Protons and Ca^{2+}: Ionic Allies in Tumor Progression?

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Ion channels and G-protein-coupled receptors (GPCRs) play a fundamental role in cancer progression by influencing Ca^{2+} influx and signaling pathways in transformed cells. Transformed cells thrive in a hostile environment that is characterized by extracellular acidosis that promotes the pathological phenotype. The pathway(s) by which extracellular protons achieve this remain unclear. Here, a role for proton-sensing ion channels and GPCRs as mediators of the effects of extracellular protons in cancer cells is discussed.

Cancer is a group of diseases that affects one in three people at some point of their life. Despite there being over 200 different types of cancer, there are certain hallmarks that are common to most cancers: self-sufficiency in growth signals, insensitivity to anti-growth signals, ability to evade apoptosis and anoikis, limitless replicative potential, altered metabolism (of which the high glucose consumption of cancer cells due to the glycolytic breakdown of glucose is a classic characteristic), sustained angiogenesis, and tissue invasion and metastasis (27, 50, 57).

One important consequence of tumor growth and altered metabolism of cancer cells is the generation of a microenvironment that differs quite substantially from the microenvironment of non-transformed cells. Tumor tissue is characterized by disorganized vasculature that includes shunts and in which blood flow is heterogeneous and may even reverse direction, resulting in transient and chronic hypoxic regions within the tumor (16, 41, 42, 51). Furthermore, the interstitial fluid of solid cancers is characterized by acidosis; in fact, interstitial pH values as low as pH 5.8 have been measured (158) although the majority of tumors are less acidic (around pH 6.5–7.0). It was originally thought that acidification of the interstitial tumor fluid was a consequence of hypoxia within the tumor tissue and resulted from glycolytic breakdown of glucose to lactate, which was then extruded from the cells, thereby acidifying the extracellular fluid. Two lines of evidence suggest that this may not be the whole story: *1*) Glycolytic breakdown of glucose to lactate also takes place under aerobic conditions, i.e., extracellular acidification does not depend on hypoxic conditions (44); and *2*) cancer cells that are not glycolytically active still acidify the extracellular milieu (59, 113, 182), suggesting that lactate extrusion is not essential for interstitial acidosis. Cancer cells, in addition to monocarboxylate transporters responsible for lactate extrusion, have highly active sodium-proton exchangers as well as bicarbonate transporters and V-type ATPases that extrude protons from cancer cells, thereby keeping the intracellular pH at physiological levels. In some tumors, there is also conversion of extracellular CO$_2$ to carbonic acid via activity of membrane-bound carbonic anhydrase 9, and this contributes to the acidification of the interstitial fluid (20, 36, 69, 155). Hence, there are a number of distinct mechanisms by which tumor cells can acidify the extracellular fluid, and a role of these transporter systems in cancer and its therapy has been extensively reviewed (e.g., Refs. 20, 36, 69).

The fact that cancer cells thrive in an acidic environment is counterintuitive since homeostasis of pH is thought to be paramount to the normal functioning of cells and tissues. Proton concentrations impact on protein structure by affecting the degree of ionization of the protein, and this may have consequences for the functional properties of that protein (be it an enzyme, receptor, channel, transporter, structural, or other protein). It is thought that cancer cells have adapted to their hypoxic and acidic environment, thereby having a selection advantage over non-transformed cells that die on prolonged exposure to extracellular acidosis and hypoxia and that this is how hypoxia and acidosis promote cancer progression and metastasis (36, 44, 149). In agreement with this, conditioning melanoma cells to an acidic environment resulted in the generation of highly invasive tumor cells with altered gene expression (108), whereas increasing tumor pH was shown to decrease spontaneous metastases in a mouse model of metastatic breast cancer (129). However, the mechanism(s) by which the acidic pH promotes tumor progression remain unclear. This review will address the hypothesis that extracellular protons contribute to cancer progression through activation of proton-sensing cell surface receptors and subsequent modulation of

intracellular Ca^{2+} signaling pathways. The focus on proton-sensing ion channels and receptors is unique in the literature and aims to draw attention to these proteins as novel targets for cancer treatment.

Intracellular Ca^{2+} Signaling and Cancer

Various cancer types differ significantly in terms of morphology, cell of origin, physiology, and pharmacology, but one thing common to all cancer cells is the requirement for intracellular Ca^{2+} signaling to maintain a proliferating phenotype (70). How changes in intracellular Ca^{2+} concentration contribute to cancer cell proliferation and tumor progression has been reviewed in a number of recent articles (70, 110, 112, 130).

In resting cells, the basal intracellular Ca^{2+} concentration is very low (between 10 and 100 nM), resulting in a steep Ca^{2+} gradient across the membrane that favors Ca^{2+} entry over Ca^{2+} extrusion even at positive membrane potentials. This cytosolic Ca^{2+} concentration is tightly regulated because increases in intracellular Ca^{2+} concentration can set about a whole host of distinct processes within cells, including cell cycle progression and proliferation (78). Cytoplasmic Ca^{2+} increases can be generated by two pathways: *1*) Ca^{2+} influx through Ca^{2+}-permeable ion channels in the plasma membrane and *2*) Ca^{2+} release from intracellular stores through Ca^{2+}-permeable ion channels in the store membrane.

Ion channels in the plasma membrane can be opened by changes in membrane potential, following ligand binding, receptor activation, and Ca^{2+} store depletion or in response to a mechanical stimulus. Just how much Ca^{2+} enters the cell through any given ion channel depends on the membrane potential of that cell, which not only controls the opening of voltage-gated ion channels but also the driving force for Ca^{2+} to enter the cell, with Ca^{2+} influx being greater at negative potentials than at more positive potentials. Ion channels on the store membrane (also called Ca^{2+} release channels) are usually activated only upon ligand binding; the intracellular ligand is Ca^{2+} [triggering so-called Ca^{2+}-induced Ca^{2+} release (CICR)] and/or inositol-1,4,5-trisphosphate (IP$_3$). IP$_3$ is generated in cells subsequent to activation of plasma membrane receptors [G-protein-coupled receptors or tyrosine kinase receptors activating phospholipase Cβ or γ, respectively, which catalyses the conversion of the membrane phospholipid phosphoinositolbisphosphate (PIP$_2$) into IP$_3$ and diacylglycerol (DAG)]. Ca^{2+} release from intracellular stores is largely unaffected by the membrane potential but will depend on the Ca^{2+} store content, which in turn is determined by the relative activity of the Ca^{2+} leak pathway from the Ca^{2+} store and Ca^{2+} store refilling via sarcoplasmic endoplasmic reticulum Ca^{2+} ATPase (SERCA) pumps, as well as the activity of Ca^{2+} release channels on the store membrane. Termination of the Ca^{2+} signal is achieved by clearance of Ca^{2+} from the cytosol via transporters either on the plasma or store membrane, and their activity therefore contributes to the shape and duration of the intracellular Ca^{2+} signal (summary of Ca^{2+} influx and clearance pathways in FIGURE 1A). Mitochondria, lysosomes, endosomes, large dense-core vesicles, the Golgi apparatus, and the nuclear envelope have all been shown to act as Ca^{2+} stores (23, 85, 106, 122), although in the case of the endosomes this may only be a transient property (122). Of these additional Ca^{2+} stores, mitochondria and lysosomes are the best-understood, and, for mitochondria, roles in cancer have been well established (53). It appears that silencing of mitochondria signaling is important for tumor cell survival (53) and that Ca^{2+} release from mitochondria induces cell death and hence counteracts cancer progression (21, 131). Similarly, lysosomes are important in mediating autophagy, which is thought to function as a tumor suppressor mechanism (55). Since neither pathway promotes cancer progression in a Ca^{2+}-dependent manner, mitochondria and lysosomes (or any of the other additional Ca^{2+} stores mentioned above, for which roles in cancer have not been determined) have not been included in the schematic.

There are numerous publications demonstrating differential expression of certain types of ion channels on the plasma and ER Ca^{2+} store membrane in cancerous tissue (increased or decreased expression in cancerous compared with healthy tissue/cells); there is also ample evidence that interfering with channel expression can impact on cancer cell proliferation in vitro as well as in vivo (13, 32, 68, 80, 109, 140). Similarly, a number of G-protein-coupled receptors (GPCRs) linking to intracellular Ca^{2+} signaling, such as P2Y purinoreceptors (P2YRs) (39, 120), calcium-sensing receptors (CaRs) (128, 134), lysophosphatidic acid receptors (LPARs) (22, 91, 127), chemokine receptors (CXCRs) (43, 159), and metabotropic glutamate receptors (mGluRs) (100, 142), have been implicated in tumor progression (24, 88, 90). Equally, Ca^{2+} release channels on Ca^{2+} store membranes and intracellular Ca^{2+} store dynamics have been recognized as important targets in cancer treatment in terms of their ability to promote proliferation, apoptosis, and angiogenesis (10, 93, 95, 174). However, it is unclear how the efficiency of these pathways is affected by the

local microenvironment in which cancer cells have to operate. A number of ion channels, for which there is compelling evidence that they are involved in cancer progression, are in fact inhibited by extracellular protons (Table 1; this table is by no means exhaustive). It is therefore difficult to reconcile how ion conducting activity of these membrane proteins can be important for cancer progression when they are inhibited by extracellular protons that accumulate during cancer progression and promote the disease (but see outlook). The focus of this review will therefore be on a group of ion channels and membrane receptors that are activated or potentiated by extracelluar protons and that should therefore constitute prime candidates for mediating proton-dependent Ca^{2+} signaling involved in tumor progression (Table 2).
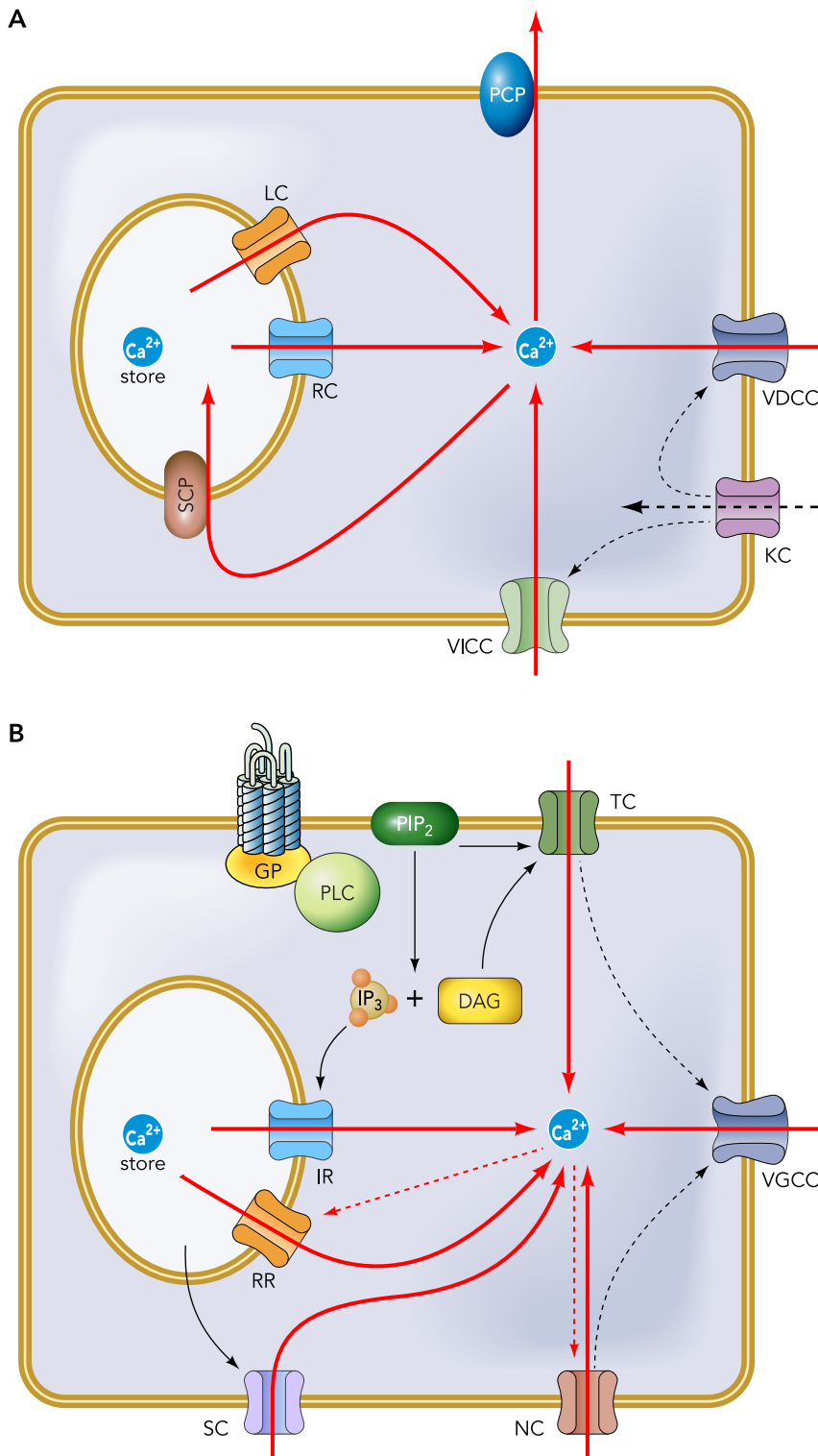

FIGURE 1. Ca^{2+} signaling pathways for changing cytoplasmic Ca^{2+} concentrations
A: Ca^{2+} entry and extrusion pathways. Ca^{2+} can enter cells through voltage-dependent and voltage-independent Ca^{2+}-permeable channels (VDCCs and VICCs, respectively). Activation of hyperpolarizing channels [generally K^+ channels (KCs)] influences voltage-dependent and -independent Ca^{2+} influx differentially (gray dotted lines): voltage-dependent Ca^{2+} influx is inhibited (closure of voltage-dependent Ca^{2+} channels due to hyperpolarization of membrane potential), whereas voltage-independent Ca^{2+} influx is enhanced (hyperpolarization increases driving force for Ca^{2+}). The same scenario applies when Cl^--permeable channels open (provided opening of Cl^--permeable channels triggers Cl^- influx), not depicted here. Cytoplasmic Ca^{2+} concentrations can also be increased by releasing Ca^{2+} from intracellular Ca^{2+} stores via release channels (RCs); these could be either IP_3 receptors or CICR channels (see text). Furthermore, Ca^{2+} leaves Ca^{2+} stores through leak channels (LCs) in the store membrane, and modulation of these channels also impacts on cytoplasmic Ca^{2+} concentrations. Ca^{2+} extrusion from the cytoplasm is achieved through activity of Ca^{2+} pumps/exchangers both on the plasma membrane [plasma membrane Ca^{2+} pump (PCP)] and store membrane [store membrane Ca^{2+} pump (SCP)], and activity of these pumps/exchangers also influences cytoplasmic Ca^{2+} concentrations.
B: phospholipase C (PLC)-mediated Ca^{2+} signaling. Activation of phospholipase C [following stimulation of a G-protein-coupled receptor (GPCR) and subsequent activation of a G protein (GP)] triggers conversion of phosphoinositolbisphosphate (PIP_2) into inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol (DAG). PIP_2 is thought to constitutively inhibit some ion channels, including members of the canonical transient receptor (TRPC) channel family (TC); breakdown of PIP_2 will hence lead to opening of these channels. DAG has also been shown to directly open TRPC channels, which are nonselective cation channels that can trigger changes in cytoplasmic Ca^{2+} concentration directly and by depolarizing the membrane potential, thereby leading to opening of voltage-gated Ca^{2+} channels (VGCCs; dotted black line). Phospholipase C-mediated IP_3 formation can also trigger Ca^{2+} release from Ca^{2+} stores through activation of IP_3 receptors (IR). This store depletion can in turn activate store-operated Ca^{2+} channels (SCs), triggering further Ca^{2+} influx. Finally, a rise in cytoplasmic Ca^{2+} can cause the opening of CICR channels [ryanodine receptors (RRs)] on the Ca^{2+} store membrane and/or open Ca^{2+}-dependent nonselective cation channels (dotted red lines) that can be Ca^{2+}-permeable and also increase cytoplasmic Ca^{2+} rises by depolarizing the membrane potential sufficiently for voltage-gated Ca^{2+} channels to open (dotted black line). A rise in intracellular Ca^{2+} concentration may also lead to the opening of Ca^{2+}-dependent, nonselective cation channels (dotted red line; NC) that may be Ca^{2+} permeable and/or trigger opening of VGCCs (dotted black line).

Table 1. Proton-inhibited ion channels implicated in cancer progression

Ion Channel	Cancer	Effect of Extracellular Acidosis
Store-operated Ca^{2+} channels	e.g., Prostate cancer (166), breast cancer (111, 183), leukemia (61)	83% inhibition at pH 6.89 in platelets (46); 80% inhibition at pH 6.4 in endothelia cells (6)
Eag1	e.g., Breast cancer, cervical cancer (Ref. 60 and references therein; Ref. 118)	Voltage-dependent inhibition; for pH 6.0, inhibition by 50% at +40 mV compared with pH 7.0 in *Xenopus* oocytes (160)
Kv1.5	Numerous different cancer tissues (11)	50% inhibition at pH 6.3 compared with pH 7.3 at +40 mV in *Xenopus* oocytes (148)
TRPC6	e.g., Glioma (33), esophageal cancer (138), gastric cancer (15), prostate cancer (161)	50% inhibition at pH 5.7 in HEK cells (138)
TRPV6	Prostate cancer (reviewed in Ref. 84)	35% inhibition at pH 6.5; 50% inhibition at pH 5.5 in *Xenopus* oocytes (121)
Cav3.1	e.g. Astrocytoma, neuroblastoma, renal cancer (reviewed in Ref. 117)	Reduced Ca^{2+} selectivity over monovalent cations (reviewed in Ref. 157)

Eag, ether-a-gogo K^+ channel; Kv, voltage-gated K^+ channel; TRPC, canonical transient receptor potential channel; TRPV, vanilloid transient receptor potential channel; Cav, voltage-gated Ca^{2+} channel.

Proton-Activated Receptors and Ion Channels in Cancer

Proton-Activated G-Protein-Coupled Receptors

A novel family of GPCRs activated upon binding of protons was recently identified, comprising ovarian cancer gene 1 (OGR1), G-protein-coupled receptor 4 (GPR4), and T-cell death-associated gene 8 (TDAG8) (94, 139, 175) (Table 3). Of these, only OGR1 has been shown to link to intracellular Ca^{2+} signaling via the phospholipase C-IP$_3$ pathway (139). These receptors are very interesting: they are active already at physiological pH values (pH 7.4) and do not desensitize. Hence, their activity mirrors extracellular proton concentrations directly and continuously. Maximal activation of OGR1 occurs at pH 6.8, and, depending on the expression system, these receptors either display a bell-shaped pH dependence [CCL39 cells (94) with still elevated IP$_3$ levels at pH 5.6] or saturating responses [for HEK293 cells determined up to pH 5.6 (94), for CHO cells determined up to pH 5.9 (175)], suggesting that the cellular environment influences how active these receptors are at pH values below 6.8. OGR1 was originally cloned from a human ovarian cancer cell line (180), but its role in cancer progression is unclear. For prostate cancer, OGR1 was reported to act as tumor suppressor gene (143), whereas OGR1 knockout mice displayed reduced melanoma tumorigenesis, indicating that OGR1 was required for melanoma tumor progression (89). Interestingly, OGR1 was shown to be highly expressed in human medulloblastoma tissue, a pediatric cerebellar cancer originating from neuronal precursor cells (65), and activation of these receptors in a human medulloblastoma cell line triggered activation of the ERK cascade in response to proton-mediated Ca^{2+} release from intracellular Ca^{2+} stores (65). This is an important finding because it demonstrates that a fall in extracellular pH can impact on gene transcription and may therefore provide a mechanistic explanation as to how the acidic environment of the tumor tissue might promote cell survival. Intriguingly, the ability of medulloblastoma cells to respond to external acidification with gene transcription was lost upon differentiation of these cells (64). The differentiation-dependent loss of ERK activation was due to significantly reduced proton-stimulated Ca^{2+} release from intracellular Ca^{2+} stores as a result of reduced IP$_3$ formation in differentiating as opposed to proliferating cells. The reason for lack of IP$_3$ production in response to an acidic stimulus in differentiating cells is unclear; there was no significant change in OGR1 mRNA levels upon differentiation, which may point toward impaired coupling of OGR1 to its G protein and/or to PLC in differentiating cells (64). Alternatively, it is possible that functional membrane OGR1 protein levels were reduced. Regardless of the reason(s) for the reduced IP$_3$ formation, these results suggest that only proliferating cells can translate an acidic extracellular pH into gene transcription, which is entirely consistent with the idea that the acidic microenvironment provides a survival advantage to proliferating (i.e., transformed) cells over non-transformed (i.e., differentiated) cells.

The fact that protons can directly activate receptors coupled to phospholipase C is not only important in terms of Ca^{2+} release from intracellular Ca^{2+} stores but also because phospholipase C activity can influence the activity of distinct families of ion channels that all have the potential to modulate Ca^{2+} influx into the cells in which they are expressed (FIGURE 1B). PIP$_2$ is the substrate for phospholipase C, and its breakdown following receptor activation has been shown to impact on the gating of a number of

Table 2. Proton-sensing ion channels and GPCRs

H$^+$-activated GPCRs	OGR1, TDAG8, GPR4 (reviewed in Ref. 139)	*OGR1* + Melanoma (blood vessel formation) (89) + Medulloblastoma (gene expression) (65) − Prostate cancer (cell migration) (143)
H$^+$-activated channels	ASIC1-3 (92)	*ASIC1* + Glioblastoma (cell cycle and migration) (9, 132) *ASIC2* + Adenoid cystic carcinoma (role unclear) (184) − Glioblastoma (inhibits ASIC1) (9) *ASIC3* + Adenoid cystic carcinoma (role unclear) (184)
	TRPV1 (170)	*TRPV1* + Prostate cancer (mechanism unclear) (26, 96, 136) − Glioma (apoptosis) (4) − Skin cancer (apoptosis) (12) − Bladder cancer (apoptosis) (79, 87) − Fibrosarcoma (apoptosis) (49)
H$^+$-potentiated channels	TRPC4β and 5 (138)	*TRPC4* + Medulloblastoma (role unclear) (65) − Renal cell carcinoma (inhibition of angiogenesis) (167) *TRPC5* − Neuronal cancer (promotion of neuronal progenitor differentiation) (141)
	TRPM7 (74)	*TRPM7* + Breast cell cancer (proliferation) (56) + Gastric cancer (cell survival) (83) + Nasopharyngeal carcinoma (cell migration) (18) + Pancreatic epithelia (proliferation) (185) + Mesenchymal stem cells (survival) (19) + Head and neck carcinoma (proliferation) (73) + Hepatoma (proliferation) (107)
	P2X$_2$ homomers P2X$_{2+3}$ heteromers P2X$_3$ homomers (for high ATP levels) (47,150,151)	Not investigated
	TREK2 (137)	Not investigated
	GIRK1/4 heteromers (99)	*GIRK1/4* + Lung cancer (role unclear) (123, 156) + Breast cancer (role unclear) (30, 124, 171)
	Kv1.3 (145)	*Kv1.3* + Lung adenocarcinoma (cell proliferation) (71)
H$^+$-potentiated GPCRs	P2Y$_4$ (176)	*P2Y4* +Colon cancer (role unclear) (116)

ASIC, acid-sensing ion channel; TRPV, transient receptor potential channel of the vanilloid family; TRPC, canonical transient receptor potential channel (β denotes splice variant of the TRPC4 channel investigated); P2X, ATP-gated ion channel; TREK, TWIK (two-pore, weakly inwardly rectifying)-related K channel; OGR1, ovarian cancer G-protein-coupled receptor 1; TDAG8, T-cell Death; GPR4, G-protein-coupled receptor 4; −, negative effect on tumor progression; +, positive effect on tumor progression and/or overexpression compared with normal tissue.

distinct ion channels including members of the transient receptor potential (TRP) family, various K$^+$ channels, and voltage-gated Ca^{2+} channels (152). Crucially, PIP$_2$ can both directly act as an activator and inhibitor of channel opening, and hence its metabolism can activate or inhibit ion channel function. Moreover, the products of phospholipase C activity (DAG and IP$_3$) both

Table 3. Proton-activated G-protein-coupled receptors

GPCR	Transduction Cascade	pH_A and $pH_{0.5}$	pH of Maximal Response	Desensitization Properties
OGR1	Phospholipase C (IP_3 + DAG)	$pH_A < 7.6$ $pH_{0.5}$ of ~7.4	6.8	Not desensitising
GPR4	Adenylate cyclase	$pH_A < 7.8$ $pH_{0.5}$ of ~7.55	6.8	n.d.
TDAG8	Adenylate cyclase	$pH_A < 7.4$ $pH_{0.5}$ of ~7.0	6.8	n.d.

Values are from Refs. 94, 175. pH_A, pH threshold for activation; $pH_{0.5}$, pH giving half-maximal response; n.d., not determined.

influence channel opening: DAG has been shown to directly open certain members of the canonical TRP (TRPC) channel family (165), whereas IP_3, by promoting Ca^{2+} release from intracellular Ca^{2+} stores, indirectly controls the activity of Ca^{2+}-activated and store-operated ion channels. Hence, activation of phospholipase C-coupled receptors can lead to modulation of a number of distinct ion channels that can either promote or reduce Ca^{2+} influx into cells.

Acid-Sensing Ion Channels

Acid-sensing ion channels (ASICs) are nonselective cation channels that open upon binding of extracellular protons and can be inhibited by amiloride (Table 4). To date, four genes coding for ASIC subunits have been identified: ASIC1 (two splice variants, a and b; b not in humans), ASIC2 (two splice variants, a and b), ASIC3 (three splice variants), and ASIC4 (two splice variants) (29). ASIC2b and ASIC4 do not form functional ion channels when expressed as homomers, but ASIC2b can form functional heteromultimers with ASIC2a and ASIC3 (29, 62, 92). ASIC1a was thought to be particularly Ca^{2+} permeable, but recent evidence suggests that this may not be true in the presence of physiological extracellular Na^+ and Ca^{2+} concentrations (135, 187). Intriguingly, serine protease activity (which can be induced following a fall in extracellular pH) was shown to shift ASIC1a proton sensitivity to higher proton concentrations (half-maximal activation of ASIC1a at pH 5.8 following trypsin treatment compared with pH 6.6 under control conditions) while leaving the desensitisation kinetics of the channel unaffected (125). Thus the pH sensitivity of ASIC channels can be shifted following proton-induced protease activity.

Different combinations of ASIC subunits yield distinct ion channels that can be distinguished in

Table 4. Proton-activated ion channels

Ion Channel Subunit	Permeability	pH_A and $pH_{0.5}$	pH of Maximal Response	Desensitization Properties
ASIC1a	Nonselective for cations	pH_A of ~7.0 $pH_{0.5}$ of ~6.8	5.5 (173)	Near complete within 10 s at pH 6.0
ASIC1b	Preference for K^+ over other cations	pH_A of ~6.5 $pH_{0.5}$ of ~6.2	5.3	Full within 5 s at pH 6.0
ASIC2a	Nonselective for cations	pH_A of ~6.0 $pH_{0.5}$ of ~4.9	n.d.	Partial within 5 s at pH 5.0 (within 100 s in Ref. 187)
ASIC2b	No functional homomers	n.d.	n.d.	n.d.
ASIC3	Nonselective for cations	$pH_A > 7.0$ $pH_{0.5}$ of ~6.6	6.0	Partial at pH 7.0 (181) or below pH 4.0 (172); full within 5 s at pH 6.0 (187)
ASIC4	No functional homomers	n.d.	n.d.	n.d.
TRPV1	Nonselective for cations with preference for Ca^{2+}	$pH_A < 6.0$ $pH_{0.5}$ of ~5.4	pH4.4	Not apparent (163)

pH values for activation threshold and half-maximal activation of the current are given for homomultimers and are extracted from Refs. 8, 29, 63, 92, 163 unless otherwise indicated.

terms of proton-sensitivity, pH optima, and de-sensitisation kinetics (Table 3). This is important since it allows cells to respond differentially to varying extracellular proton concentrations. Small changes in extracellular pH activate ASIC channels that give rise to fully desensitizing currents (containing ASIC1a/b,2b), whereas large proton concentration changes activate ASIC channels that generate biphasic currents with a rapidly desensitizing and a sustained component [ASIC2a (187)], meaning that a large drop in extracellular pH will result in persistent activation of channels that contain the ASIC2a subunit. Regarding desensitization properties of ASIC3 channels, there are contradictory reports in the literature, which may reflect the use of different expression systems and/or extracellular proton concentrations: ASIC3 homomers expressed in oocytes were found to be rapidly desensitizing at pH 6.0 (187), whereas ASIC3 homomers expressed in COS7 cells (below pH 4.0) and CHO cells (pH 7.0) were non-inactivating (172, 181).

There are only few reports looking at a potential role for ASICs in cancer progression. The functional expression of ASIC2a and 3 in adenoid cystic carcinoma but not healthy control cells has been suggested as a marker for these cancer cells (184); however, their functional role in these cells remains unclear. Human high-grade glioma cells were found to have a constitutively active Na^+ conductance that could be blocked by amiloride and that was not present in cells from normal brain tissue or low-grade or benign tumors (9). Interestingly, ASIC1 was expressed in all tissues under investigation (normal human brain tissue, glioblastoma tissue, glioma-derived cell lines), whereas ASIC2 was only expressed in normal tissue and in less than half of the malignant tissue/cell lines. The constitutive amiloride-sensitive current was shown to be mediated by ASIC1 and is thought to result from lack of plasma membrane expression of ASIC2, suggesting that ASIC2 acts as an inhibitor of constitutive ASIC1 activity in these cells (9, 14, 169). Importantly, pharmacological block or knockdown of ASIC1 inhibited acid-induced currents and cell migration in glioblastoma cells (81, 169). This probably reflects a role for ASIC1 channels in volume regulation during the cell cycle and migration in these cells (132). The ability to change shape and volume is thought to be a crucial property of cancer cells since it enables them to migrate through narrow spaces (i.e., promotes invasion) as well as enhances cell proliferation (86, 146). Hence, the increasing proton concentrations may progressively stimulate proliferation rates and support tissue invasion by facilitating shape changes in isolated tumor cells. An important implication of these studies is that ASICs can be constitutively active and that increasing proton concentrations may then potentiate the constitutive channel function, thereby matching channel activity to environmental conditions. ASICs might hence be able to contribute to cancer progression by keeping the membrane potential at more depolarized potentials, thereby affecting the opening of voltage-gated ion channels and/or the Ca^{2+} driving force. Furthermore, ASICs have been proposed to be involved in the perception of pain in tumors, suggesting that neuronal ASICs can sense and respond to proton concentrations in or around tumor tissue (92, 98, 186).

Transient Receptor Potential Channel Vanilloid Subfamily 1

Transient receptor potential channel vanilloid subfamily 1 (TRPV1) channels are cation channels with a high permeability for Ca^{2+} (17) that are activated by a number of distinct stimuli including heat, vanilloid compounds (most notably capsaicin), camphor, piperine, garlic, and the endocannabinoid anandamide (168). Intriguingly, extracellular protons exert both potentiating and activating effects on TRPV1: protons potentiate the effect of capsaicin (at pH 6.3) (17) as well as directly open TRPV1 channels in the absence of any other stimulus provided the pH falls below pH 5.9 (163) (Table 2). These two proton-dependent effects are not mediated by the same amino acid residues, suggesting that, depending on the stimulus, TRPV1 channels can utilize distinct opening states with different properties that may convey distinct signals to cells (76, 163). TRPV1 channels are thought to be the only members of the TRPV subfamily that are activated by protons (35), although heterologously expressed TRPV4 was also shown to be activated by protons in the absence of extracellular Ca^{2+} (154).

There is substantial evidence that TRPV1 is involved in the mediation of cancer pain; this is particularly true for bone cancer pain: inhibitors of TRPV1 channels reduce bone cancer pain (48, 82, 104, 114, 115), and importantly it was found that the acidic microenvironment of the bone cancer was in part responsible for the TRPV1-mediated pain perception (164, 186), crucially demonstrating that the high proton concentration found in and around solid cancers can be sensed by proton-sensing ion channels on nearby neurons. It is, however, unclear what role TRPV1 expressed in cancer cells plays in the progression of cancer. TRPV1 is functionally expressed in human prostate cells (136), its expression is upregulated in transformed cells (26), and its activation induces Akt and ERK activation (96), suggesting that TRPV1 activation promotes prostate cancer progression (however, see Ref. 188 for TRPV1-mediated apoptosis of

prostate cancer cells). In contrast, other reports looking at a number of different types of cancers find that activation of TRPV1 leads to induction of apoptosis in these cancer cells and that high levels of expression suggest a better prognosis for patients (4, 12, 49, 79, 87, 105), which raises concerns for the use of TRPV1 antagonists in controlling cancer pain (12). In agreement with this dual and contradictory role for TRPV1 channels in cancer progression, the TRPV1 agonist capsaicin has been reported to act both as inducer of apoptosis in cancer cells as well as carcinogen or co-carcinogen promoting tumor progression, and more recently it was suggested that capsaicin may not always mediate its pro-cancerogenic effects through TRPV1 (see Ref. 67 and references therein).

Most studies looking at a role for TRPV1 in cancer use capsaicin for activation of the channel. As mentioned above, this gives rise to an open state that is distinct from that activated by protons, and this may in part explain the lack of evidence of involvement and/or the contradictory results obtained in different cell types.

Proton-Potentiated Ion Channels and Receptors in Cancer

Apart from directly gating ion channel opening, extracellular protons can facilitate ion channel function by binding to allosteric sites, thereby promoting ion flux through the channel protein. These channels may influence intracellular Ca^{2+} signaling by either being Ca^{2+} permeable or by changing the driving force for Ca^{2+} entry (see introduction).

Transient Receptor Potential Channels

Canonical transient receptor potential channels (TRPC) are nonselective cation channels that are activated following stimulation of receptors coupling to phospholipase C and D (52, 168). Two of the seven members of this channel family, TRPC4β (short TRPC4 splice variant) and TRPC5, have a bell-shaped dependence on extracellular pH with maximal responses around pH 6.5 and potentiation of the TRPC-mediated current already at physiological pH (pH 7.4) (138). In contrast, the long TRPC4α splice variant is inhibited by increases in extracellular proton concentration (153), as is TRPC6 (138) (Table 1). Hence, depending on the TRPC subunit (and/or splice variant) expression, extracellular acidosis can either potentiate or inhibit current flow through these channels.

TRPC4 downregulation is thought to advance angiogenesis (which is required for tumor progression) in renal cell carcinoma (167), and TRPC5 promotes differentiation of proliferating neural progenitor cells (141), suggesting that neither channel supports cancer progression. However, in human medulloblastoma cells, activation of proton-sensing GPCRs led to activation of TRPC-like ion channels and subsequent Ca^{2+} influx (65) that was lost upon differentiation of these cells (64). Medulloblastoma cells express TRPC1, 3, 4, 6, and 7, with TRPC4 being the dominant TRPC subunit (64), and importantly TRPC4 channels were downregulated following differentiation, suggesting that these channels play a role in the proliferative state of these cancer cells. This notion is supported by the finding that TRPC4 channels are highly expressed in native granule precursor cells (the cells of origin for the medulloblastoma type under investigation in Refs. 65, 66) in the proliferative state but that their expression decreases dramatically during/following differentiation of these cells (66). This is interesting because, just like in proliferating transformed tissue, there are also acidotic conditions in proliferating normal tissue during normal development (103), which may point toward a common mechanism through which external acidosis influences proliferation in transformed and developing tissue. Furthermore, it seems plausible that proton-sensing GPCRs should trigger opening of ion channels that are potentiated (rather than inhibited) by extracellular acidosis.

TRPM7, a member of the melastatin TRP channel family, has been reported to be a Ca^{2+}- and Mg^{2+}-permeable, constitutively open ion channel that is ubiquitously expressed and responsible for Mg^{2+} uptake into cells (Refs. 38, 45, but see Ref. 75 and reply in Ref. 133). Inward currents through these channels were shown to be dramatically increased by decreasing the extracellular pH; maximal potentiation was seen at pH 3.0 with currents already increased at pH 7.0 (74). Mg^{2+}, just like Ca^{2+}, has been shown to be involved in tumor growth and progression, and tumor cell Mg^{2+} content correlates positively with proliferation rates (reviewed in Refs. 5, 178). The fact that influx of both of these metal ions can be potentiated by extracellular protons is important, since it supports the idea that protons promote cancer progression and provides a mechanism whereby this might be achieved (Mg^{2+} and Ca^{2+} influx). In agreement with this, TRPM7 channels have been implicated in breast cancer cell proliferation, gastric cancer cell survival, migration of nasopharyngeal carcinoma cells, pancreatic epithelial and hepatoma cell proliferation, head and neck carcinoma cell proliferation and mesenchymal stem cell survival (18, 19, 56, 73, 83, 107, 185). However, it is possible that the cancer-promoting effect of TRPM7 is independent of Mg^{2+} influx through these channels and that other properties of this intriguing protein are important.

ATP-Gated Ion Channels

P2X receptors are ATP-gated nonselective cation channels, and a role for ATP-sensing receptors in cancer progression is supported by a large body of evidence in the literature (147). Of the seven P2X subunits (P2X1–7), $P2X_2$ and $P2X_3$ homomers as well as $P2X_{2/3}$ heteromers are potentiated by extracellular protons (Refs. 47, 150, 151; Table 2). The two P2X subunits differ in their pattern of pH dependence: $P2X_2$ homomers displayed an increase in current size between extracellular pH values of pH 8.3–6.3 (151), whereas for $P2X_3$ the main potentiating proton effect occurs between pH 7.4 and 6.4 for this receptor (47). Both $P2X_3$ and $P2X_{2/3}$ receptors have been implicated in cancer pain (54, 77, 177); however, it is unclear whether their ability to sense extracellular protons is involved in this process.

K^+ Channels

TREK2, GIRK1/4, and Kv1.3 are all K^+ channels that can profoundly influence intracellular Ca^{2+} signaling by hyperpolarizing the membrane potential, resulting in potentiation of voltage-independent Ca^{2+} influx pathways and inhibition of voltage-dependent ones. K^+ channels are thought to play a key role in cancer progression (179), and it appears that many if not most K^+ channels are inhibited rather than potentiated by extracellular acidosis (62).

TREK 2 channels are members of the two pore-domaine K^+ channel family, and currents through these channels were significantly increased at pH 6.0 compared with pH 7.4, and TREK2 currents are already potentiated at physiological pH. This effect was dependent on a histidine residue, confirming that it was mediated by proton biding to the channel protein itself (137). There are no reports on TREK2 and cancer progression.

GIRK1/4 heteromers belong to the family of G-protein-coupled, inwardly rectifying K^+ channels that are potentiated by a drop in extracellular pH through binding of protons to a histidine residue on the channel protein. The potentiating effect of extracellular protons was already apparent at pH 7.4 compared with pH 8.4 and saturated between pH 6.2 and pH 5 (99). Intriguingly, GIRK1 is expressed in human lung cancer cell lines and tissue, where its expression levels correlated with malignancy of the disease (123, 156), and both GIRK1 and 4 are expressed in human breast cancer cell lines (30, 124) and form functional channels (171), suggesting a role for these channels in lung and breast cancer progression.

Voltage-gated Kv1.3 channels were shown to display decreased inactivation but also reduced current amplitude at low external pH (pH 6.5 and 5.5), resulting in prolonged opening (albeit with smaller amplitude) under conditions of external acidosis (144). Expression of these channels has been investigated in a number of different cancer tissues, including breast cancer, lung cancer, prostate cancer, and glioma cells (1–3, 11, 40, 72, 126), but reports differ quite substantially with regard to expression levels and correlation with malignancy of the tumor tissue. It appears that for a number of cancers, Kv1.3 expression either does not change with increased tumor malignancy or is in fact downregulated (1, 11, 40, 126). Furthermore, some studies used broad-spectrum K^+ channel blockers rather than Kv1.3-specific inhibitors to assess impact of inhibition of Kv1.3 channel activity on cancer cell proliferation. Importantly, however, in human lung adenocarcinoma, it was shown that either pharmacological block of Kv1.3 channel function using a selective Kv1.3 blocker or knockdown of Kv1.3 significantly decreased cell proliferation and, in the case of the Kv1.3 blocker, tumor volume in vivo (71).

Not only ion channels but also GPCRs can be boosted in their function by extracellular protons. $P2Y_4$ receptors belong to the family of metabotropic ATP receptors and are potentiated by decreasing extracellular pH [increase in efficiency between pH 7.5, 6.5, and 5.5 (176)]. These receptors are found in human colon cancer cells (25, 28) and are overexpressed in human colon cancer tissue with respect to
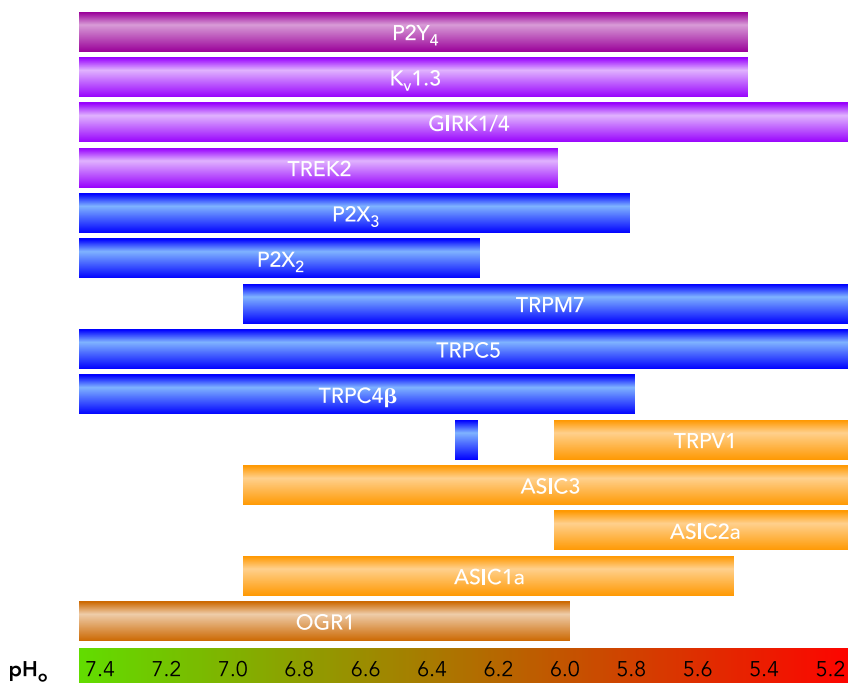

FIGURE 2. pH-profile of proton-sensing ion channels and GPCRs
Proton-sensing ion channels have different degrees of dependence on extracellular pH (pHo). Brown/orange indicates proton-activated receptor or channel; purple/blue symbolizes proton-potentiated receptor or channel. TRPV1 responses to heat or capsaicin can be potentiated by extracellular acidosis (only pH value tested was pH 6.3), and a further significant decrease in pH activates TRPV1 channels independently of the presence of other stimuli. Different channels/receptors will respond to distinct extents of external acidosis, thereby providing a unique pH-dependent profile of active channels in cells. For some channels, not the whole pH spectrum shown here was analyzed: for TREK2, the lowest pH level investigated was pH 6.0; for P2X3, the lowest pH value was pH 5.8, whereas for P2X2 it was pH 6.3.

tumour-free tissue (116), which points toward a role for these receptors in tumor cells.

Outlook

Different cancer cells express distinct subsets of channels and receptors that define the properties of those cells. Depending on the extracellular pH, distinct ion channels and/or receptors may be recruited or potentiated–and, indeed, inhibited (FIGURE 2). This raises the intriguing possibility that the presence of increasing concentrations of extracellular protons selectively switches on and off distinct ion channels and/or receptors, thereby translating the extracellular pH into spatio-temporally distinct intracellular Ca^{2+} signals that induce pH-dependent, distinguishable responses in the cells in which they occur. It is well established that different Ca^{2+} signals can give rise to expression of diverse sets of genes (31, 34, 37) and that Ca^{2+} microdomains are key in determining which intracellular processes are initiated (101, 119). These microdomains will be determined to a significant extent by the channel proteins responsible for Ca^{2+} influx, which, in turn, depend in their activity on the local microenvironment. However, the impact of local acidosis on receptor and ion channel-mediated signaling in tumor cells is only rarely addressed. For cancers that do not form solid tumors (e.g., leukaemias), acidosis of the microenvironment is unlikely to occur, but for cancers generating solid tumors extracellular acidosis is a factor that likely impacts on cell surface receptors of the cancer cells.

A number of ion channels and receptors that are inhibited by protons are overexpressed in cancerous tissue, and it could be that this overexpression compensates for diminished channel or receptor function in an increasingly acidic environment. Likewise, a lack of change in expression levels of a channel or receptor protein does not necessarily mean lack of change in activity of that protein: if its function is potentiated by extracellular protons, then there is no need for upregulating its expression as the increasing acidification will achieve augmented responses from these proteins by default. There is also evidence that ion channels have functions beyond their ion-transporting ability, and it may be that these ion-independent functions help promote cancer progression: Voltage-gated Ca^{2+} channel subunits can function as transcription factors (7), and ion channels can also be expressed on intracellular membranes (45), which would mean that they are not affected by the acidotic extracellular conditions. Furthermore, ion channels may not need to conduct ions to exert effects at the plasma membrane. For EAG K^+ channels, it was shown that a conformational change of the channel protein was sufficient to activate

intracellular signaling cascades leading to cell proliferation (58), and it is thought that the β-subunit of Na^+ channels can promote cell-cell adhesion (97). All these ion-independent channel functions can at least in part explain the seemingly contradictory finding that ion channels, which are inhibited by extracellular protons, play a crucial role in cancer progression that is accompanied by acidosis of the tumor tissue. However, if a key function of proton-inhibited ion channels is ion-transfer-independent, then this requires that Ca^{2+} influx, which is necessary for cancer progression, is managed by proton-independent and/or proton-activated (or proton-potentiated) channels. It is likely that proton-sensing receptors and ion channels play a pivotal role here since they have the ability to faithfully report increases in extracellular proton concentration by translating them into intracellular Ca^{2+} signaling. It should be noted, however, that the impact of external acidosis on ion channels (such as channels given in Table 2) is generally not tested in native cancer cells but in expression systems transfected with the channel protein of interest, and it can therefore not be ruled out that channels that are inhibited by extracellular protons in expression system may not be inhibited by acidic extracellular conditions in native cancer tissue.

One limitation of proton-sensing receptors and ion channels is that they may exhibit desensitization upon prolonged exposure to protons (see Table 4). However, this does not pose a problem for all channels or receptors: they do not all (fully) desensitize (e.g., OGR1, ASIC2a, ASIC3, TRPV1) or may change their conductance state with increasing pH (ASIC1). Additionally, constitutively active channels monitor extracellular acidification continuously [e.g., TRPM7; constitutive activity also found in TRPC5 (138), TRPC4 (102), ASIC1]. Constitutive channel activity combined with proton sensitivity of this channel is hence a very efficient way for cancer cells to gradually give more weight to this channel, as ion flux through it will increase with rising extracellular proton concentrations. Finally, it is possible that intermittent blood flow, which is observed in tumor tissue, plays an important role in changing local proton concentrations, thereby allowing (partial or full) recovery from desensitization. Intermittent blood flow is crucial for tumor progression since it permits reoxygenation of hypoxic regions [which is important for cell survival of non-transformed host stromal cells such as endothelia, fibroblasts, macrophages, lymphocytes, mast cells, myofibroblasts, etc. (42)] (162). However, protons are very small and hence have a very high charge density. It therefore remains to be established whether extracellular proton concentrations change significantly with time and to a similar extent as O_2 levels to allow recovery from desensitization.

More research is needed to understand the impact that protons have on tumor cells and how increasing proton concentrations can promote the transformed phenotype. It seems plausible that increasing proton concentrations trigger expression of genes that support cell survival under increasingly hostile conditions through activation and/or potentiation of proton-sensing receptors and channels that are located in the plasma membrane and hence sense the acidic environment. Expression of proton-sensing proteins/signaling cascades may therefore be considered as a contributing factor of transformation of cancer cells. ■

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