Uncovering the interplay between pH receptors and immune cells: Potential drug targets (Review)

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Abstract. Extracellular acidosis is associated with various immunopathological states. The microenvironment of numerous solid tumours and inflammatory responses during acute or chronic infection are all related to a pH range of 5.5-7.0. The relationship between inflammation and immune escape, cancer metabolism, and immunologic suppression drives researchers to focus on the effects of low pH on diverse components of disease immune monitoring. The potential effect of low extracellular pH on the immune function reveals the importance of pH in inflammatory and immunoreactive processes. In this review, the mechanism of how pH receptors, including monocarboxylate transporters (MCTs), Na^+/H^+ exchanger 1, carbonic anhydrases (CAs), vacuolar-ATPase, and proton-sensing G-protein coupled receptors (GPCRs), modulate the immune system in disease, especially in cancer, were studied. Their role in immunocyte growth and signal transduction as part of the immune response, as well as cytokine production, have been documented in great detail. Currently, immunotherapy strategies have positive therapeutic effects for patients. However, the acidic microenvironment may block the effect of immunotherapy through compensatory feedback mechanisms, leading to drug resistance. Therefore, we highlight promising therapeutic developments regarding pH manipulation and provide a framework for future research.

Contents

1. Introduction

The accumulation of lactic acid and protons, products of cancer metabolites together with acute and chronic inflammatory diseases, reduces the extracellular pH level. Inflammatory cells and tumour cells themselves usually show an increase in metabolic activity, causing tissue hypoxia, leading to glycolytic metabolism transition and subsequent lactic acid accumulation (1,2). In addition, the disruption of the vasculature due to hypoxia prevents protons from being efficiently flushed from the extracellular space, exacerbating extracellular acidification (3). Numerous studies have shown that acidosis has a variety of effects on the inflammation/immune response (4) (Fig. 1). On the one hand, acidosis drives T lymphocytes and natural killer (NK) cells toward deprivation of their functions, and they remain in a reversible paralysis condition followed by apoptosis and reduction of interleukin (IL)-2, interferon (IFN)-γ, perforin and granzyme secretion (5-7). On the other hand, the acidity of the tumour microenvironment (TME) can change the differentiation of dendritic cells (DCs) from haematopoietic stem cells and impair the ability of both antigen presentation and induce specific T cell responses by inhibiting the maturation and differentiation of DCs (8,9).

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Abbreviations: ADCC, antigen-dependent cellular cytotoxicity; CA, carbonic anhydrase; CAR, chimeric antigen receptor; CTL, cytotoxic T lymphocyte; EIPA, ethyl isopropyl amiloride; GPCRs, G-protein coupled receptors; HSCs, haematopoietic stem cells; MAPK, mitogen-activated protein kinase; MCT, monocarboxylate transporter; MDSCs, myeloid-derived suppressor cells; NK, natural killer; PD-1, programmed cell death protein 1; PPIs, proton pump inhibitors; TAMs, tumour-associated macrophages; VEGF, vascular endothelial growth factor

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Paradoxically, myeloid-derived suppressor cells (MDSCs) (10) and regulatory T cells (Tregs) (11) can be activated and recruited via acidosis, as well as by neutrophils, to produce a series of pro-inflammatory mediators (12). Extracellular acidosis also causes tumour-associated macrophages (TAMs) to undergo M1 to M2 phenotype transformation induced by hypoxia-inducible factor-1α (*HIF1A*, HIF-1α), and increases the expression of M2-like phenotype-related genes, such as arginase 1 (*ARG1*), mannose receptor C-type 1 (*MRC1*) and chitinase-3-like protein (*CHI3L1*) (13-15). To neutralise metabolic acid overload, immune cells use pH-sensing proteins, transporters, and proton pumps and promote their survival in an acidic environment (16). These transporters or pumps include monocarboxylate transporters (MCTs), such as MCT1-4, $Na^+/HCO3^-$ co-transporters, such as sodium-hydrogen exchanger 1 (NHE1), and carbonic anhydrase (CA) family of proteins, such as CA1-2, CA4, CA9, and CA12, in addition to vacuolar-ATPase (V-ATPase) that co-transports lactate and protons (15,17). The proton-sensing G-protein coupled receptor (GPCR) family has four members: GPR4, TDAG8 (GPR65), OGR1 (GPR68), and G2A (GPR132), which can be activated by acidic extracellular pH. GPCRs are expressed on a variety of immune cells, acting on the PLC/Ca^{2+} signaling pathway via Gq/11 proteins or cAMP signaling pathways through Gs (18,19). It is worth noting that although pH-sensitive regulators are present in T cells or NK cells, they are also widely expressed on cancer-related myeloid cells, which means that immune cells may use pH transporters to balance local acidic sites to survive in uncongenial environments.

Although low pH is a common feature in inflammatory environments and tumours, little attention has been paid to how pH receptors modulate immune cell function in acidic environments. Most of the current reviews focus on the mechanism of different pH receptors regulating tumor metabolism in acidic environment. In this review, we innovatively summarized the bidirectional regulation mechanism of pH receptors on immune cells in the acidic environment of tumours and inflammation, and listed the effects of pH receptor inhibitors on the immune system in the preclinical model, as well as the therapeutic efficacy and problems of the latest clinical drugs at present. Interestingly, according to the dissimilarities in the structure and expression levels of pH-sensitive regulators on immune cells, immune evasion may be driven by inactivating T lymphoctyes or NK cells, boosting the accumulation and activity of pro-inflammatory factors, including macrophages and neutrophils. Targeted drugs based on this development have shown promising clinical applications and expanded the number of people who benefit from immunotherapy (Table I).

2. Proton (H^+) transporters

MCTs. MCTs consist of 14 members, and MCT1-4 promote the passive transport of monocarboxylates, such as lactate, pyruvate, and ketone bodies, as well as protons across the cell membrane (20) (Fig. 2). As an important regulator of intracellular lactic acid and pH, MCTs contribute to the production of lactic acid by hyperglycolytic cells, such as cancer cells and immune cells (21). Lymphocytes and NK cells may be biased against immunosuppressive phenotypes and function at lower pH values (22). As early as 2004, Merezhinskaya *et al* found the expression of three monocarboxylate transporters (MCT1, MCT2, and MCT4) in isolated human monocytes and lymphocytes, and assumed that leukocytes express lactate transporters to promote their efflux under acidic conditions to reduce intracellular acidosis (23,24). Inhibition of MCT4 can help to enhance the cytotoxicity of NK cells and their ability to kill tumour cells by inducing autophagy to prevent lactic acid rejection by tumour cells (25). One of the characteristics of the transformation of lymphocytes from primitive cells to effector ones is aerobic glycolysis, which promotes the process of proliferation and differentiation (5). Recent studies indicate that this metabolic switch could cause memory $CD8^+$ T cells to undergo terminal differentiation, increase lactate production and reduce mitochondrial consumed oxygen (26). Therefore, suppression of glycolysis can augment the number of memory $CD8^+$ T cells in conjunction with their antitumour function (26). Fischer *et al* proposed that lymphocytes utilise glycolysis and produce lactic acid via MCT1 during activation (27). An acidic environment may block the lactate transport by MCT1 on cytotoxic T lymphocytes (CTLs), resulting in the impairment of their effector function, causing an increased apoptosis rate and the decreased production of IFN-γ, IL-2, perforin and granzyme B (28). It was later reported that high concentrations of lactic acid are detected by MCT1 when $CD4^+$ and $CD8^+$ T subsets enter the site of inflammation. Moreover, it interferes with glycolysis by downregulating hexokinase-1 (HK1) or inhibiting phosphofructokinase (PFK), producing a large amount of IL-17 and losing its cytolytic activity, which is adversely inhibited by T cell movement (29). A recent study using a variety of tumour mouse models, reported that MCT1 inhibitors effectively inhibit tumour growth by enhancing T cell infiltration and reversing the immunosuppressive microenvironment of solid tumours (30). In addition to MCT1, T lymphocytes also express MCT2 and MCT4, which participate in lactic acid transport by CTLs to inhibit proliferation and cytokine production (23,27,28).

Tumour-derived lactic acid is also an important factor regulating DC phenotypes through MCT1 in the tumour environment, which significantly inhibits the differentiation of DCs characterised by low expression of CD1a and low secretion of IL-12 (31,32). Extracellular lactic acid levels also affect plasmacytoid DCs (pDCs) through the function of cytoplasmic MCTs. Raychaudhuri *et al* found that MCT1 expression is significantly elevated in human pDCs. Lactic acid weakens the reaction of human pDCs to the TLR9 ligand, hinders TLR9-induced glycolysis, which leads to the production of type I IFN, and finally reduces the extracellular acidification significantly. Meanwhile, lactate promotes kynurenine and tryptophan metabolism of pDCs, which helps activate $Foxp3^+$ $CD4^+$ Tregs, the main immunosuppressive immune cell subsets in the TME (31).

An increasing number of studies have shown that the disruption of the glycolytic metabolism of inflammatory cells in the TME is critical to the development and progression of cancer (33). The ATP transfer mechanism of MCTs in the tumour inflammatory microenvironment was studied by co-culturing colorectal cancer cells with monocyte macrophages (THP-1). The lactic acid in the microenvironment is absorbed by THP-1 monocytes through MCT1, which promotes the positive regulation of cyclooxygenase-2 and

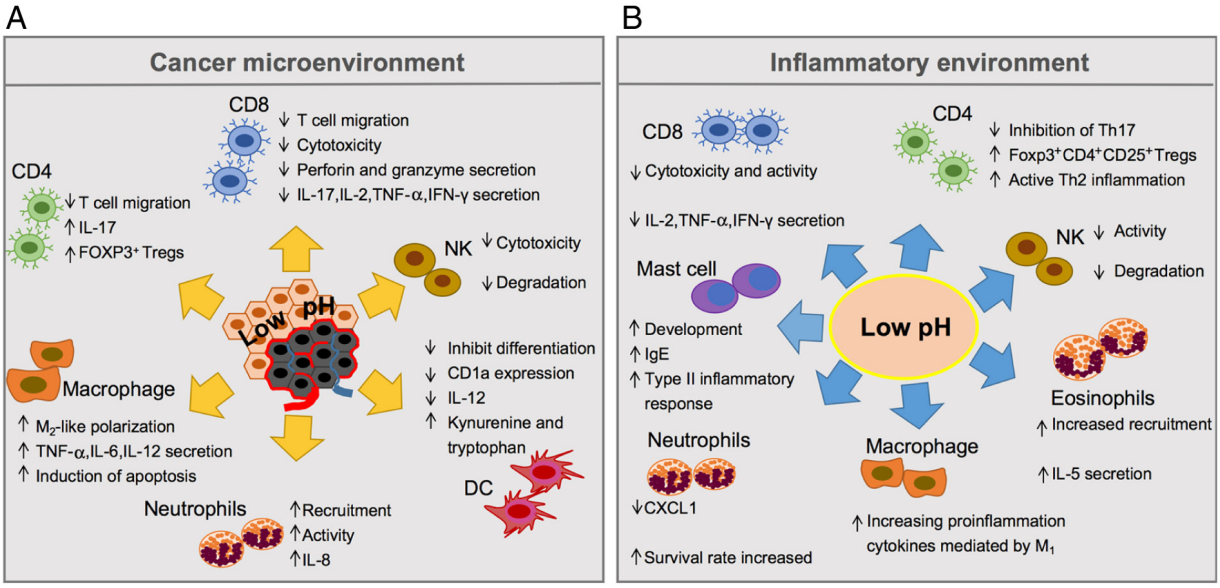

Figure 1. Effects of pH regulators on immune cell function in the acidic tumour microenvironment (TME) and inflamed tissues. (A) Acidification in TME is an extensive immune escape mechanism by which cancer cells eliminate the activity of all antitumour immune effectors, such as T cells, natural killer (NK) cells, and dendritic cells (DCs). At the same time, regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs) accumulate and transform into immunosuppressive cells. (B) Extracellular acidosis may be caused by bacterial inflammation in peripheral tissues, activation of respiratory burst, or proton aggregation caused by autoimmune and allergic diseases. Low pH delays the apoptosis of neutrophils, induces the activation of inflammatory bodies in eosinophils and macrophages, and induces type II inflammatory response caused by mast cell activation. However, exposure of $CD8^+$ T and NK cells to low pH reduce their activity.

PEPCK transcription by HIF-1α, which in turn accelerates tumour growth (34). Aerobic glycolysis also enhances and mediates inflammatory responses in activated macrophages. To understand the effects of tumour-derived lactic acid on the functional polarisation of TAMs, Colegio *et al* established lung and melanoma cancer mouse models and proved that MCTs are involved in the cellular uptake of lactic acid, inducing vascular endothelial growth factor (VEGF) and M2-like polarisation of TAMs (35). A study found that MCT4 is upregulated in lipopolysaccharide (LPS)-stimulated macrophages, which is mediated by MyD88 in an NF-κB-dependent manner. MCT4 knockdown weakens the secretion of pro-inflammatory mediators, such as tumor necrosis factor (TNF)-α, IL-6 and IL-12, in macrophages, increases lactic acid accumulation and decreases glycolysis (36). Similarly, lactate promotes macrophage polarisation in gastric and cervical cancer through MCT-HIF-1α signal transduction (37,38). Microglia in the central nervous system upregulate MCT1 and MCT4 expression under LPS stimulation, which is similar to that of macrophages in peripheral tissues. Studies have shown that MCT1 and MCT4 may enhance glycolysis through HIF-1 and ultimately promote microglial polarisation and pro-inflammatory effects (39,40). However, some studies also found that MCT4, rather than the related transporters MCT1 and MCT2, confers the ability of macrophages to export lactic acid in a high lactic acid microenvironment (41). High expression of MCT4, rather than MCT1, in TAMs is a marker of high metabolic heterogeneity between Hodgkin's lymphoma and the TME (42).

The metabolic cooperation between tumour cells and inflammatory/immune cells in the microenvironment is mediated by MCT1 and MCT4 (43). MCT inhibitors are considered an attractive therapeutic strategy. The lack of targeted MCT specificity and associated toxicity in first-generation MCT inhibitors is not feasible in clinical treatment. However, the second-generation MCT1 inhibitor, AZD3965, from AstraZeneca has displayed an effective result. AZD3965 was initially used in the phenotypic screening of immunosuppression and is currently being tested in a clinical trial. AZD3965 slows down choline metabolism after the accumulation of lactic acid in Burkitt's lymphoma and diffuse large B-cell lymphoma, and increases the infiltration of monocytes, DCs, and NK cells (44). Oral administration of AZD3965 also shows benefits in the treatment of Burkitt's lymphoma and diffuse large B-cell lymphoma with low expression of MCT4 (45,46). However, the efficacy of these agents in reducing lactate outflow from tumour cells may be limited due to the co-expression of MCT4. As the increase in glycolysis impair the effector function of T cells, new drugs are needed to target not only MCT4, but also immune cells to improve their metabolic function. The first-class inhibitor of MCT1 and MCT4, 7-amino-carboxy coumarins (7ACC), has recently been developed to prevent the influx but not the efflux of lactic acid in tumour cells. 7ACC delayed cervical SIHA tumour growth and inhibited tumour recurrence after cisplatin treatment. Moreover, it was also found to inhibit the growth of colorectal HCT116 tumours and *in situ* MCF-7 breast tumours (47). BAY-8002, as a new class of MCT1 inhibitors, significantly increased the lactate levels and transient regulation of pyruvate levels in tumours, and provides a new treatment for patients with MCT1 inhibitor resistance (48). Diclofenac, which blocks the activity of MCT1 and MCT4 as well as lactate secretion in tumour cell lines and primary T cells, can improve the killing effect of T cell-mediated tumour cell death by increasing the number of tumour-infiltrating leukocytes ($CD45^+$) and T cell subsets ($CD3^+$, $CD3^+CD8^+$) (49). Furthermore, AS2495674, an MCT1 inhibitor, was found to impede the transportation of lactic acid in $CD4^+$ T lymphocytes, which inhibited the proliferation of lymphocytes, thereby alleviating acute rejection and

Table I. pH receptors act as pharmacological targets by regulation of immune cells.

Isoform	Compound	Disease	Target cells	Results	Authors, year (Refs.)
MCT1	AS2495674	After organ transplantation	T cells	-Aggregation of $CD4^+$ T cells and inhibits the proliferation of lymphocytes with acute allograft rejection	Cho *et al*, 2010 (50)
	AZD3965	Solid tumors	DCs and NK cells	-Increased abundance of both monocyte-derived and conventional DCs and NK cells	Beloueche-Babari *et al*, 2020 (44)
MCT1 and MCT4	Diclofenac	Melanoma	Lymphocytes	-Blocks lactic acid secretion in T cells and increases the number of T cells and leukocyte infiltration	Renner, 2019 (49)
Na^+/H^+ exchanger-1 (NHE1)	Mifepristone (RU486)	Contraception leukemia	T cells	-RU486 blocks the inhibition of T cell proliferation induced by progesterone and glucocorticoid -Inhibition of PD-1 expression on T cells induced by high dose of dexamethasone	Chien *et al*, 2016 (55) Xing *et al*, 2015 (56) Lai *et al*, 2012 (57)
	Cariporide (HOE642)	Glioma	Microglia/ TAMs	-Pro-inflammatory polarization of microglia by downregulating iNOS and Arg1 in microglia -Reduces HIF-1α expression -Connection with T cell infiltration and immune response	Shi *et al*, 2011 (68) Zhu *et al*, 2016 (62) Guan *et al*, 2018 (70) Liu *et al*, 2020 (69)
V-ATPase	PPIs	Solid tumors	Lymphocytes	-Inhibition of acidification in extracellular environment and intracellular vesicles -Enhances the antitumor effect of immune cells	Corbet and Feron, 2017 (104)
CA1, CA2	Methimazole	Mast cell-mediated inflammatory diseases	Mast cells	-Reduces the development of mast cells in stem cells and type 2 inflammatory response	Henry *et al*, 2016 (111) Noti *et al*, 2014 (112) Winum, 2018 (113) Supuran, 2018 (114) Supuran *et al*, 2019 (115)
CA9	SLC-0111	Advanced solid tumors	T cells	-Increases the frequency of T cells secreting granzyme B, decreases the presence of Tregs and Th17 cells, and increases the frequency of Th1 cells	Chafe *et al*, 2019 (121) McDonald *et al*, 2020 (122)
	Girentuximab (cG250)	Metatstatic kidney cancer	NK cells	-Triggers ADCC immune response of NK cells	Dubois *et al*, 2015 (133)
	DNA vaccine	Renal cancer	T cells	-Activates CTL responses in renal cancer	Chai *et al*, 2019 (129)
	CA9-specific CAR-T cells	Renal cancer	T cells	-Decreases frequency of myeloid-derived suppressor cells in the tumor microenvironment of renal cancer	Li *et al*, 2020 (131)
	CA9-specific-CAR-NK cells	Renal cancer	NK cells	-Enhances NKs' cytotoxicity, releases IFN-γ, granzyme B and perforin in renal cancer	Zhang *et al*, 2018 (130)
CA12	U-104	T-cell acute lymphoblastic leukemia/ lymphoma	T cells	-Reduces cell proliferation and induces cell death in T-lymphoma cells	Lounnas *et al*, 2013 (119)

Table I. Continued.

Isoform	Compound	Disease	Target cells	Results	Authors, year (Refs.)
G-protein coupled receptor 4 (GPCR4)	NE-52-QQ57	Intestinal and joint inflammation	Lymphocytes	-The degree of leukocyte infiltration is reduced; -Reduces the ability of neutrophils, macrophages and T cells to infiltrate into the inflammatory site; -Relieves intestinal and joint inflammation	Sanderlin *et al*, 2019 (157) Velcicky *et al*, 2017 (158) Miltz *et al*, 2017 (159)
G-protein coupled receptor 65 (TDAG8)	BTB09089	Intestinal inflammation and allergic asthma	T cells Macrophages	-Inhibits the production of IL-2 stimulated by anti-CD3 and anti-CD28 antibodies; -Inhibits the production of TNF-α and IL-6 in macrophages; -Reduces immune-mediated inflammation	Pilon-Thomas *et al*, 2016 (138) Tcymbarevich *et al*, 2019 (151) Kottyan *et al*, 2009 (156)

ADCC, antigen-dependent cellular cytotoxicity; CA, carbonic anhydrase; CAR, chimeric antigen receptor; CTL, cytotoxic T lymphocyte; GPCR, G-protein coupled receptor; MCT, monocarboxylate transporter; NK, natural killer; PD-1, programmed cell death protein 1; PPIs, proton pump inhibitors; TAM, tumour-associated macrophage.

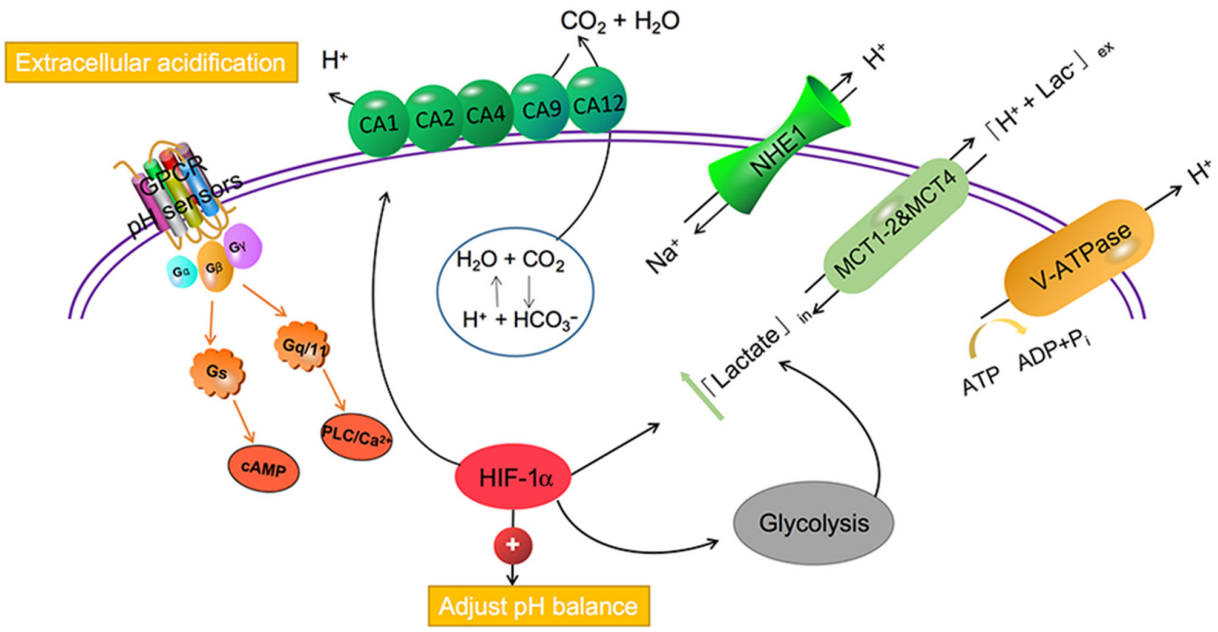

Figure 2. Major pH regulators in immune cells. After the activation of immune cells, metabolic changes occur, favouring increased glutamine metabolism and glycolysis, with increased glucose entering the pentose phosphate pathway. Glutamine metabolism promotes acidosis and produces CO_2 under hypoxia. Carbonic anhydrases (CAs) rely on CO_2 hydration to produce H^+ and hydrogen carbonate (HCO_3^-) ions. Similarly, in the absence of oxygen, pyruvate is reduced to lactic acid, which is exported to the extracellular space through MCTs. It should be noted that both processes produce H^+, which leads to extracellular acidification. This figure represents the major proteins that regulate the pH of immune cells, including the monocarboxylate transporter (MCT), Na^+/H^+ exchanger 1 (NHE1), CAs, V-ATPase and proton-sensing G-protein coupled receptor (GPCRs).

improving the survival rate of allografts (50). The combination of the targeted immune system and pH regulation can inhibit tumour progression and drug resistance more effectively, thereby, improving the therapeutic effect.

Na^+/H^+ exchanger-1 (NHE1). As a reversible reverse transporter, NHE1 belongs to the solute carrier coupled transporter family 9A (slc9a) and uses ATP provided by the Na^+ gradient to excrete H^+ from the cytoplasm. NHE1 activity is very low under neutral pH conditions but can be rapidly activated by cytoplasmic acidification (51) (Fig. 2). NHE1 regulates intracellular pH and cell volume, maintains the cavity microenvironment, and affects nutrient absorption, but also plays an important role in cell proliferation, migration, and apoptosis (52).

Previous studies in human lymphocytes demonstrated that IL-2 stimulation increases the abundance of pHi and NHE1, thereby affecting cell proliferation and cytokine production. In contrast, inhibiting the activity of NHE1 results in the rapid acidification of T cells which leads to apoptosis (53,54). It has been further proposed that glucocorticoid and progesterone can inhibit T cell activation through intracellular acidification, increase in free calcium concentration ($[Ca^{2+}]_i$) and rapid non-genomic inhibition of membrane NHE1 activity. Mifepristone (RU486), an antagonist of glucocorticoids in T cells, reportedly restrains the rapid decline of NHE1 activity induced by glucocorticoids and blocks PHA-stimulated T cells at the G0/G1 phase, indicating that RU486 antagonises NHE1 in the plasma membrane of T cells (55-57). As a subtype of $CD4^+$ T cells, Th9 cells highly express NHE1. siRNA-silencing of NHE1 was found to downregulate the production of IL-9 and ATP, and the increased activity of the Na^+/H^+ exchanger depends on Akt/rictor/mTOR signal transduction, which can protect the acidic environment (58). Similarly, inhibition of NHE1 activity in DCs resulted in cell swelling and oxidative burst with ROS formation, which is dependent on phosphoinositide 3 kinase (PI3K) activity and directly proportional to Akt phosphorylation (59-61).

NHE1 plays a significant role in the regulation of pHi homeostasis, as well as activation and migration of microglia (62). NHE1 is expressed in microglia/TAMs and participates in the pretumour communication between glioblastoma and TAMs. Co-culturing glioma-conditioned medium with microglia stimulates the activity of NHE1 on microglia and promotes the proliferation and migration of glioma by regulating microglia-derived factors, such as matrix metalloproteinase (MMP)-9, inducible nitric oxide synthase (iNOS), tumour growth factor (TGF)-β and IL-6. The NHE1-specific inhibitor, HOE642, stimulates the pro-inflammatory polarisation of microglia by downregulating iNOS and Arg1. Moreover, it also increases the infiltration of $CD8^+$, $CD4^+$ T cells, and Th1 cells in the tumour core and margin, while decreasing the infiltration of Treg cells, thus improving the microenvironment of immunosuppression in glioma (62). However, in one study, it was found that selective NHE1-knockout mice did not have inhibited microglia/macrophages pro-inflammatory response, but had improved neural repair function after ischaemic stroke (63). In addition to nervous system tumours, NHE1 also regulates macrophage function in other diseases. For example, in atherosclerotic disease, the activation of NHE1 on macrophages by IgE can reduce the extracellular pH value and induce apoptosis of macrophages, which leads to an increase in apoptosis in atherosclerotic lesions (64). A study examined NHE1 expression in macrophages of inflammatory diseases. Long-term inflammatory stimulation, such as LPS exposure, can activate TLR4 on intestinal macrophages, leading to inflammation through the MYD88-dependent pathway, thereby accelerating intracellular degranulation of NHE1 mediated by the ubiquitin-proteasome system (65).

Amilolol was the first NHE inhibitor developed to reduce VEGF production and the activity of MMPs, as well as other proteases which aid tumour metastasis, and notably increase the infiltration of T cells into the tumour core (66). It is safe and well-tolerated when used for chronic disease treatment in pharmacological dosages with the common side effect of an occasional increase in plasma K^+ levels (67). Potential analogues of amiloride have been prepared, including ethyl isopropyl amiloride, hexamethyl amiloride (HMA), and dimethyl amiloride (DMA) (67). The only clinically tested amiloride with strong NHE1 inhibitory activity is cariporide, which is useful in overcoming cancer and cardiovascular diseases. Some studies have shown that treatment with cariporide (HOE642) inhibits microglial activation and pro-inflammatory responses in the brain tissue after transient ischaemic stroke (68,69). NHE1 inhibition can change the glioma microenvironment by stimulating the pro-inflammatory polarisation of TAMs, increasing the activation of cytotoxic T cells, and reducing the number of Treg cells. The combination of anti-PD-1 therapy with cariporide minimised GL26 glioma volume and improved the survival rate of animals with glioma (62,70). Cariporide also diminished hypoxia-mediated tumour invasion in human tongue squamous cell carcinoma (67). Similarly, inhibition of NHE1 noticeably downregulated CCAAT enhancer-binding protein (C/EBPα) expression under hypoxic conditions via the pharmacological suppression of p38 mitogen-activated protein kinase (MAPK), suggesting that NHE1 may be a target for leukaemia treatment in a hypoxic microenvironment (71,72). It was found equally important in heart disease and ischaemia-reperfusion injury, and was used in 1,590 patients with unstable angina pectoris or myocardial infarction to gain clinical benefits in the early stage of the disease and improve the 6-month survival rate (64).

V-ATPase. V-ATPase is a multi-subunit H^+ pump approximately 800 kDa in size and consists of two regions. The V0 domain is found on the plasma membrane vesicles or plasma membrane vesicles, while the V1 domain is present in the cytoplasmic or extracellular environment of cells. The V1 domain produces protons during the conversion of ADP to ATP (73,74). The channel formed by connecting two domains of the V0a subunit can transfer H^+ from the cytoplasm to the inner or outer surface of the cell membrane. There are four subtypes of the V0a subunits: a1V, a2V, a3V, and a4V, which are responsible for the transportation of V-ATPase to different organelles, as well as membrane fusion of V0 and V1 (75,76) (Fig. 2). Remarkably, the specific subtype a2V of V-ATPase is expressed on the surface of host immune cells, such as monocytes or activated lymphocytes, which acidifies the extracellular environment and promotes growth, metastasis, and chemoresistance of cancer cells (77-79). Contrary to their research, Rao *et al* showed that the loss of a2V in haematopoietic stem cells led to a decrease in the number of $CD4^+$ and $CD8^+$ T cells in the peripheral blood (79), thereby accelerating the growth and metastasis of breast tumours (80). Inhibiting a2V reduced the number of T cells. Peterson *et al* further traced this phenomenon to the thymus and found that a2V-deficient thymocytes partially impeded the double negative phase of T cell development, resulting in the complete failure of their ability to proliferate and differentiate into double-positive cells (81), which is partly due to the poor signal transduction via Notch1 (82). Increased V-ATPase aggregation and lysosomal acidification are also characteristics of dendritic cell maturation, which depend on PI3K and mTORC1 (83).

Cancer cells secrete an a2V peptide (a2NTD), which has vital immunomodulatory properties in the TME. It was

found to increase the recruitment of neutrophils at the tumour site and promote the tumourigenicity of neutrophils and macrophages (84,85). In addition, it can enhances tumour angiogenesis and cancer cell invasion (86,87). Recent studies have shown that a2NTD activates the NF-κB pathway in neutrophils, leading to increased secretion of IL-8, which, in turn, prolongs the life of neutrophils and stimulates their migration to tumour sites via autocrine signaling (84,87). Granule-associated a2V subtypes play a role in maintaining the pH gradient between cytoplasmic and granular neutrophils and may serve as a biomarker for activated neutrophils (88).

A V-ATPase, ATP6V0D2/subunit d2, is a key component of the macrophage-specific autophagy-lysosome fusion mechanism, which can maintain the homeostasis of macrophage organelles, thus limiting inflammation and bacterial infection (89). In an established macrophage cell line lacking ATP6V0D2, the expression of TLR4 on the cell surface was prolonged, which enhanced the inflammatory response to LPS and decreased the response of I-IFN (90). In addition, ATP6V0D2 deficiency can lead to the accumulation of damaged mitochondria in macrophages. As shown in a previous study, deficiency of ATP6V0D2 resulted in decreased *Salmonella* clearance and increased DSS-induced colitis *in vivo* (89). V-ATPase is the core for the transport and secretion of inflammatory cytokines, and its activation is essential for the polarization of macrophages toward the M2-like phenotype and is required for the suppression of innate immune response (91-93). The inhibition of V-ATPase was found to selectively upregulate the production of TNF-α and the activation of NF-κB as well as SAPK/JNK in macrophages, which led to the M1-like phenotype (94).

Bafilomycin A1 (Baf-A1) is a specific inhibitor of the V-ATPase C subunit. Baf-A1 has been used for the treatment of osteoporosis and antiviral infection in the clinic (95). Although it is still in the primary research stage for cancer treatment, many studies have reported that Baf-A1 can inhibit the proliferation and metastasis of cancer cells. For example, high concentrations of Baf-A1 were found to inhibit cell growth in prostate cancer (PC3), liver cancer (BEL-7402), and ovarian cancer (HO-8910), and to induce the apoptosis of glioblastoma (U87MG) (96,97). Recent research has demonstrated that Baf-A1 is a promising candidate for the treatment of B-cell acute lymphoblastic leukaemia (B-ALL) and mantle cell lymphoma (MCL) (98,99). The reason why it has not been used in the clinic is that a high concentration of Baf-A1 may act on all vesicular V-ATPases in eukaryotic cells, thus acidosis and oxygen deficiency may occur under normal physiological conditions, resulting in strong side effects *in vivo*.

The similarities between V-ATPases and H^+/K^+ ATPases (enzymes involved in gastric proton secretion) have led to the study of proton pump inhibitors (PPIs). These compounds include omeprazole, pantoprazole, lansoprazole and rabeprazole, which are widely used as antacids in clinical practice (100). *In vivo* studies demonstrated that PPIs inhibit tumour growth in line with changes in the pH gradients (increased extracellular pH, decreased cytoplasmic pH), thereby eliminating the tumour-dependent reversal of the pH gradients. Also, early, repeated *in vivo* treatment with high doses of PPIs significantly improved survival in tumour-bearing mice, without any evidence of systemic toxicity (101). Since tumour acidity represents a mechanism of chemical resistance (102), Luciani *et al* used omeprazole and esomeprazole to enhance the antitumour sensitivity of chemotherapeutic agents (CDDP, 5-Fu, and vinblastine) by inhibiting V-ATPase in human melanoma-, adenocarcinoma-, and lymphoma-derived cell lines (103). In addition, two clinical trials are being conducted to test PPIs in patients with chemotherapy-sensitised melanoma and osteosarcoma (100,104). PPI is a prodrug that requires acidity to be fully activated, and when used in combination with weakly basic cytotoxic drugs, it is expected to increase the rapid extracellular protonation of cytotoxic drugs before they reach their specific cellular targets (100,105).

3. CO_2/HCO_3^- regulating proteins: Carbonic anhydrases (CAs)

Glutamine metabolism promotes acidosis and produces carbon dioxide under hypoxia (106). Carbon dioxide is hydrated by CA and converted into bicarbonate and protons to acidify the extracellular environment. At the same time, hypoxia induces HCO_3^- ions to combine with intracellular acids, resulting in the diffusion of CO_2 out of the cell, which is maintained by CA (2,107) (Fig. 2). CAs and metabolic acidosis are further known to modulate immune cell activation. The human asthmatic airway is an acidic microenvironment, in which infiltrated leukocytes expressing surface CAs may regulate local pH (108). When comparing eosinophils from lung samples exposed to allergens or saline (control) using genome-wide mRNA microarray analysis, it was found that CA4 is overexpressed on the plasma membrane of IL-5-activated eosinophils, which also regulate the lung transcriptome associated with allergic airway inflammation. Therefore, CA4 has a potential value in diagnosing and monitoring the eosinophilic reaction (109). In addition, allergic inflammation is also associated with type 2 cytokine responses, in which elevated levels of CA are expressed (110). Henry *et al* reported that CA1 and CA2 are significantly upregulated in mature mast cells. Methimazole (MZ), an inhibitor of CA1 and Ca2, was used to reduce the development of mast cells in stem cells and type 2 inflammatory response (111). Furthermore, a mouse model of food allergy-like disease caused by chicken ovalbumin was treated with MZ, which significantly reduced intestinal mastocytosis and effectively halted the allergic response (112). In conclusion, these data suggest that CA1 and CA2 are positive regulators of mast cell development and targeting them may prove to be effective in the treatment of mast cell-mediated inflammation. Currently, there are patent applications for CA inhibitors for the treatment of allergic diseases, bacterial infections, virus infections, mastocytosis, and mast cell-mediated inflammation (113-115).

The change in pH is usually accompanied by infectious and inflammatory injury, and CAs may also serve as a sensory mechanism for immune cell haematopoiesis under inflammatory conditions. CA2 and CA4 are related to the pathogenesis of diarrhea (116) and pulmonary diseases caused by infection. CA1 can induce antigen-specific immune tolerance by producing $Foxp3^+CD4^+CD25^+$ Tregs and inhibiting Th17 cells, resulting in a negative effect on the pathogenesis of inflammatory bowel disease (117). CA4 was identified by genome-wide assays as a specific regulatory element expressed in lung macrophages, which can be used

to distinguish different tissue-resident macrophages and is essential for understanding their role in alerting the immune system to lung disease (118).

In addition to CA1, CA2 and CA4, CA9 and CA12, which support the pH regulation mechanism, are considered as therapeutic targets. Lounnas *et al* observed that CA12 is overexpressed in T-cell acute lymphoblastic leukaemia/lymphoma (T-ALL/LL) cells, and its inhibitor was found to reduce cell proliferation and induce cell death in a T-lymphoma cell line (119). The analysis of some solid tumours has confirmed that the tumour margin has higher proliferation, lower apoptosis rate, and more immune cells infiltration than the core area. Cells at the edge of invasion also expressed more CA9 and less CA12 (120). In a cohort of 449 patients with metastatic melanoma and basal-like breast cancer, hypoxia-induced expression of the pH regulator CA9 was associated with poor overall survival. The ureido-sulphonamide CA9 inhibitor (SLC-0111) was found to reduce tumour glycolytic metabolism and extracellular acidification, increase the conversion of T cells to cytotoxic phenotypes, reduce the number of Tregs, and eliminate Th17 cells (121). SLC-0111 has been used in the phase 1 evaluation of 17 patients with advanced solid tumours and shown safe and effective results (122). Nasu *et al* also found that CA9 enhanced the tumourigenicity of ST1 cells isolated from human T cell leukaemia/lymphoma (ATL), and successfully established a xenograft model of leukaemia (123). Similarly, B-cell lymphoma cells also express CA9, which is associated with extracellular acidosis in xenograft tumours (124).

Research on CA9 has now been extended to the field of tumour immunotherapy including the production of vaccines and adoptive chimeric antigen receptor (CAR) T or NK cell therapy. DNA vaccination can be used as a potential method to induce antigen-specific T cell immune responses to enhance anti-tumour therapy (125,126). CA9 has obvious tumour specificity and can be used as an ideal target for the immunotherapy of renal cell carcinoma (127). To date, there have been many studies on the vaccines of renal cell carcinoma based on CA9 antigens, including peptide vaccines, DCs containing CA9 antigen, and DNA vaccine (128). A new study designed a DNA vaccine containing CA9 antigen with the result of a definite inhibition of CA9-Renca tumour compared with the control group. The vaccine activated CTL responses, and activated $CD8^+$ T cells with the expression of IFN-γ, IL-2, and TNF-α (129). In addition, CA9-specific CAR was transduced into T or NK92 cells, which showed specific cytotoxicity *in vitro* and *in vivo*, including the release of IFN-γ, granzyme B and perforin (130,131). Antigen-dependent cellular cytotoxicity (ADCC) induces the production of NK cells. The monoclonal antibody, girentuximab, can specifically bind to CA9 expressed in tumour cells and trigger the ADCC immune response to kill tumour cells. This monoclonal antibody is currently in phase III clinical trials for patients with clear cell renal cell carcinoma (132,133).

4. G-protein coupled receptors (GPCR): pH sensors

The proton-sensitive GPCR family has four members: GPR4, TDAG8 (GPR65), OGR1 (GPR68), and G2A (GPR132), which help cells respond to extracellular acidosis (134) (Fig. 2). A growing body of research suggests that proton-sensing GPCRs are expressed on a variety of immune cells. As lysophosphatidic receptors, they can be activated in a pH range of 6.4 to 6.8, via the protonation of histidine residues located in the extracellular domain (135,136). Their activated signaling pathways such as activation of the protein kinase A/ERK signaling pathway and stimulation of phospholipase C- and adenylate cyclase-induced cAMP accumulation, enable them to regulate the role of immune cells in inflammation, allergic reaction and tumour biology (137-139). Inflammation is attributed to an increase in local proton concentration, lactic acid production, and subsequent pro-inflammatory cytokine production, while acidic environments, in turn, influence the progression and regression of inflammation (140).

Upon activation of NF-κB by TNF, myeloid cells, including monocytes and macrophages, overexpress OGR1, which indicates the pathological role of the pH-sensitive receptor OGR1 in precancerous mucositis. Many studies have shown that an acidic environment can trigger the production of pro-inflammatory cytokines (including TNF, IL-6, IFN-γ, and IL-1β) in macrophages by increasing proton concentration and lactate production. In such an environment, OGR1 can induce extensive activation, including phospholipase C, and the formation of inositol triphosphate and Ca^{2+}. Interestingly, TNF induces OGR1 expression in monocytes, thus playing a key role in chronic colitis, and OGR1 deficiency was found to prevent spontaneous inflammation in preclinical models (141). Similarly, GPR4 knockout was found to effectively alleviate colitis in mice (142). In a $G2A^{-/-}$ mouse model of enteritis, it was found that monocyte and eosinophil recruitment to the injured colon was increased, while the number of $CD4^+$ lymphocytes, such as IFN-γ, were decreased (143). Similarly, the $G2A^{-/-}$ sepsis mouse model showed higher lethality, as well as lower plasma cytokine levels and bacterial scavenging capacity (144). G2A also acts as a threshold regulator of neurons. In nerve injury sites, G2A overexpression reduced the release of pro-inflammatory cytokines and growth factors to alleviate the inflammatory reaction, thereby alleviating hyperalgesia (145,146). In acute inflammation, G2A and TDAG8 indirectly regulate the M1/M2 polarisation by increasing proinflammatory cytokine levels (produced by M1 macrophages) and reducing anti-inflammatory cytokine levels (produced by M2 macrophages) to relieve joint inflammation and ease pain (147-149). In other inflammatory diseases, such as acute lung injury with large neutrophil infiltration, TDAG8, as a negative regulator of lung neutrophilic inflammation and injury, significantly reduced the expression of CXCL1 (150). However, the role of TDAG8 in neutrophil survival was found to augment, rather than attenuate, intestinal inflammation in an adoptive transfer colitis model (151).

Furthermore, in the TME, G2A on macrophages can sense and respond to the lactic acid signal from tumour cells and induce M2-like macrophage polarisation, thereby increasing the risk of breast cancer metastasis (152). OGR1 expression in bone marrow-derived $CD11b^+$ $GR1^+$ DP cells can promote M2 type macrophage polarisation and inhibit the infiltration of T cells, which is essential for tumour cell-induced immunosuppression and tumour development (153). The above data support the hypothesis that pH sensors expressed by immune

cells may be involved in maintaining precancerous inflammation at an early stage, and thus represent a potential target for tumour prevention based on recurrent inflammation-induced carcinogenesis.

GPCRs also play a variety of roles in airway response. Allergic asthma is an inflammatory airway disease that is mediated by Th2 lymphocytes. Due to the stimulation of glycolysis and respiratory burst, lactic acid is produced around the bronchial tube where inflammatory cells aggregate (154). In the ovalbumin (OVA)-induced asthma model, OGR1 regulates the migration of DCs to draining lymph nodes after exposure to antigens and stimulates Th2 phenotype changes, which subsequently induces airway inflammation and airway hyperresponsiveness (155). TDAG8 is expressed on eosinophils in two different allergic asthma models and regulates allergen-induced airway eosinophilia (156).

The proton-sensitive GPCR family as orphan GPCRs, whose endogenous ligands are yet to be discovered, can be used as a potential new target for the treatment of various indications. For example, G2A acts as a threshold regulator of neurons for treatment of intestinal inflammation. TDAG8 was used as an antispasmodic target for the treatment of allergic inflammation and OGR1 was used for the treatment of melanoma and prostate cancer. Only GPR4 has a small molecule inhibitor, GPR4 antagonist 13 (NE-52-QQ57), which can reduce the ability of neutrophils, macrophages, and T cells to infiltrate into the inflammatory site, thereby reducing intestinal and joint inflammation (157-159). Compound 3b is also a selective antagonist of GPR4. It is a suitable pharmacological tool for *in vitro* and *in vivo* studies on the role of GPR4 in tissue acidosis and consequential pathological tissue damage (160). Although OGR1 inhibitors have not been explored, dibenzazine derivatives, a GPR4 antagonist, have been reported to have antagonistic effects on OGR1 at high concentrations (160).

5. Clinical implications

As mentioned above, extracellular acidosis is associated with the duration and severity of tumours, allergies, and infectious diseases. Targeting extracellular acidosis can not only deprive tumours of local progression, metastasis, and resistance to cytotoxic drugs, but also may serve as a key factor in reversing immune cell effector function.

Table I shows the inhibitors of pH receptors currently in clinical development or in use. The key role played by MCT in the metabolic use of lactic acid in controlling lactic acid exchange and its use as a signal transduction molecule has led to the development of MCT inhibitors (161). Clinically, AZD-3965, an antitumour drug, which does not specifically target the MCT1-dependent lactic acid uptake, may have dose-limiting toxicity in MCT2-expressing tissues, such as the brain, liver, kidney, or immune cells, that use MCT1 to perform physiological functions. As a substitute for AZD-3965, 7ACC2 does not have any anticoagulant activity (47,162), but this compound is under preclinical evaluation and extensive studies are needed to confirm its efficacy. NHE1 inhibitors, including ciliperate from the amiloride family, and selective NHE1 inhibitors from the non-amiloride family, such as PHX-3 and compound 9T, have shown minimal toxicity and efficacy in preclinical models of glioma and leukemia (163,164). However, the use of this drug in a wide range of cancer treatments has not been explored and therefore has not yet been translated into the clinic. Only amiloride compounds with NHE1 inhibitory activity have been tested in clinical trials, focusing on cardiology and ischaemia-reperfusion injury. Although cariporide had a cardioprotective effect in reducing myocardial infarction in the EXPEDITION and early GUARDIAN trials, the increased cumulative dose increased the risk of cerebrovascular events and thus increased mortality (165-167). Clearly, in an oncology context, the initial approach that is clinically rational is to minimise the systemic dose of the drug in order to separate undesirable and potentially off-target effects from beneficial effects. A 3-methyl-4-fluorine analogue of 5-aryl-4-pyrimidine (compound 9T), as a selective NHE1 inhibitor, is 1,400 times more selective than cariporide and 500 times more effective than cariporide in inhibiting NHE1 (168,169). In addition, the compound has low toxicity in a mouse model upon oral administration but has never been used in cancer patients to date. Finally, PHX-3 (APO) is another promising NHE inhibitor and anticancer agent (163,170).

V-ATPase inhibitors can improve the acidity of the extracellular microenvironment and lysosomal compartment so that the acidity gradient inside and outside the cell is unbalanced, to achieve tumour cell death. PPIs are widely used in clinical practice. They have low toxicity and improve the recruitment of the host immune system to control tumours better (171). The same effect can be achieved by CA to regulate the extracellular pH gradient. Small molecule inhibitors of CA, such as sulphonamide U-104 and SLC-0111, are in preclinical evaluation for solid tumours and metastases with CA9 overexpression (172). It is worth noting that, compared with small-molecule inhibitors, antibodies are currently the main drugs in clinical development. For example, the monoclonal antibody, girentuximab, not only inhibits the growth of primary and metastatic tumours by reversing tumour acidification, but also circumvents some toxicity and selectivity problems encountered by small-molecule compounds. It is clinically used to treat kidney cancer, brain metastases or arthritis (172).

6. Summary

Acidosis is commonly developed in several diseases via multiple pathways, such as an acidic TME caused by inadequate hemoperfusion, hypoxia and fatty acid metabolism, a respiratory anaphylactic reaction caused by airway vapour condensate acidification, and accumulation of lactic acid secreted by inflammatory mediators in inflammation. Importantly, acidosis affects the phenotype and activity of immune cells, and pH-sensitive transporters have become excellent targets for regulating immune cell function (Fig. 1). However, the cellular composition and functional state of tumor-infiltrating immunocytes show significant differences in different tumors. For example, brain tumors show the lowest level of immunocyte infiltration, with macrophages as the dominant cells over lymphocytes and NK cells, which might be the reason for the limited response to immune checkpoint

blockade. Therefore, NHE1 inhibitor, cariporide, which impells the polarization of TAMs towards pro-inflammatory M1-type has become an effective treatment. Malignant tumors containing a higher proportion of immune cells including lung cancer, renal cancer, and skin melanoma, are the ones most sensitive to immunotherapy. They show a high frequency of T cells in the immunology compartment, for which the combination with pH receptor inhibitors may further enhance the effect of immunotherapy (173).

The main purpose of this review was to clarify the importance of the molecular mechanisms involved in pH dysregulation in different diseases, as well as the recent advances and applications of pH regulator inhibitors. It is suggested that targeting extracellular acidosis in combination with other conventional therapies can selectively sensitise cancer cells to combination therapies, thereby reducing drug resistance. This could improve the survival rates of patients with cancer. To understand the clinical application of pH-regulator-related inhibitors better and facilitate the development of modern cancer research in this new field, improvement in the current chemotherapy, surgery, and radiotherapy models is warranted.

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Availability of data and materials

All information provided in this review is documented by relevant references.

Authors' contributions

Conceptualization of the review was accomplished by LC, RG and XC. Data curation was carried out by LC, WL and TH. Formal analysis was conducted by LC, TH, WZ, XY and ML. Funding acquisition was the responsibility of RG. Writing of original draft was carried out by LC and TH. Writing, review and editing were the responsibility of LC, WL and TH. Project administration was the responsibility of RG and XC.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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