/Na^+ exit) mode (148, 149). NCX reverse mode action has been implicated as a key mediator of transforming growth factor β (TGF-β)-induced Ca^{2+}-dependent migration in hepatocellular and pancreatic cancer cells (150, 151). NCX may thus provide a mechanism linking elevations in $[Na^+]_i$ to protumour Ca^{2+} signalling (149, 152). Interestingly, NCX inhibition has also been reported to decrease the intracellular accumulation of ^{11}C-choline in cancer cells. This has important implications for positron emission tomography (PET) imaging, since alterations in tumour Na^+ content might compromise NCX action and thus lead to poor contrast agent accumulation within tumours (153).

Invasion

A cancer hallmark commonly associated with aberrant Na^+ homeostasis is the ability of cancer cells to invade into healthy tissues and migrate around the body to form metastases (49). Invasion requires proteolytic breakdown of the extracellular matrix by enzymes such as matrix metalloproteases and cathepsins. Cathepsins in particular are activated by low pH (154) so Na^+-dependent tumour acidification may thus facilitate invasion. NHE1 is expressed in the invadopodia of migrating breast cancer cells and colocalises with the invadopodial

marker cortactin (155). NHE1 activity in the invadopodia leads to local acidification of the extracellular compartment, providing the ideal pH conditions at the leading edge of an invading cell for digestion of the extracellular matrix (87, 120, 156-159).

VGSCs also promote the invasiveness and metastatic ability of a range of different cancer cell types (26, 141, 160, 161) and VGSC expression correlates with lymph node metastasis and a poor prognosis in breast cancer patients (54, 160). This appears to rely on the Na^+ conductance properties of VGSCs, since silencing VGSC expression or specific blockade results in decreased invasion in vitro (22, 161) and metastasis in vivo (162, 163). VGSCs facilitate an invasive phenotype by inducing transcriptional changes in genes contributing to Wnt, MAPK and Ca^{2+} signalling (161, 164). VGSC activity may also increase cancer cell invasiveness through altering pH homeostasis. VGSC-dependent Na^+ influx in caveolae promotes H^+ extrusion by NHE1, thus acidifying the extracellular space and increasing cathepsin B protease activity (120, 142, 152). The authors proposed a putative allosteric interaction between VGSCs and NHE1 as the mechanism by which this interaction is mediated, since Na^+ influx would otherwise be expected to reduce the Na^+ gradient driving H^+ export by NHE1 (142).

ASICs are also upregulated in tumour cells where they promote pH-dependent migration and invasion (165). For example, ASIC1a increases $[Ca^{2+}]_i$ and promotes migration as a result of pH_e acidification (166) and ASIC2 mediates acidosis-induced invasion and metastasis (56). ASIC opening also results in activation of RhoA and induction of the epithelial-mesenchymal transition in pancreatic cancer cells (167). Co-expression of NMDA receptors and glutamate transporters (vGlut1–3) in glioma, pancreatic and ovarian cancer cells correlates with poor prognosis, suggesting an autocrine signalling mechanism that drives disease progression (60). Moreover, *in vitro* invasion and *in vivo* tumour burden are both decreased following treatment with a selective non-competitive NMDA receptor antagonist (60). Although these effects were attributed to Ca^{2+} entry-induced activation of the Ca^{2+}/calmodulin-dependent

protein kinase (CaMK) and mitogen-activated protein kinase kinase (MEK)-MAPK pathways, it remains to be determined whether elevated Na^+ entry via NMDA receptors also contributes to their pro-invasive potential.

NKCC1 is also localised to the leading processes of migrating glioma cells, where it facilitates invasive behaviour by regulating focal adhesions, Cl^- accumulation and cell volume (47, 117, 168). The cell shrinkage that results from Na^+-linked Cl^- accumulation allows the invading cell to navigate narrow gaps in the peritumoural space. Interestingly, NKCC1 expression in hepatoma cells is upregulated in response to hyperosmolarity (169), suggesting that an elevation in tumour $[Na^+]_e$ could promote an aggressive cell phenotype overexpressing NKCC1. These findings implicate NKCC1 as an important mediator of invasive potential and suggest that elevated Na^+ may exacerbate metastatic behaviour by upregulating NKCC1 expression and Na^+-linked Cl^- accumulation.

Tumour inflammation

In sites of acute inflammation induced by inoculation with complete Freund adjuvant, BCG, or *Leishmania*, $[Na^+]_e$ is increased (170, 171), leading to alterations in immune cell function (172, 173). The $[Na^+]_e$ within inflamed solid tumours has not been studied, but it may be similarly altered. Chronic inflammation plays a critical role in cancer progression due to the abundance of cytokines and chemokines which stimulate proliferation and angiogenesis, and the release of reactive oxygen and nitrogen species from inflammatory cells which can cause DNA damage (174). Inflamed tissue has an increased extracellular osmolarity (171, 175, 176), and hyperosmolar conditions (e.g. high $[Na^+]_e$) exacerbate inflammation (177, 178). Hyperosmotic stress is detected by many cell types including epithelial cells where it leads to activation of the nuclear factor of activated T-cells (NFAT-5) transcription factor. NFAT-5 is responsible for mediating integrin-induced breast cancer cell invasion (122). In macrophages, NFAT-5 activation in response to a hyperosmolar extracellular environment results in secretion of VEGF-C which stimulates angiogenesis (179). Hyperosmotic stress

upregulates production of inflammatory cytokines including interleukin (IL)-1β, IL-6, IL-8 and tumour necrosis factor-α (TNF-α) via the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (171). High Na^+ in the microenvironment also provides a mitogenic stimulus to macrophages via activation of the p38 MAPK cascade (180). In addition, hyperosmolarity prolongs survival of macrophages by reducing production of the pro-apoptotic molecules p53 and bax (171). Tumour-associated macrophages (TAMs), which promote many aspects of cancer progression (181), may therefore be more prevalent in hyperosmotic tumour microenvironments. However, while evidence indicates that high $[Na^+]_e$ stimulates an immune response in the skin via p38/MAPK and NFAT5 signalling and classical (M1) macrophage activation (170), studies have also shown that high $[Na^+]_e$ induces peripheral macrophages to switch to an anti-inflammatory M2 (alternative activation) phenotype with poor phagocytic capacity, which would be expected to facilitate tumour progression rather than inhibit it (182). Based on these data, it appears that the regulation of the anti-tumour immune response by Na^+ is highly complex and may be tissue-specific (183).

Many pro-inflammatory effects of elevated $[Na^+]_e$ are mediated by the cytokine IL-17. IL-17 is produced by CD4+ T-helper 17 (Th17) cells in response to high $[Na^+]_e$, downstream of NFAT-5 activation (177). IL-17 signalling facilitates tumour development and progression (184-186). Thus, IL-17 induction by hyperosmotic stress links high $[Na^+]_e$ to both tumour cell survival and metastasis. Indeed, elevated $[Na^+]_e$ and IL-17 have been shown to induce expression of the promigratory VEGF-A (187). The synergistic proinflammatory effects of high $[Na^+]_e$ and IL-17 can also be mediated by SIK3 to increase arginine metabolism, reactive nitrogen species production, CXCL-12 expression and matrix metalloproteinase (MMP9) activation (129). Interestingly, the high $[Na^+]_e$-induced component of the inflammatory response could be blocked by inhibiting Ca^{2+} influx or by knockdown of the store-operated Ca^{2+} entry (SOCE) regulatory molecules stromal interaction molecule

(STIM1) and Orai1, suggesting that these changes are governed by store-operated Ca^{2+} entry (188). These studies show that elevated $[Na^+]_e$ can drive tumourigenicity via NFAT5, NF-κB, SIK3 activation and SOCE mechanisms.

Diagnostic implications

The accumulating body of evidence implicating altered Na^+ handling in regulating cancer cell behaviour, tumour metabolism, acidosis, growth, inflammation and invasion raises the intriguing possibility that the tumour $[Na^+]$ may have value as a diagnostic or predictive biomarker in response to treatment. For example, measurement of $[Na^+]_i$ may serve as a biomarker for both hypoxia-induced necrosis and cell death in response to successful chemotherapy treatments. Earlier detection of treatment response using techniques such as ^{23}Na MRI (Box 1) may thus facilitate more timely selection of optimal therapies for individual patients, thereby improving clinical outcomes (189). ^{23}Na MRI was first applied to supratentorial brain tumours in patients (190). Subsequently, this under-represented imaging methodology was used to show that the tissue $[Na^+]$ is higher in malignant gliomas compared to normal brain tissue (4). A similar pattern was observed for breast (9, 191) and prostate tumours (25). This elevated tissue $[Na^+]$ is present in both tumour tissue and oedema. Furthermore, $[Na^+]$ is heterogeneous across the peritumoral region, suggesting that altered tissue $[Na^+]$ may be demonstrating local physiological or biochemical changes within the tumour microenvironment (Figure 1) (9). Additionally, relaxation-weighted ^{23}Na-MRI can differentiate between brain tumours of grades I-III and grade IV (192). However, while similar trends linking tumour grade to $[Na^+]$ have been observed in breast and prostate cancers, any differences between tumour grades were below the threshold of statistical significance (25, 193). As such, future clinical studies with larger cohorts are needed to better determine the correlation between tumour $[Na^+]$ and tumour type and grade.

A limitation with these observations using ^{23}Na MRI is that they did not differentiate between Na^+ located within the intracellular and extracellular compartments. To investigate this, Neto et,al. (2018) determined $[Na^+]_i$ and $[Na^+]_e$ in brain tumours (194). This revealed that although lesions have higher total $[Na^+]$ than the normal appearing white matter, the extracellular volume fraction is also consistently elevated, whereas the apparent $[Na^+]_i$ is lower than in white matter. This would imply that that increased extracellular volume may underlie the majority of the elevated tissue $[Na^+]$. However, relaxation-weighted ^{23}Na MRI has shown that $[Na^+]_i$ is elevated in glioblastomas and cerebral metastases (192) and is supported by in vitro data (22, 26). This observation of elevated $[Na^+]_i$ has since been supported by additional studies in breast and prostate tumours using fluid suppression by inversion recovery and diffusion-weighted MRI approaches (25, 193, 195). Taken together, these studies suggest that both increases in extracellular volume fraction and $[Na^+]_i$ can contribute to elevated total tissue $[Na^+]$ in cancer, although the relative contributions of these two compartments may vary between tumour type and location.

^{23}Na MRI may have the potential to complement standard of care radiological imaging approaches including positron emission tomography (PET). Elevated $[Na^+]_i$ in brain tumours correlates with the proliferation marker MIB-1 (192), raising the possibility that ^{23}Na MRI may complement ^{18}F-fluorothymidine positron emission tomography ([^{18}F]FLT-PET) for proliferation assessment in CNS tumours (196). In addition, ^{23}Na MRI measurement of total tumour $[Na^+]$ has been shown to be superior to isocitrate dehydrogenase (IDH) mutation status in predicting progression-free survival, suggesting that tumour $[Na^+]$ may be a promising tool for non-invasive outcome prediction (197). Furthermore, ^{23}Na MRI may also have value for detecting changes in real-time during treatment as a potential early biomarker for therapy assessment (198). However, it must be noted that $[Na^+]$ is likely to change following initiation of treatment as a result of physiological changes in the tumour and so the relationship between $[Na^+]$ and therapy response may be complex. For example, in a preclinical mouse xenograft model of prostate cancer, $[Na^+]_i$ increased within 24 h of

initiation of chemotherapy treatment (24), whereas in a rat glioma model, $[Na^+]_i$ was significantly reduced 5 days after onset of chemotherapy (199). In broad agreement with the latter, total tumour $[Na^+]$ is reduced in breast cancer patients responding to neoadjuvant chemotherapy (189, 200). However, the heterogeneity of $[Na^+]$ within the tumour following therapy is likely to be critical. For example, chemotherapy-induced cellular necrosis would be expected to increase the extracellular volume fraction, likely underpinning the early increase in total tissue $[Na^+]$ observed in preclinical tumour models following onset of therapy (201-203). Clearly, it is necessary to evaluate changes in both $[Na^+]_i$ and $[Na^+]_e$ in response to therapeutic intervention in order to evaluate predictive value of Na^+ in the clinical setting. Whether MRI can do this remains to be explored across both preclinical and clinical theatres.

Therapeutic potential of manipulating Na^+ levels

Directly or indirectly manipulating tumour Na^+ levels, for example by using pharmacological tools to manipulate transporter activity, may present novel treatment options to complement existing therapeutics. Indeed, numerous studies are presently ongoing to evaluate targeting tumour $[Na^+]$ (Table 2). Given that tumour acidification can induce drug resistance (1, 81, 204) and Na^+ and pH are very closely linked, pharmacological modification of tumour $[Na^+]$ may be a useful adjunct to other chemotherapeutics. For example, the ENaC/NHE1 blocker amiloride strongly synergises with doxorubicin to induce apoptosis and reduce glycolysis in osteosarcoma cells (205). On the other hand, Na^+/K^+ ATPase activity is increased in hepatocarcinoma cells upon development of resistance to chemotherapeutics (206). Similarly, high $[Na^+]_e$ increases expression of the multi-drug resistance protein P-glycoprotein in breast cancer cells in a Ca^{2+}-dependent manner (188). In addition, elevated $[Na^+]_i$-mediated acidification of the tumour microenvironment may help cancer cells to evade immune surveillance. Acidotic conditions correlate with low leukocyte counts (207) and decrease cytotoxicity of natural killer (NK) cells (208) and cytotoxic T-lymphocytes (209).

Thus, tumour $[Na^+]_e$ may modulate response to existing chemotherapeutics and emerging immunotherapeutics via pH- and non pH-dependent mechanisms.

Extracellular $[Na^+]$ in tumours would be expected to follow serum $[Na^+]$ and indeed, hypertonic interstitial fluid accumulates in the skin of rats fed on a high salt diet (179). Since elevated tumour $[Na^+]_e$ may promote tumour progression, serum $[Na^+]$ is likely critical in cancer patients. Hypernatremia, which has a high mortality rate, is an uncommon side effect of some chemotherapy regimes (210). Significantly, $NaHCO_3$ infusions, currently under test as a treatment to increase extracellular pH in cancer (Table 2), should be considered in the light of these findings. The risks associated with elevating $[Na^+]_e$ (211) will need to be balanced against any advantages of reducing tumour acidity. Recently TRIS-base has been identified as a well-tolerated and effective anti-metastatic oral pH buffer in mice which does not require a counter-ion and would therefore not be expected raise $[Na^+]_e$ (212).

Several studies have examined whether normalising tumour $[Na^+]$ might be a useful treatment strategy. The use of VGSC inhibitors to prevent cancer growth and metastasis has been investigated in several preclinical studies (162, 213-215), and is currently the subject of several ongoing clinical trials (Table 2). In support of this, in retrospective observational studies, VGSC-inhibiting tricyclic antidepressants and antiepileptic medications have been shown to associate with reduced incidence of several common cancers including lung and colorectal cancer and glioma (216, 217). On the other hand, antiepileptic medications associate with increased mortality in breast, bowel and prostate cancer patients, although this may be a result of confounding by indication (218, 219). Significant improvements in cancer outcomes have been associated with local anaesthetic drugs such as lidocaine (220) and the anti-epileptic drug valproate in combination with doxorubicin (221) or a topoisomerase inhibitor (222); however the benefits may be attributed to other mechanisms in addition to VGSC inhibition. For example, regional anaesthesia reduces the need for general anaesthetic drugs and opioids, which have various deleterious effects on immunity

(223). The local anaesthetic lidocaine stimulates natural killer cell cytolytic activity (224) and several local anaesthetics inhibit src activity independently of VGSC blockade (225). As well as inhibiting VGSCs, valproic acid acts as a histone deacetylase inhibitor, and this action is commonly considered responsible for its anti-cancer properties (226). VGSCs are also inhibited by omega 3 fatty acids, which may explain some of the beneficial effects shown by these molecules in the diet of cancer patients (227, 228). A low Na^+ diet, which reduces the risk of hypertension, has been hypothesised to be beneficial in preventing cancer (229). While speculative, this is supported by data from a meta-analysis of prospective studies examining habitual salt intake (230). However, a low Na^+ diet may be difficult to achieve as a therapeutic intervention in the clinic as factors such as patient nutrition and blood pressure are already very difficult to control in patients with advanced disease. Furthermore, a complicating factor when considering VGSC inhibition as a therapeutic strategy is that these channels may also play a role in regulating immune cell function (231).

The reverse approach has also been considered: given that apoptosis is initiated by a large influx of Na^+ into cells (232), attempts have been made to replicate this mechanism to kill cancer cells. Viral vector delivery of a constitutively open ASIC channel into culture glioma cells caused Na^+ entry and cell death (233). However, viral delivery of Na^+ channels would need to be precisely targeted to neoplastic cells in order for this method to be of use. In a different study, targeted osmotic lysis of VGSC-expressing tumours in mice was demonstrated by systemic administration of the Na^+/K^+ ATPase-inhibiting cardiac glycoside ouabain in conjunction with electrical pulses to open VGSCs, leading to cytotoxic influx of Na^+ into tumour cells (234). Blocking the Na^+ efflux activity of Na^+/K^+ ATPase with cardiac glycosides has the added advantage of decreasing inflammation and increasing specific anti-tumour immunity (235). A retrospective study showed improved survival in cancer patients that were being prescribed cardiac glycosides despite these patients having cardiac conditions (236). This has led to the development of several clinical trials examining the effect of adding the cardiac glycosides to various chemotherapy protocols (Table 2).

Conclusion

Na^+ homeostasis is disrupted in cancer, leading to accumulation of Na^+ in solid tumours. At the cellular level, Na^+ transport is linked to pH and Ca^{2+} regulation and it alters plasma membrane potential, metabolism and proliferation. At the tissue level, high $[Na^+]_i$ aids proliferation, migration and invasion of cancer cells and high $[Na^+]_e$ induces an inflammatory microenvironment which promotes tumour progression. Systemic changes in $[Na^+]$ affect blood pressure and immune function, together with secretion of pro-angiogenic mediators. Given that Na^+ is the predominant extracellular cation and its distribution can be affected by diet and many drugs in common use, it is imperative that we further improve understanding of how Na^+ regulation affects cancer progression. There is plenty of evidence that by doing so, we will uncover new modes of cancer detection and monitoring, e.g. through use of ^{23}Na-MRI, and may also improve cancer treatment via pharmacological and dietary modulation of Na^+ homeostasis.

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Conflicts of interest statement

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Table 1. Na^+ transport mechanisms with altered expression in cancer.

Transporter	Subtype	Cancer	Change	Effects	References
Na^+/K^+ ATPase	α3 subunit	Liver, gastric	↑	↑ proliferation ↑ migration ↑ invasion ↓ apoptosis	(206, 237)
ENaC	αENaC, γENaC	Glioblastoma, HCC, melanoma, placenta	↑	↑ migration ↑ proliferation	(121, 137, 238, 239)
ASIC	ASIC1, ASIC1a, ASIC2, ASIC2a, ASIC3	Liver, glioblastoma, PDAC, colorectal, adenoid	↑	↑ invasion ↑ migration ↑ EMT	(56, 165, 167, 238, 240)
VGSC	$Na_v1.2$, $Na_v1.4$, $Na_v1.5$, $Na_v1.6$, $Na_v1.7$	NSCLC, prostate, cervical, colorectal, breast, ovarian	↑	↑ invasion ↑ migration	(26, 140, 144, 160, 161, 241-243)
NKCC	NKCC1	HCC, glioblastoma, NSCLC	↑	↑ invasion ↑ migration ↑ proliferation	(47, 244, 245)
NMDA-R	NMDAR2B	Glioblastoma NSCLC ESCC gastric colorectal breast	↓/↑	↓ proliferation/ ↑ proliferation	(246)
Na^+/H^+ Exchanger	NHE1	Glioma, HNSCC, breast, HCC, cervical	↑	↑ acid extrusion ↑ invasion	(247-250)
$Na^+/HCO3^-$ transporter	NBCn1	Breast	↑	↑ acid extrusion	(42, 251, 252)
Na^+/glucose cotransporter	SGLT2	Breast	↑	↑ glucose uptake?	(46)
Amino acid transporters	SLC1a5/ASCT2	NSCLC, glioblastoma, eye, kidney, liver, lymph node, breast, muscle, placenta, skin, gastric, colorectal	↑	↑ amino acid/glutamine metabolism	(253-257)
	SLC6A14	Cervical, colon, PDAC, breast	↑	↑ amino acid uptake?	(114, 258, 259)
	SLC38a3/SNAT3	Glioma	↑	↑ amino acid uptake?	(260)
	SLC38a1/SNAT1	HCC, bile duct	↑	↑ growth ↑ survival	(261, 262)
NCX		Kidney	↓	↓ EMT	(263)

Abbreviations: ENaC, epithelial Na^+ channel; VGSC, voltage-gated Na^+ channel; NKCC, $Na^+/K^+/2Cl^-$ co-transporter; NMDA-R, N-methyl D-aspartate receptor; NCX, sodium calcium exchanger; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; ESCC, oesophageal squamous cell carcinoma; NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma, EMT, epithelial-mesenchymal transition.

Table 2. Na^+-regulating mechanisms as potential therapeutic targets[1].

Transporter	Compound	Category	Cancer types	Highest phase	Clinical trial NCT numbers and references
ENaC	Amiloride	ENaC inhibitor	Solid tumours	Preclinical	(264)
NHE1	Cariporide	NHE1 inhibitor	NA	Phase III (stopped)	(265)
VGSC	Lidocaine	VGSC blocker, local anaesthetic	Breast, colorectal	Phase III	NCT01916317, NCT02786329, NCT01841294, NCT02839668, NCT02647385, (266)
	Ropivacaine	VGSC blocker, local anaesthetic	Abdominal/thoracic	NA	NCT03134430
	Bupivacaine	VGSC blocker, local anaesthetic	Breast, colon	Phase III	NCT00938171, (267)
	Valproate	VGSC blocker, HDAC inhibitor, antiepileptic	HNSCC, cervical, melanoma, mesothelioma, bladder, thyroid, NSCLC	Phase III	NCT01695122, NCT01738815, (222, 268-270)
	Phenytoin	VGSC blocker, antiepileptic	Breast	Preclinical	(213)
	Ranolazine	VGSC blocker, antianginal	Breast	Preclinical	(162)
Na^+/K^+ ATPase	Digoxin	Cardiac glycoside, Na^+/K^+ ATPase inhibitor	Prostate, breast, melanoma, acute myeloid leukaemia/ myelodyspastic syndromes, HNSCC	Phase II	NCT02138292, NCT03113071, NCT02906800, NCT01763931, NCT01887288, (221, 271)

	Ouabain	Cardiac glycoside, Na^+/K^+ ATPase inhibitor	Breast	Preclinical	(234)
NKCC	Bumetanide	NKCC1 inhibitor	HCC	Preclinical	(244)
NMDA-R	Memantine & MK-801	NMDA-R blocker	Breast	Preclinical	(272)
NCX	KB-R7943	NCX reverse mode inhibitor	Prostate	Preclinical	(273)
SGLT2	Empagliflozin and others	SGLT2 inhibitors	Urinary tract	Observational	NCT03464045
N/A	$NaHCO_3$	Neutral pH buffer	Any	Phase I	NCT02531919

[1]ENaC, epithelial Na^+ channel; VGSC, voltage-gated Na^+ channel; NKCC, $Na^+/K^+/2Cl^-$ co-transporter; NMDA-R, N-methyl D-aspartate receptor; NCX, sodium calcium exchanger; HDAC, histone deacetylase; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; NSCLC, non-small cell lung cancer; EMT, epithelial-mesenchymal transition.

Box 1: Development of ^{23}Na MRI

^{23}Na MRI is non-invasive and presents a unique mechanism for measuring Na^+ in tissue. ^{23}Na MRI does not disturb the tissue state, unlike Na^+ measurements that require physical tissue sampling. Na^+ is endogenous to human tissue, which enables imaging without external contrast. ^{23}Na MRI can differentiate intracellular Na^+ from total tissue Na^+. The non-invasive quantification of cancer Na^+ content with MRI has the potential to provide a large amount of information on tissue microstructure and function that could improve our understanding of the changes occurring in this disease both early on in formation and in response to therapy. ^{23}Na MRI is currently primarily used for research due to the non-standard hardware necessary to enable Na^+ signal acquisition. The signal from ^{23}Na MRI is much lower than the signal in conventionally used H^+ MRI for several reasons: Na^+ has a lower abundance in the human body compared to H^+, which proportionally reduces the available magnetization and therefore signal-to-noise; the gyromagnetic ratio of Na^+ (11.26 MHz/Tesla) is 4 times smaller than that of H^+, which results in 25 % fewer ^{23}Na spins being magnetized; and Na^+ has a spin of 3/2, which causes electrostatic field sensitivity and fast T2* signal decay. Thus, the total Na^+ signal available on MRI in human tissue is only about $1/22{,}000^{th}$ the size of the H^+ signal (274). High static magnetic fields are commonly used with Na^+ imaging to improve the signal to noise ratio. Moving to higher MRI field strengths, such as 7 Tesla, further increases the signal-to-noise, enabling higher spatial resolutions in faster acquisition times, which improves the probing of the tumour Na^+ microenvironment (193).

Interest in performing ^{23}Na MRI dates back as far as the 1980s. It was postulated that ^{23}Na MRI would allow for superior contrast in distinguishing features of brain tumours such as oedema, necrosis and non-necrotic tumour, compared to conventional proton spin density imaging (275). Initial studies focused on healthy volunteers and animal models of stroke and myocardial infarction (275, 276), followed by the first images of brain tumour patients (277).

A set of publications followed (278-280) after which the focus turned to spectroscopic and animal model studies and small investigative human studies (190, 281). It was shown that ^{23}Na MRI reliably revealed brain tumour lesions, albeit no correlation to histology or grading (190). Imaging developments in pulse sequence design and quantification methods were subsequently made to overcome shortcomings of previous studies, namely to increase spatial resolution and to capture the Na^+ signal in its entirety by using ultra-short echo time imaging (282-284). Further detailed ^{23}Na MRI studies followed in human brain and breast tumours (4, 9, 285). More recent advances in sodium imaging have shown that ^{23}Na MRI can both predict IDH status (197) and show early response to pre-clinical therapeutic interventions (198). Furthermore, relaxation-weighted ^{23}Na MRI has now been shown to differentiate between brain tumours of grades I-III and grade IV when spin-weighted ^{23}Na MRI was unable to do so (286).

Figure legends

Figure 1. Accumulation of Na^+ in tumours. Converse to the reported decrease in pO_2 and pH, many tumours (such as breast cancer) exhibit elevated [Na^+] (9). This elevated tumour [Na^+] may be due to increases in the extracellular volume fraction relative to the intracellular volume fraction, or due to increases in the [Na^+] concentration within either compartment. Moreover, tumour [Na^+] is likely influenced by the heterogeneity of the tumour microenvironment. Factors that could increase the extracellular volume fraction (interstitial volume) include increases in colloidal and interstitial pressure (13) due to vasculature permeabilisation, blood plasma protein release and the formation of fibrin (11, 12), and cancer cell death as a result of targeted chemotherapy or poor vascularisation within the necrotic tumour core. Moreover, Na^+ binding to protein sequestered within the desmoplastic tumour microenvironment (e.g. glycosaminoglycans) could contribute to an increase in the extracellular Na^+ content (287). Alternatively, the increased cellularity observed within poorly vascularised tumours (193) suggests that increases in intracellular volume fraction can contribute to elevated tumour [Na^+]; indeed, $[Na^+]_i$ has been reported to be elevated in cultured cancer cells (20, 22, 23), potentially due to altered transporter expression (Table 1).

Figure 2. Cellular Na^+ import and export mechanisms. Cells exhibit a diverse repertoire of Na^+ channels and transporters, many of which exhibit altered expression in cancer (Table 1) and are being explored as potential therapeutic targets (Table 2). The activity and conductance of these channels is regulated by $[Na^+]_i$, $[Na^+]_e$, membrane potential and auxiliary regulatory proteins. Channels that facilitate Na^+ influx include voltage gated Na^+ channels (VGSC), epithelial Na^+ channels (ENaC), acid-sensing channels (ASIC), glutamate-activated N-methyl-D-aspartate receptors (NMDA), ATP-activated P2X purinoceptor 7 ($P2X_7$) and the G protein-coupled Na^+ leak channel, non-selective (NALCN). The inward Na^+ gradient and a hyperpolarised membrane potential are maintained by the ATP-driven Na^+/K^+ ATPase. Na^+ influx is also linked to the transport of numerous other ions

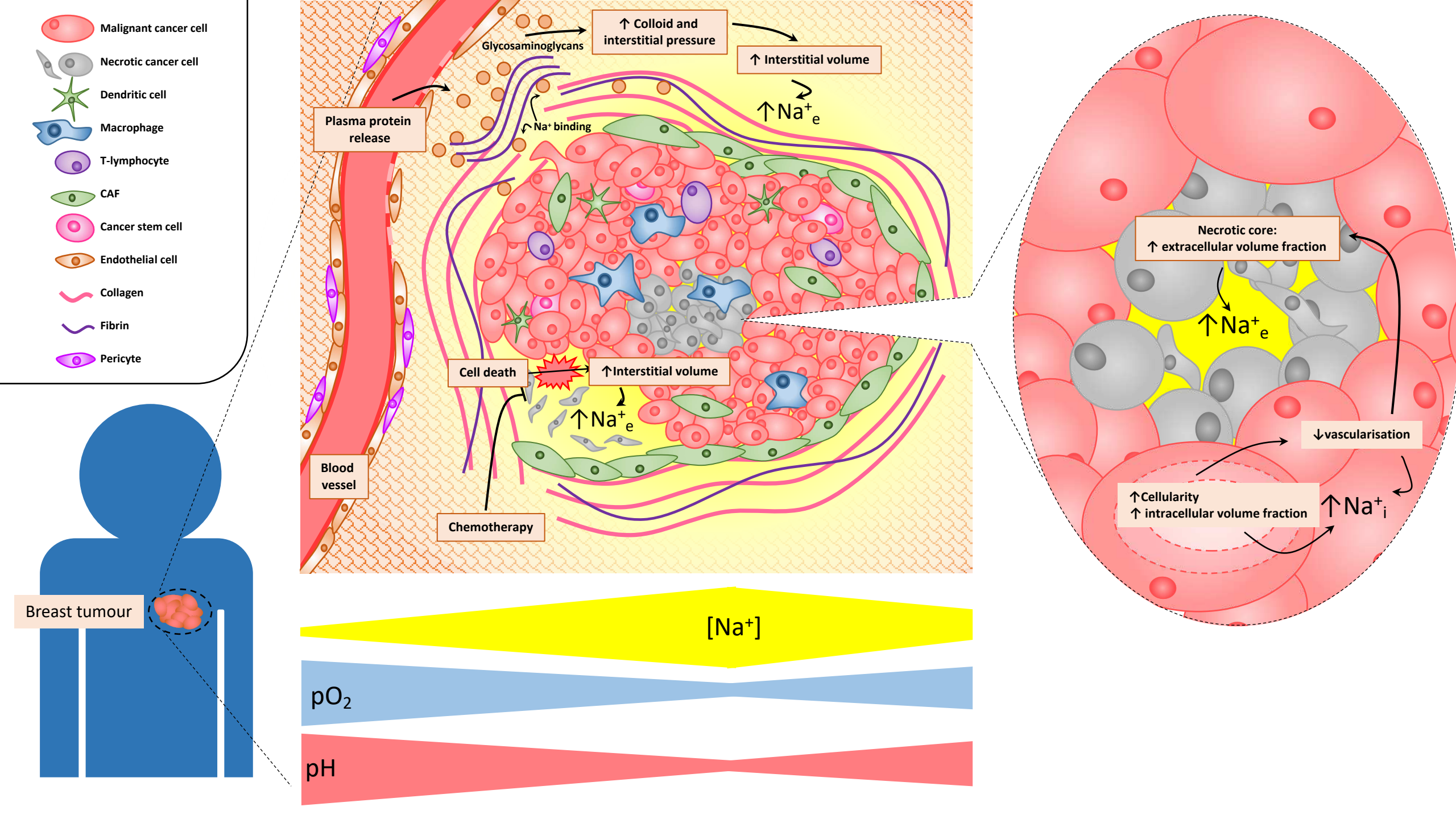
and substrates, namely H^+ efflux (Na^+/H^+ exchanger 1, NHE1), Cl^- and K^+ influx (Na^+-K^+-Cl^- cotransporter, NKCC), cytosolic and mitochondrial Ca^{2+} efflux (Na^+/Ca^{2+} exchanger, NCX and mitochondrial Na^+-Ca^{2+}(Li^+) exchanger, NCLX, respectively) glucose uptake (sodium-glucose linked transporter, SGLT) and amino acid (AA) uptake. Na^+/H^+ exchangers (NHE) are also present on both mitochondria and lysosomes, the latter of which achieve Na^+ efflux into the cytosol via two-pore channels (TPC) and transient receptor potential mucolipin (TRPML) channels.

Figure 3. Physiological consequences of Na^+ accumulation within tumours. Dashed arrows indicate putative mechanisms which remain to be fully characterised. Red arrows indicate denote movement/conversion of metabolites. *A: Elevated [Na^+] is linked to alterations in tumour metabolism and pH regulation.* Elevations in $[Na^+]_i$ activate the Na^+/K^+ ATPase, thereby raising ATP demand and driving a high glycolytic rate (34). To maintain a high pH_i, the resulting H^+ is rapidly extruded by NHE, which is driven by the inward Na^+ gradient. Increased $[Na^+]_i$ could also facilitate depletion of mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) via NCLX, thereby altering mitochondrial metabolism. Conversely, changes to the Na^+ gradient across the plasma membrane will likely alter the driving force for transporters importing key metabolic substrates such as glutamine (SLC1A5) and glucose (SGLT), thereby influencing anabolic and anapleurotic processes. *B: Elevated tumour Na^+ and membrane potential (V_m).* V_m is generated by the electrogenic Na^+/K^+ ATPase; Na^+ influx via VGSC/ENaC/ASICs results in a depolarised membrane potential (V_m) in cancer cells. A depolarised V_m can lead to the activation of proliferative signalling cascades (such as KRas), cytoskeletal reorganisation facilitating migration, and accelerates the cell cycle. Conversely, most healthy differentiated cells exhibit a more hyperpolarised V_m that tightly controls cell cycle progression. *C: Cell volume regulation by Na^+-linked transport mechanisms.* Elevations in tumour [Na^+] are linked to changes in cell volume regulation. NKCC sequentially facilitates the accumulation of intracellular Cl^-, H_2O uptake (aquaporins, AQP, and osmosis) and cell swelling (288). Conversely, K^+-Cl^- cotransporters (KCC) mediate Cl^- efflux, promoting H_2O

exit from the cell (289). NHE1 activity results in a net osmotic gain due to Na^+ influx; the osmolar contribution of intracellular H^+ ions is compensated due to the dissociation of intracellular buffers. The resulting increase in pH_i and $[HCO_3^-]_i$ drives Cl^- import via anion exchangers (AE), leading to H_2O uptake and cell swelling (290). NHE1 can also operate to in reverse mode to resist compressive forces (118).

Figure 4. Effect of elevated Na^+ on cancer progression and the tumour microenvironment. Dashed arrows indicate putative mechanisms which remain to be fully characterised**.** *A: Elevated tumour Na^+ and migration/invasion.* VGSCs have been correlated with activation of a proinvasive gene transcription network upregulating Wnt, MAPK and Ca^{2+} signalling (161, 164). NHE1 localises to the leading edge of invading cells (136); VGSC colocalisation with NHE1 within cavaeolae leads to activation of NHE1, acidification of the extracellular environment and digestion of the extracellular matrix by cathepsins and matrix metalloproteinases (120, 142, 152). Interestingly, the β subunit of VGSCs acts as an adhesion molecule that interacts with the extracellular matrix to regulate migration and invasion (291). Extracellular acidification can also activate ASIC and ENaC channels (58, 59), leading to further increases in $[Na^+]_i$. Na^+-linked Ca^{2+} influx via reverse-mode NCX action has been linked to cancer cell motility via Ca^{2+} signalling-activated TGF-β signalling (151). NKCC regulates cell swelling required for migratory behaviour by facilitating $[Cl^-]_i$ accumulation and H_2O uptake via osmosis and aquaporins (288). NKCC also acts as a scaffold for cofilin within invadopodia, which facilitates cytoskeletal remodelling (292). *B: Elevated $[Na^+]_e$ and cancer cell proliferation.* The inward Na^+ gradient drives the uptake of anabolic substrates such as glucose and glutamine (SGLT and SLC1A5), respectively (44-46); altered tumour $[Na^+]$ might regulate the uptake of these substrates. Via glycolysis/pentose phosphate pathway (PPP) and glutaminolysis, glucose and glutamine are utilised as substrates for redox homeostasis, biosynthesis, and cell proliferation (i.e. reducing equivalents, nucleotides, and fatty acids) (293). SLC1A5 also regulates mTORC1, a key regulator of protein translation and cell growth (254). Moreover, salt inducible kinase 3

is activated by elevated $[Na^+]_e$, promoting G1/S phase transition (129), and $[Cl^-]$ accumulation due to upregulated NKCC activity can promote cell cycle progression (48, 117, 130). Elevated $[Na^+]_e$ leads to DNA breaks with significant implications for oncogenic mutations/tumour suppressor silencing (132), and a high osmolality and VGSC activity also activates the MAPK signalling cascade, potentially via Rac1 activation (127, 128). *C: Elevated Na^+ and osmolality drives inflammation in the tumour microenvironment.* Increased $[Na^+]_e$ and osmolality promote proliferative and antiapoptotic signalling in tumour associated macrophages (171) and by increasing the production of proinflammatory cytokines by local endothelial cells and Th-17 helper cells (177). Together these factors also promote the further recruitment of proinflammatory immune cells (171). These factors lead to extracellular matrix breakdown, neovascularisation and tumour remodelling, thereby promoting tumour progression and metastasis (129, 179, 187, 294).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Endothelial cell
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Plasma protein release
Na+ binding
Cell death
↑Interstitial volume
↑Na+e
Blood vessel
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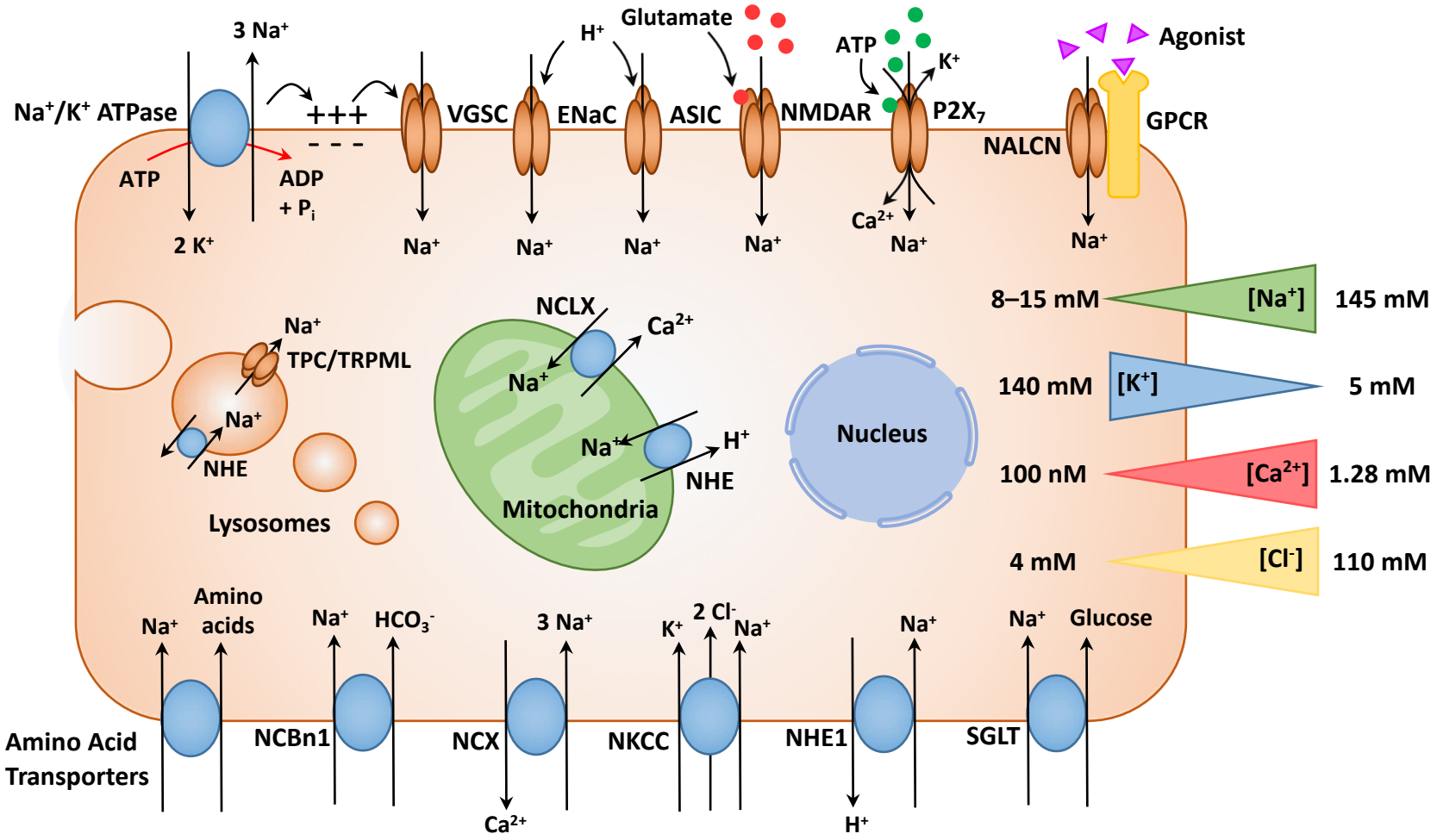
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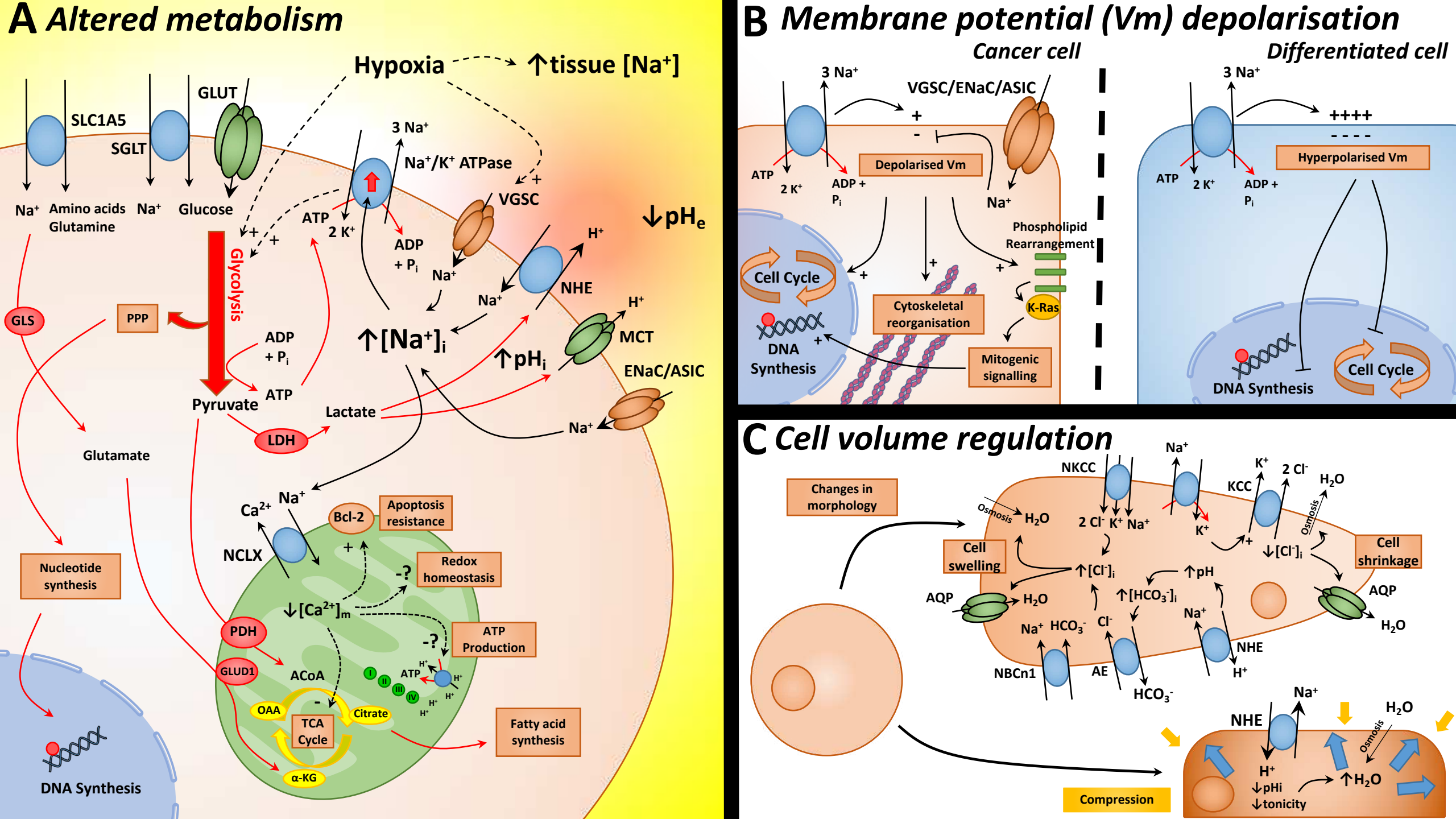
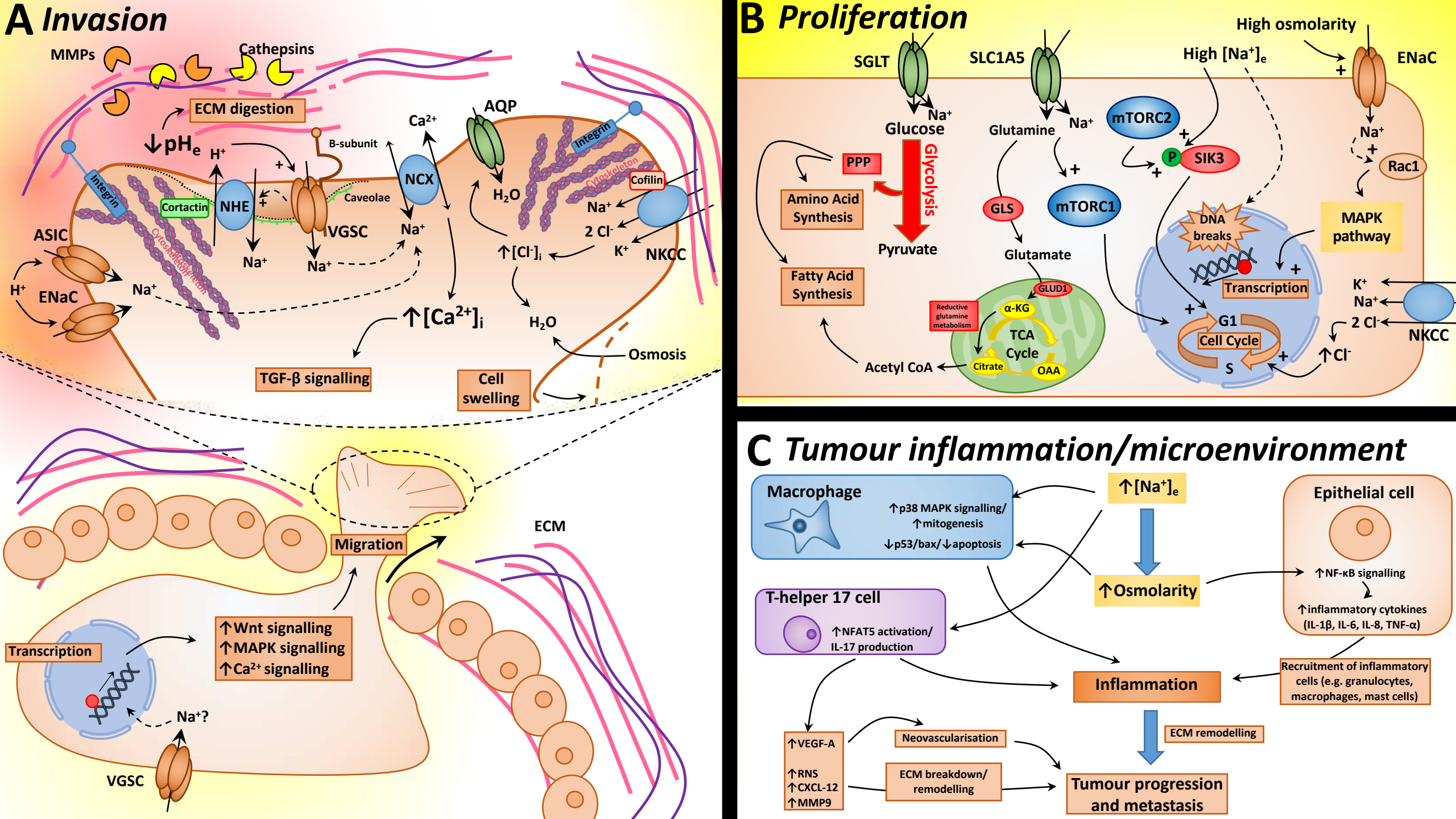
110 mM
Na+
TPC/TRPML
Na+
NHE
Lysosomes
NCLX
Ca2+
Na+
Na+
H+
NHE
Mitochondria
Nucleus
Na+
Amino acids
Amino Acid Transporters
Na+
HCO3-
NCBn1
3 Na+
Ca2+
NCX
K+
2 Cl-
Na+
NKCC
Na+
H+
NHE1
Na+
Glucose
SGLT


A Altered metabolism
Hypoxia
↑tissue [Na+]
SLC1A5
SGLT
GLUT
Na+
Amino acids
Glutamine
Na+
Glucose
3 Na+
Na+/K+ ATPase
ATP
2 K+
ADP
+ Pi
VGSC
↓pHe
H+
NHE
Na+
Glycolysis
GLS
PPP
ADP
+ Pi
ATP
↑[Na+]i
↑pHi
MCT
H+
ENaC/ASIC
Na+
Pyruvate
Lactate
LDH
Glutamate
Nucleotide synthesis
Ca2+
Na+
NCLX
Bcl-2
Apoptosis resistance
Redox homeostasis
↓[Ca2+]m
PDH
GLUD1
ACoA
OAA
Citrate
TCA Cycle
α-KG
ATP Production
Fatty acid synthesis
DNA Synthesis
B Membrane potential (Vm) depolarisation
Cancer cell
Differentiated cell
3 Na+
VGSC/ENaC/ASIC
ATP
2 K+
ADP + Pi
Depolarised Vm
Na+
Phospholipid Rearrangement
Cell Cycle
Cytoskeletal reorganisation
K-Ras
DNA Synthesis
Mitogenic signalling
3 Na+
++++
- - - -
Hyperpolarised Vm
ATP
2 K+
ADP + Pi
DNA Synthesis
Cell Cycle
C Cell volume regulation
Changes in morphology
Osmosis
H2O
Cell swelling
↑[Cl-]i
AQP
H2O
NKCC
2 Cl- K+ Na+
Na+
K+
KCC
K+
2 Cl-
H2O
Osmosis
↓[Cl-]i
Cell shrinkage
AQP
H2O
↑pH
↑[HCO3-]i
Na+ HCO3-
Cl-
Na+
NHE
H+
NBCn1
AE
HCO3-
Compression
NHE
Na+
H+
↓pHi
↓tonicity
H2O
Osmosis
↑H2O


A Invasion
MMPs
Cathepsins
ECM digestion
↓pHe
H+
B-subunit
Ca2+
AQP
Integrin
Cortactin
NHE
Caveolae
NCX
H2O
Cytoskeleton
Cofilin
Na+
2 Cl-
K+
NKCC
VGSC
ASIC
ENaC
↑[Cl-]i
↑[Ca2+]i
Osmosis
TGF-β signalling
Cell swelling
B Proliferation
SGLT
SLC1A5
High [Na+]e
High osmolarity
ENaC
Glucose
Glutamine
mTORC2
SIK3
Rac1
PPP
Glycolysis
Amino Acid Synthesis
Pyruvate
GLS
mTORC1
DNA breaks
MAPK pathway
Glutamate
Transcription
Fatty Acid Synthesis
GLUD1
Reductive glutamine metabolism
α-KG
TCA Cycle
Citrate
OAA
Acetyl CoA
G1
Cell Cycle
S
↑Cl-
C Tumour inflammation/microenvironment
Macrophage
↑p38 MAPK signalling/ ↑mitogenesis
↓p53/bax/↓apoptosis
↑[Na+]e
Epithelial cell
↑NF-κB signalling
↑inflammatory cytokines (IL-1β, IL-6, IL-8, TNF-α)
↑Osmolarity
T-helper 17 cell
↑NFAT5 activation/ IL-17 production
Recruitment of inflammatory cells (e.g. granulocytes, macrophages, mast cells)
Inflammation
ECM remodelling
↑VEGF-A
↑RNS
↑CXCL-12
↑MMP9
Neovascularisation
ECM breakdown/ remodelling
Tumour progression and metastasis
Migration
ECM
↑Wnt signalling
↑MAPK signalling
↑Ca2+ signalling
Transcription
Na+?
VGSC