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

LIN CAO^{1,2}, TIANQIAO HUANG³, XIAOHONG CHEN², WEISHA LI¹, XINGJIU YANG¹, WENLONG ZHANG¹, MENGYUAN LI¹ and RAN GAO¹

¹Department of The Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Peking Union Medical College (CAMS & PUMC), Beijing 100020; ²Department of Otolaryngology-Head and Neck Surgery, Beijing Tongren Hospital, Beijing 100010; ³The Affiliated Hospital of Qingdao University, Qingdao, Shandong 266000, P.R. China

Received December 30, 2020; Accepted May 10, 2021

DOI: 10.3892/or.2021.8179

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

Correspondence to: Professor Ran Gao, Department of The Institute of Laboratory Animal Science, Chinese Academy of Medical Sciences and Peking Union Medical College (CAMS & PUMC), Panjiayuan Nanli 5 Hao, Beijing 100020, P.R. China E-mail: gaoran@cnilas.org

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

Key words: pH regulation, immunity, acidosis, microenvironment, therapeutic inhibitors

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
- 2. Proton (H⁺) transporters
- 3. CO₂/HCO₃⁻ regulating proteins: Carbonic anhydrases (CAs)
- 4. G-protein coupled receptors (GPCRs): pH sensors
- 5. Clinical implications
- 6. Summary

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).

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-1a (HIF1A, HIF-1a), 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/Ca2+ 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-y, 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-1a, 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-kB-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-1a 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

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	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 <i>et al</i> , 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 <i>et al</i> , 2016 (55) Xing <i>et al</i> , 2015 (56) Lai <i>et al</i> , 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 <i>et al</i> , 2011 (68) Zhu <i>et al</i> , 2016 (62) Guan <i>et al</i> , 2018 (70) Liu <i>et al</i> , 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 <i>et al</i> , 2016 (111) Noti <i>et al</i> , 2014 (112) Winum, 2018 (113) Supuran, 2018 (114) Supuran <i>et al</i> , 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 <i>et al</i> , 2019 (121) McDonald <i>et al</i> , 2020 (122)
	Girentuximab (cG250)	Metatstatic kidney cancer	NK cells	-Triggers ADCC immune response of NK cells	Dubois <i>et al</i> , 2015 (133)
	DNA vaccine	Renal cancer	T cells	-Activates CTL responses in renal cancer	Chai <i>et al</i> , 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 <i>et al</i> , 2013 (119)

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



Table I. Continued.

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





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 (HCO₃⁻) 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/EBPa) 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- κ B 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-KB 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.CO₂/HCO₃ 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 HCO₃ ions to combine with intracellular acids, resulting in the diffusion of CO₂ 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-y, 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 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-y, and IL-1 β) 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 Ca²⁺. 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.

Acknowledgements

We thank all the individuals who took part in this research.

Funding

Our work was supported by grants from the CAMS initiative for Innovative Medicine of China (2016-12M-3-019), and the National Mega Projects of China for Major Infectious Diseases (2017ZX10304402).

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.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- 1. Imtiyaz HZ and Simon MC: Hypoxia-inducible factors as essential regulators of inflammation. Curr Top Microbiol Immunol 345: 105-120, 2010.
- Jing X, Yang F, Shao C, Wei K, Xie M, Shen H and Shu Y: Role of hypoxia in cancer therapy by regulating the tumor microenvironment. Mol Cancer 18: 157, 2019.
- 3. Koltai T: The Ph paradigm in cancer. Eur J Clin Nutr 74 (Suppl 1): S14-S19, 2020.
- 4. Jancic CC, Cabrini M, Gabelloni ML, Rodríguez Rodrigues C, Salamone G, Trevani AS and Geffner J: Low extracellular pH stimulates the production of IL-1beta by human monocytes. Cytokine 57: 258-268, 2012.
- Chang CH, Curtis JD, Maggi LB Jr, Faubert B, Villarino AV, O'Sullivan D, Huang SC, van der Windt GJ, Blagih J, Qiu J, *et al*: Posttranscriptional control of T cell effector function by aerobic glycolysis. Cell 153: 1239-1251, 2013.
- 6. Nakagawa Y, Negishi Y, Shimizu M, Takahashi M, Ichikawa M and Takahashi H: Effects of extracellular pH and hypoxia on the function and development of antigen-specific cytotoxic T lymphocytes. Immunol Lett 167: 72-86, 2015.
- Chalmin F, Bruchard M, Vegran F and Ghiringhelli F: Regulation of T cell antitumor immune response by tumor induced metabolic stress. Cell Stress 3: 9-18, 2018.
- 8. Gardner A and Ruffell B: Dendritic cells and cancer immunity. Trends Immunol 37: 855-865, 2016.
- Hargadon KM: Tumor-altered dendritic cell function: Implications for anti-tumor immunity. Front Immunol 4: 192, 2013.
- Cramer T, Yamanishi Y, Clausen BE, Förster I, Pawlinski R, Mackman N, Haase VH, Jaenisch R, Corr M, Nizet V, *et al*: HIF-1alpha is essential for myeloid cell-mediated inflammation. Cell 112: 645-657, 2003.
- Lee JH, Elly C, Park Y and Liu YC: E3 ubiquitin ligase VHL regulates hypoxia-inducible factor-1α to maintain regulatory T cell stability and suppressive capacity. Immunity 42: 1062-1074, 2015.
- stability and suppressive capacity. Immunity 42: 1062-1074, 2015.
 12. Martínez D, Vermeulen M, Trevani A, Ceballos A, Sabatté J, Gamberale R, Alvarez ME, Salamone G, Tanos T, Coso OA and Geffner J: Extracellular acidosis induces neutrophil activation by a mechanism dependent on activation of phosphatidylinositol 3-kinase/Akt and ERK pathways. J Immunol 176: 1163-1171, 2006.
- Franklin RA, Liao W, Sarkar A, Kim MV, Bivona MR, Liu K, Pamer EG and Li MO: The cellular and molecular origin of tumor-associated macrophages. Science 344: 921-925, 2014.
- Erra Díaz F, Dantas E and Geffner J: Unravelling the interplay between extracellular acidosis and immune cells. Mediators Inflamm 2018: 1218297, 2018.
- Roma-Rodrigues C, Mendes R, Baptista PV and Fernandes AR: Targeting tumor microenvironment for cancer therapy. Int J Mol Sci 20: 840, 2019.
- Huber V, Camisaschi C, Berzi A, Ferro S, Lugini L, Triulzi T, Tuccitto A, Tagliabue E, Castelli C and Rivoltini L: Cancer acidity: An ultimate frontier of tumor immune escape and a novel target of immunomodulation. Semin Cancer Biol 43: 74-89, 2017.
- McDonald PC, Chafe SC and Dedhar S: Overcoming hypoxiamediated tumor progression: Combinatorial approaches targeting pH regulation, angiogenesis and immune dysfunction. Front Cell Dev Biol 4: 27, 2016.
- Li J, Guo B, Wang J, Cheng X, Xu Y and Sang J: Ovarian cancer G protein coupled receptor 1 suppresses cell migration of MCF7 breast cancer cells via a Gα12/13-Rho-Rac1 pathway. J Mol Signal 8: 6, 2013.
- Wiley SZ, Sriram K, Liang W, Chang SE, French R, McCann T, Sicklick J, Nishihara H, Lowy AM and Insel PA: GPR68, a proton-sensing GPCR, mediates interaction of cancer-associated fibroblasts and cancer cells. FASEB J 32: 1170-1183, 2018.
- Pérez-Escuredo J, Van Hée VF, Sboarina M, Falces J, Payen VL, Pellerin L and Sonveaux P: Monocarboxylate transporters in the brain and in cancer. Biochim Biophys Acta 1863: 2481-2497, 2016.
- 21. Halestrap AP and Wilson MC: The monocarboxylate transporter family-role and regulation. IUBMB Life 64: 109-119, 2012.
- 22. Alfarouk KO: Tumor metabolism, cancer cell transporters, and microenvironmental resistance. J Enzyme Inhib Med Chem 31: 859-866, 2016.
- Merezhinskaya N, Ogunwuyi SA, Mullick FG and Fishbein WN: Presence and localization of three lactic acid transporters (MCT1, -2, and -4) in separated human granulocytes, lymphocytes, and monocytes. J Histochem Cytochem 52: 1483-1493, 2004.



- 24. Merezhinskaya N, Ogunwuyi SA and Fishbein WN: Expression of monocarboxylate transporter 4 in human platelets, leukocytes, and tissues assessed by antibodies raised against terminal versus pre-terminal peptides. Mol Genet Metab 87: 152-161, 2006.
- 25. Long Y, Gao Z, Hu X, Xiang F, Wu Z, Zhang J, Han X, Yin L, Qin J, Lan L, *et al*: Downregulation of MCT4 for lactate exchange promotes the cytotoxicity of NK cells in breast carcinoma. Cancer Med 7: 4690-4700, 2018.
- 26. Sukumar M, Liu J, Ji Y, Subramanian M, Crompton JG, Yu Z, Roychoudhuri R, Palmer DC, Muranski P, Karoly ED, *et al*: Inhibiting glycolytic metabolism enhances CD8+ T cell memory and antitumor function. J Clin Invest 123: 4479-4488, 2013.
- 27. Fischer K, Hoffmann P, Voelkl S, Meidenbauer N, Ammer J, Edinger M, Gottfried E, Schwarz S, Rothe G, Hoves S, *et al*: Inhibitory effect of tumor cell-derived lactic acid on human T cells. Blood 109: 3812-3819, 2007.
- Murray CM, Hutchinson R, Bantick JR, Belfield GP, Benjamin AD, Brazma D, Bundick RV, Cook ID, Craggs RI, Edwards S, *et al*: Monocarboxylate transporter MCT1 is a target for immunosuppression. Nat Chem Biol 1: 371-376, 2005.
- Végran F, Boidot R, Michiels C, Sonveaux P and Feron O: Lactate influx through the endothelial cell monocarboxylate transporter MCT1 supports an NF-κB/IL-8 pathway that drives tumor angiogenesis. Cancer Res 71: 2550-2560, 2011.
- 30. Huang T, Feng Q, Wang Z, Li W, Sun Z, Wilhelm J, Huang G, Vo T, Sumer BD and Gao J: Tumor-targeted inhibition of monocarboxylate transporter 1 improves T-cell immunotherapy of solid tumors. Adv Healthc Mater 10: e2000549, 2021.
- Raychaudhuri D, Bhattacharya R, Sinha BP, Liu CSC, Ghosh AR, Rahaman O, Bandopadhyay P, Sarif J, D'Rozario R, Paul S, *et al*: Lactate induces pro-tumor reprogramming in intratumoral plasmacytoid dendritic cells. Front Immunol 10: 1878, 2019.
- 32. Gottfried E, Kunz-Schughart LA, Ebner S, Mueller-Klieser W, Hoves S, Andreesen R, Mackensen A and Kreutz M: Tumor-derived lactic acid modulates dendritic cell activation and antigen expression. Blood 107: 2013-2021, 2006.
- 33. Bonuccelli G, Whitaker-Menezes D, Castello-Cros R, Pavlides S, Pestell RG, Fatatis A, Witkiewicz AK, Vander Heiden MG, Migneco G, Chiavarina B, *et al*: The reverse Warburg effect: Glycolysis inhibitors prevent the tumor promoting effects of caveolin-1 deficient cancer associated fibroblasts. Cell Cycle 9: 1960-1971, 2010.
- Wei L, Zhou Y, Yao J, Qiao C, Ni T, Guo R, Guo Q and Lu N: Lactate promotes PGE2 synthesis and gluconeogenesis in monocytes to benefit the growth of inflammation-associated colorectal tumor. Oncotarget 6: 16198-16214, 2015.
 Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V,
- 35. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, Cyrus N, Brokowski CE, Eisenbarth SC, Phillips GM, *et al*: Functional polarization of tumour-associated macrophages by tumour-derived lactic acid. Nature 513: 559-563, 2014.
- 36. Tan Z, Xie N, Banerjee S, Cui H, Fu M, Thannickal VJ and Liu G: The monocarboxylate transporter 4 is required for glycolytic reprogramming and inflammatory response in macrophages. J Biol Chem 290: 46-55, 2015.
- Zhang L and Li S: Lactic acid promotes macrophage polarization through MCT-HIF1α signaling in gastric cancer. Exp Cell Res 388: 111846, 2020.
- Res 388: 111846, 2020.
 38. Stone SC, Rossetti RA, Alvarez KL, Carvalho JP, Margarido PF, Baracat EC, Tacla M, Boccardo E, Yokochi K, Lorenzi NP and Lepique AP: Lactate secreted by cervical cancer cells modulates macrophage phenotype. J Leukoc Biol 105: 1041-1054, 2019.
- KongL, WangZ, LiangX, WangY, GaoLandMaC: Monocarboxylate transporter 1 promotes classical microglial activation and pro-inflammatory effect via 6-phosphofructo-2-kinase/fructose-2, 6-biphosphatase 3. J Neuroinflammation 16: 240, 2019.
- 40. Silva LS, Poschet G, Nonnenmacher Y, Becker HM, Sapcariu S, Gaupel AC, Schlotter M, Wu Y, Kneisel N, Seiffert M, et al: Branched-chain ketoacids secreted by glioblastoma cells via MCT1 modulate macrophage phenotype. EMBO Rep 18: 2172-2185, 2017.
- 41. Contreras-Baeza Y, Sandoval PY, Alarcón R, Galaz A, Cortés-Molina F, Alegría K, Baeza-Lehnert F, Arce-Molina R, Guequén A, Flores CA, *et al*: Monocarboxylate transporter 4 (MCT4) is a high affinity transporter capable of exporting lactate in high-lactate microenvironments. J Biol Chem 294: 20135-20147, 2019.
- 42. Mikkilineni L, Whitaker-Menezes D, Domingo-Vidal M, Sprandio J, Avena P, Cotzia P, Dulau-Florea A, Gong J, Uppal G, Zhan T, *et al*: Hodgkin lymphoma: A complex metabolic ecosystem with glycolytic reprogramming of the tumor microenvironment. Semin Oncol 44: 218-225, 2017.

- 43. Afonso J, Pinto T, Simões-Sousa S, Schmitt F, Longatto-Filho A, Pinheiro C, Marques H and Baltazar F: Clinical significance of metabolism-related biomarkers in non-Hodgkin lymphoma-MCT1 as potential target in diffuse large B cell lymphoma. Cell Oncol (Dordr) 42: 303-318, 2019.
- 44. Beloueche-Babari M, Casals Galobart T, Delgado-Goni T, Wantuch S, Parkes HG, Tandy D, Harker JA and Leach MO: Monocarboxylate transporter 1 blockade with AZD3965 inhibits lipid biosynthesis and increases tumour immune cell infiltration. Br J Cancer 122: 895-903, 2020.
- 45. Noble RA, Bell N, Blair H, Sikka A, Thomas H, Phillips N, Nakjang S, Miwa S, Crossland R, Rand V, et al: Inhibition of monocarboxyate transporter 1 by AZD3965 as a novel therapeutic approach for diffuse large B-cell lymphoma and Burkitt lymphoma. Haematologica 102: 1247-1257, 2017.
- 46. Braga M, Kaliszczak M, Carroll L, Schug ZT, Heinzmann K, Baxan N, Benito A, Valbuena GN, Stribbling S, Beckley A, *et al*: Tracing nutrient flux following monocarboxylate transporter-1 inhibition with AZD3965. Cancers (Basel) 12: 1703, 2020.
- 47. Draoui N, Schicke O, Seront E, Bouzin C, Sonveaux P, Riant O and Feron O: Antitumor activity of 7-aminocarboxycoumarin derivatives, a new class of potent inhibitors of lactate influx but not efflux. Mol Cancer Ther 13: 1410-1418, 2014.
- 48. Quanz M, Bender E, Kopitz C, Grünewald S, Schlicker A, Schwede W, Eheim A, Toschi L, Neuhaus R, Richter C, *et al:* Preclinical efficacy of the novel monocarboxylate transporter 1 inhibitor BAY-8002 and associated markers of resistance. Mol Cancer Ther 17: 2285-2296, 2018.
- 49. Renner K, Bruss C, Schnell A, Koehl G, Becker HM, Fante M, Menevse AN, Kauer N, Blazquez R, Hacker L, *et al*: Restricting glycolysis preserves T cell effector functions and augments checkpoint therapy. Cell Rep 29: 135-150.e9, 2019.
- checkpoint therapy. Cell Rep 29: 135-150.e9, 2019.
 50. Cho KS, Yamada T, Wynn C, Behanna HA, Hong IC, Manaves V, Nakanishi T, Hirose J, Abe Y, Jiang H, *et al*: Mechanism analysis of long-term graft survival by monocarboxylate transporter-1 inhibition. Transplantation 90: 1299-1306, 2010.
- Kondapalli KC, Prasad H and Rao R: An inside job: How endosomal Na(+)/H(+) exchangers link to autism and neurological disease. Front Cell Neurosci 8: 172, 2014.
- De Vito P: The sodium/hydrogen exchanger: A possible mediator of immunity. Cell Immunol 240: 69-85, 2006.
- 53. Vereninov AA, Vassilieva IO, Yurinskaya VE, Matveev VV, Glushankova LN, Lang F and Matskevitch JA: Differential transcription of ion transporters, NHE1, ATP1B1, NKCC1 in human peripheral blood lymphocytes activated to proliferation. Cell Physiol Biochem 11: 19-26, 2001.
- 54. Chang CP, Wang SW, Huang ZL, Wang OY, Huang MI, Lu LM, Tarng DC, Chien CH and Chien EJ: Non-genomic rapid inhibition of Na⁺/H⁺-exchange 1 and apoptotic immunosuppression in human T cells by glucocorticoids. J Cell Physiol 223: 679-686, 2010.
- 55. Chien EJ, Hsu CH, Chang VH, Lin EP, Kuo TP, Chien CH and Lin HY: In human T cells mifepristone antagonizes glucocorticoid non-genomic rapid responses in terms of Na(+)/H(+)-exchange 1 activity, but not ezrin/radixin/moesin phosphorylation. Steroids 111: 29-36, 2016.
- Xing K, Gu B, Zhang P and Wu X: Dexamethasone enhances programmed cell death 1 (PD-1) expression during T cell activation: An insight into the optimum application of glucocorticoids in anti-cancer therapy. BMC Immunol 16: 39, 2015.
 Lai JN, Wang OY, Lin VH, Liao CF, Tarng DC and Chien EJ:
- 57. Lai JN, Wang OY, Lin VH, Liao CF, Tarng DC and Chien EJ: The non-genomic rapid acidification in peripheral T cells by progesterone depends on intracellular calcium increase and not on Na⁺/H⁺-exchange inhibition. Steroids 77: 1017-1024, 2012.
- 58. Singh Y, Zhou Y, Shi X, Zhang S, Umbach AT, Salker MS, Lang KS and Lang F: Alkaline cytosolic pH and high sodium hydrogen exchanger 1 (NHE1) activity in Th9 cells. J Biol Chem 291: 23662-23671, 2016.
- Rotte A, Pasham V, Eichenmüller M, Yang W, Bhandaru M and Lang F: Influence of dexamethasone on Na⁺/H⁺ exchanger activity in dendritic cells. Cell Physiol Biochem 28: 305-314, 2011.
- 60. Yang W, Bhandaru M, Pasham V, Bobbala D, Zelenak C, Jilani K, Rotte A and Lang F: Effect of thymoquinone on cytosolic pH and Na⁺/H⁺ exchanger activity in mouse dendritic cells. Cell Physiol Biochem 29: 21-30, 2012.
- Zhou Y, Pasham V, Chatterjee S, Rotte A, Yang W, Bhandaru M, Singh Y and Lang F: Regulation of Na⁺/H⁺ exchanger in dendritic cells by Akt1. Cell Physiol Biochem 36: 1237-1249, 2015.

- 62. Zhu W, Carney KE, Pigott VM, Falgoust LM, Clark PA, Kuo JS and Sun D: Glioma-mediated microglial activation promotes glioma proliferation and migration: Roles of Na+/H+ exchanger isoform 1. Carcinogenesis 37: 839-851, 2016.
- 63. Song S, Wang S, Pigott VM, Jiang T, Foley LM, Mishra A, Nayak R, Zhu W, Begum G, Shi Y, *et al*: Selective role of Na⁺/H⁺ exchanger in Cx3crl⁺ microglial activation, white matter demyelination, and post-stroke function recovery. Glia 66: 2279-2298, 2018.
- 64. Liu CL, Zhang X, Liu J, Wang Y, Sukhova GK, Wojtkiewicz GR, Liu T, Tang R, Achilefu S, Nahrendorf M, et al: Na⁺-H⁺ exchanger 1 determines atherosclerotic lesion acidification and promotes atherogenesis. Nat Commun 10: 3978, 2019.
- 65. Takakuwa S, Mizuno N, Takano T, Asakawa S, Sato T, Hiratsuka M and Hirasawa N: Down-regulation of Na⁺/H⁺ exchanger 1 by Toll-like receptor stimulation in macrophages. Immunobiology 222: 176-182, 2017.
- 66. Provost JJ and Wallert MA: Inside out: Targeting NHE1 as an intracellular and extracellular regulator of cancer progression. Chem Biol Drug Des 81: 85-101, 2013.
- 67. Asgharzadeh MR, Barar J, Pourseif MM, Eskandani M, Jafari Niya M, Mashayekhi MR and Omidi Y: Molecular machineries of pH dysregulation in tumor microenvironment: Potential targets for cancer therapy. Bioimpacts 7: 115-133, 2017.
- 68. Shi Y, Chanana V, Watters JJ, Ferrazzano P and Sun D: Role of sodium/hydrogen exchanger isoform 1 in microglial activation and proinflammatory responses in ischemic brains. J Neurochem 119: 124-135, 2011.
- J Neurochem 119: 124-135, 2011.
 69. Liu CL, Liu X, Wang Y, Deng Z, Liu T, Sukhova GK, Wojtkiewicz GR, Tang R, Zhang JY, Achilefu S, *et al*: Reduced Nhe1 (Na⁺-H⁺ Exchanger-1) function protects ApoE-deficient mice From Ang II (Angiotensin II)-induced abdominal aortic aneurysms. Hypertension 76: 87-100, 2020.
- 70. Guan X, Hasan MN, Begum G, Kohanbash G, Carney KE, Pigott VM, Persson AI, Castro MG, Jia W and Sun D: Blockade of Na/H exchanger stimulates glioma tumor immunogenicity and enhances combinatorial TMZ and anti-PD-1 therapy. Cell Death Dis 9: 1010, 2018.
- 71. Jin W, Li Q, Wang J, Chang G, Lin Y, Li H, Wang L, Gao W and Pang T: Na⁺/H⁺ exchanger 1 inhibition contributes to K562 leukaemic cell differentiation. Cell Biol Int 36: 739-745, 2012.
- 72. Fliegel L: Structural and functional changes in the Na⁺/H⁺ exchanger isoform 1, induced by Erk1/2 phosphorylation. Int J Mol Sci 20: 2378, 2019.
- 73. Smith AN, Lovering RC, Futai M, Takeda J, Brown D and Karet FE: Revised nomenclature for mammalian vacuolar-type H⁺ -ATPase subunit genes. Mol Cell 12: 801-803, 2003.
- Forgac M: Vacuolar ATPases: Rotary proton pumps in physiology and pathophysiology. Nat Rev Mol Cell Biol 8: 917-929, 2007.
- McGuire C, Stransky L, Cotter K and Forgac M: Regulation of V-ATPase activity. Front Biosci (Landmark Ed) 22: 609-622, 2017.
- 76. Kulshrestha A, Katara GK, Ginter J, Pamarthy S, Ibrahim SA, Jaiswal MK, Sandulescu C, Periakaruppan R, Dolan J, Gilman-Sachs A and Beaman KD: Selective inhibition of tumor cell associated Vacuolar-ATPase 'a2' isoform overcomes cisplatin resistance in ovarian cancer cells. Mol Oncol 10: 789-805, 2016.
- 77. Smith GA, Howell GJ, Phillips C, Muench SP, Ponnambalam S and Harrison MA: Extracellular and luminal pH regulation by vacuolar H⁺-ATPase isoform expression and targeting to the plasma membrane and endosomes. J Biol Chem 291: 8500-8515, 2016.
- Stransky L, Cotter K and Forgac M: The function of V-ATPases in cancer. Physiol Rev 96: 1071-1091, 2016.
- 79. Rao Z, Jordan PM, Wang Y, Menche D, Pace S, Gerstmeier J and Werz O: Differential role of vacuolar (H⁺)-ATPase in the expression and activity of cyclooxygenase-2 in human monocytes. Biochem Pharmacol 175: 113858, 2020.
- 80. Sahoo M, Katara GK, Bilal MY, Ibrahim SA, Kulshrestha A, Fleetwood S, Suzue K and Beaman KD: Hematopoietic stem cell specific V-ATPase controls breast cancer progression and metastasis via cytotoxic T cells. Oncotarget 9: 33215-33231, 2018.
- Peterson TV, Jaiswal MK, Beaman KD and Reynolds JM: Conditional deletion of the V-ATPase a2-subunit disrupts intrathymic T cell development. Front Immunol 10: 1911, 2019.
- Rothenberg EV, Ungerbäck J and Champhekar A: Forging T-lymphocyte identity: Intersecting networks of transcriptional control. Adv Immunol 129: 109-174, 2016.

- McGuire C, Cotter K, Stransky L and Forgac M: Regulation of V-ATPase assembly and function of V-ATPases in tumor cell invasiveness. Biochim Biophys Acta 1857: 1213-1218, 2016.
- 84. Ibrahim SA, Kulshrestha A, Katara GK, Amin MA and Beaman KD: Cancer derived peptide of vacuolar ATPase 'a2' isoform promotes neutrophil migration by autocrine secretion of IL-8. Sci Rep 6: 36865, 2016.
 85. Katara GK, Jaiswal MK, Kulshrestha A, Kolli B,
- 85. Katara GK, Jaiswal MK, Kulshrestha A, Kolli B, Gilman-Sachs A and Beaman KD: Tumor-associated vacuolar ATPase subunit promotes tumorigenic characteristics in macrophages. Oncogene 33: 5649-5654, 2014.
- 86. Katara GK, Kulshrestha A, Mao L, Wang X, Sahoo M, Ibrahim S, Pamarthy S, Suzue K, Shekhawat GS, Gilman-Sachs A and Beaman KD: Mammary epithelium-specific inactivation of V-ATPase reduces stiffness of extracellular matrix and enhances metastasis of breast cancer. Mol Oncol 12: 208-223, 2018.
- Ibrahim SA, Kulshrestha A, Katara GK, Riehl V, Sahoo M and Beaman KD: Cancer-associated V-ATPase induces delayed apoptosis of protumorigenic neutrophils. Mol Oncol 14: 590-610, 2020.
- 88. Gilman-Sachs A, Tikoo A, Akman-Anderson L, Jaiswal M, Ntrivalas E and Beaman K: Expression and role of a2 vacuolar-ATPase (a2V) in trafficking of human neutrophil granules and exocytosis. J Leukoc Biol 97: 1121-1131, 2015.
- 89. Xia Y, Liu N, Xie X, Bi G, Ba H, Li L, Zhang J, Deng X, Yao Y, Tang Z, et al: The macrophage-specific V-ATPase subunit ATP6V0D2 restricts inflammasome activation and bacterial infection by facilitating autophagosome-lysosome fusion. Autophagy 15: 960-975, 2019.
- 90. Murase M, Kawasaki T, Hakozaki R, Sueyoshi T, Putri DD, Kitai Y, Sato S, Ikawa M and Kawai T: Intravesicular acidification regulates lipopolysaccharide inflammation and tolerance through TLR4 trafficking. J Immunol 200: 2798-2808, 2018.
- 91. Kimura T, Nada S, Takegahara N, Okuno T, Nojima S, Kang S, Ito D, Morimoto K, Hosokawa T, Hayama Y, *et al*: Polarization of M2 macrophages requires Lamtorl that integrates cytokine and amino-acid signals. Nat Commun 7: 13130, 2016.
- 92. Rao Z, Pace S, Jordan PM, Bilancia R, Troisi F, Börner F, Andreas N, Kamradt T, Menche D, Rossi A, *et al*: Vacuolar (H⁺)-ATPase critically regulates specialized proresolving mediator pathways in human M2-like monocyte-derived macrophages and has a crucial role in resolution of inflammation. J Immunol 203: 1031-1043, 2019.
- 93. Kuchuk O, Tuccitto A, Citterio D, Huber V, Camisaschi C, Milione M, Vergani B, Villa A, Alison MR, Carradori S, *et al*: pH regulators to target the tumor immune microenvironment in human hepatocellular carcinoma. OncoImmunology 7: e1445452, 2018.
- 94. Thomas L, Rao Z, Gerstmeier J, Raasch M, Weinigel C, Rummler S, Menche D, Müller R, Pergola C, Mosig A and Werz O: Selective upregulation of TNFα expression in classically-activated human monocyte-derived macrophages (M1) through pharmacological interference with V-ATPase. Biochem Pharmacol 130: 71-82, 2017.
- 95. Bowman EJ, Graham LA, Stevens TH and Bowman BJ: The bafilomycin/concanamycin binding site in subunit c of the V-ATPases from Neurospora crassa and Saccharomyces cerevisiae. J Biol Chem 279: 33131-33138, 2004.
- 96.Lu X, Chen L, Chen Y, Shao Q and Qin W: Bafilomycin A1 inhibits the growth and metastatic potential of the BEL-7402 liver cancer and HO-8910 ovarian cancer cell lines and induces alterations in their microRNA expression. Exp Ther Med 10: 1829-1834, 2015.
- 97. Halcrow P, Khan N, Datta G, Ohm JE, Chen X and Geiger JD: Importance of measuring endolysosome, cytosolic, and extracellular pH in understanding the pathogenesis of and possible treatments for glioblastoma multiforme. Cancer Rep 2: e1193, 2019.
- 98. Yuan N, Song L, Zhang S, Lin W, Cao Y, Xu F, Fang Y, Wang Z, Zhang H, Li X, *et al*: Bafilomycin A1 targets both autophagy and apoptosis pathways in pediatric B-cell acute lymphoblastic leukemia. Haematologica 100: 345-356, 2015.
- 99. Emruli VK, Olsson R, Ek F and Ek S: Identification of V-ATPase as a molecular sensor of SOX11-levels and potential therapeutic target for mantle cell lymphoma. BMC Cancer 16: 493, 2016.
- 100. Fais S: Proton pump inhibitor-induced tumour cell death by inhibition of a detoxification mechanism. J Intern Med 267: 515-525, 2010.
- 101. De Milito A, Canese R, Marino ML, Borghi M, Iero M, Villa A, Venturi G, Lozupone F, Iessi E, Logozzi M, *et al*: pH-dependent antitumor activity of proton pump inhibitors against human melanoma is mediated by inhibition of tumor acidity. Int J Cancer 127: 207-219, 2010.



13

- 102. Denny WA and Wilson WR: Considerations for the design of nitrophenyl mustards as agents with selective toxicity for hypoxic tumor cells. J Med Chem 29: 879-887, 1986.
- 103. Luciani F, Spada M, De Milito A, Molinari A, Rivoltini L, Montinaro A, Marra M, Lugini L, Logozzi M, Lozupone F, *et al*: Effect of proton pump inhibitor pretreatment on resistance of solid tumors to cytotoxic drugs. J Natl Cancer Inst 96: 1702-1713, 2004.
- 104. Corbet C and Feron O: Tumour acidosis: From the passenger to the driver's seat. Nat Rev Cancer 17: 577-593, 2017.
- 105. Neri D and Supuran CT: Interfering with pH regulation in tumours as a therapeutic strategy. Nat Rev Drug Discov 10: 767-777, 2011.
- 106. Fan J, Kamphorst JJ, Mathew R, Chung MK, White E, Shlomi T and Rabinowitz JD: Glutamine-driven oxidative phosphorylation is a major ATP source in transformed mammalian cells in both normoxia and hypoxia. Mol Syst Biol 9: 712, 2013.
- 107. Sedlakova O, Svastova E, Takacova M, Kopacek J, Pastorek J and Pastorekova S: Carbonic anhydrase IX, a hypoxia-induced catalytic component of the pH regulating machinery in tumors. Front Physiol 4: 400, 2014.
- 108. Hunt JF, Fang K, Malik R, Snyder A, Malhotra N, Platts-Mills TA and Gaston B: Endogenous airway acidification. Implications for asthma pathophysiology. Am J Respir Crit Care Med 161 (3 Pt 1): 694-699, 2000.
- 109. Wen T, Mingler MK, Wahl B, Khorki ME, Pabst O, Zimmermann N and Rothenberg ME: Carbonic anhydrase IV is expressed on IL-5-activated murine eosinophils. J Immunol 192: 5481-5489, 2014.
- 110. Pulendran B and Artis D: New paradigms in type 2 immunity. Science 337: 431-435, 2012.
- 111. Henry EK, Sy CB, Inclan-Rico JM, Espinosa V, Ghanny SS, Dwyer DF, Soteropoulos P, Rivera A and Siracusa MC: Carbonic anhydrase enzymes regulate mast cell-mediated inflammation. J Exp Med 213: 1663-1673, 2016.
- 112. Noti M, Kim BS, Siracusa MC, Rak GD, Kubo M, Moghaddam AE, Sattentau QA, Comeau MR, Spergel JM and Artis D: Exposure to food allergens through inflamed skin promotes intestinal food allergy through the thymic stromal lymphopoietin-basophil axis. J Allergy Clin Immunol 133: 1390-1399, 1399.e1-6, 2014.
- 113. Winum JY: Carbonic anhydrase enzymes for regulating mast cell hematopoiesis and type-2 inflammation: A patent evaluation (WO2017/058370). Expert Opin Ther Pat 28: 741-743, 2018.
- 114. Supuran CT: Carbonic anhydrase inhibitors and their potential in a range of therapeutic areas. Expert Opin Ther Pat 28: 709-712, 2018.
- 115. Supuran CT, Altamimi ASA and Carta F: Carbonic anhydrase inhibition and the management of glaucoma: A literature and patent review 2013-2019. Expert Opin Ther Pat 29: 781-792, 2019.
- 116. Borenshtein D, Schlieper KA, Rickman BH, Chapman JM, Schweinfest CW, Fox JG and Schauer DB: Decreased expression of colonic Slc26a3 and carbonic anhydrase iv as a cause of fatal infectious diarrhea in mice. Infect Immun 77: 3639-3650, 2009.
- 117. Mori K, Yamanishi H, Ikeda Y, Kumagi T, Hiasa Y, Matsuura B, Abe M and Onji M: Oral administration of carbonic anhydrase I ameliorates murine experimental colitis induced by Foxp3-CD4+CD25-T cells. J Leukoc Biol 93: 963-972, 2013.
- 118. Lavin Y, Winter D, Blecher-Gonen R, David E, Keren-Shaul H, Merad M, Jung S and Amit I: Tissue-resident macrophage enhancer landscapes are shaped by the local microenvironment. Cell 159: 1312-1326, 2014.
- 119. Lounnas N, Rosilio C, Nebout M, Mary D, Griessinger E, Neffati Z, Chiche J, Spits H, Hagenbeek TJ, Asnafi V, *et al*: Pharmacological inhibition of carbonic anhydrase XII interferes with cell proliferation and induces cell apoptosis in T-cell lymphomas. Cancer Lett 333: 76-88, 2013.
- 120. Ibrahim-Hashim A and Estrella V: Acidosis and cancer: From mechanism to neutralization. Cancer Metastasis Rev 38: 149-155, 2019.
- 121. Chafe SC, McDonald PC, Saberi S, Nemirovsky O, Venkateswaran G, Burugu S, Gao D, Delaidelli A, Kyle AH, Baker JHE, *et al*: Targeting hypoxia-induced carbonic anhydrase IX enhances immune-checkpoint blockade locally and systemically. Cancer Immunol Res 7: 1064-1078, 2019.
- 122. McDonald PC, Chia S, Bedard PL, Chu Q, Lyle M, Tang L, Singh M, Zhang Z, Supuran CT, Renouf DJ and Dedhar S: A phase 1 study of SLC-0111, a novel inhibitor of carbonic anhydrase IX, in patients with advanced solid tumors. Am J Clin Oncol 43: 484-490, 2020.

- 123. Nasu K, Yamaguchi K, Takanashi T, Tamai K, Sato I, Ine S, Sasaki O, Satoh K, Tanaka N, Tanaka Y, *et al*: Crucial role of carbonic anhydrase IX in tumorigenicity of xenotransplanted adult T-cell leukemia-derived cells. Cancer Sci 108: 435-443, 2017.
- 124. Chen LQ, Howison CM, Spier C, Stopeck AT, Malm SW, Pagel MD and Baker AF: Assessment of carbonic anhydrase IX expression and extracellular pH in B-cell lymphoma cell line models. Leuk Lymphoma 56: 1432-1439, 2015.
- 125. Mei Y, Zhao L, Liu Y, Gong H, Song Y, Lei L, Zhu Y, Jin Z, Ma S, Hu B, *et al*: Combining DNA vaccine and AIDA-1 in attenuated Salmonella activates tumor-specific CD4⁺ and CD8⁺ T-cell responses. Cancer Immunol Res 5: 503-514, 2017.
- 126. Sun YY, Peng S, Han L, Qiu J, Song L, Tsai Y, Yang B, Roden RB, Trimble CL, Hung CF and Wu TC: Local HPV Recombinant vaccinia boost following priming with an HPV DNA vaccine enhances local HPV-specific CD8⁺ T-cell-mediated tumor control in the genital tract. Clin Cancer Res 22: 657-669, 2016.
- 127. Duan Y, Yang C, Zhang Z, Liu J, Zheng J and Kong D: Poly(ethylene glycol)-grafted polyethylenimine modified with G250 monoclonal antibody for tumor gene therapy. Hum Gene Ther 21: 191-198, 2010.
- 128. Zhao Y, Wei Z, Yang H, Li X, Wang Q, Wang L and Li S: Enhance the anti-renca carcinoma effect of a DNA vaccine targeting G250 gene by co-expression with cytotoxic T-lymphocyte associated antigen-4(CTLA-4). Biomed Pharmacother 90: 147-152, 2017.
- 129. Chai D, Shan H, Wang G, Zhang Q, Li H, Fang L, Song J, Liu N, Zhang Q, Yao H and Zheng J: Combining DNA vaccine and AIM2 in H1 nanoparticles exert anti-renal carcinoma effects via enhancing tumor-specific multi-functional CD8⁺ T-cell responses. Mol Cancer Ther 18: 323-334, 2019.
- 130. Zhang Q, Xu J, Ding J, Liu H, Li H, Li H, Lu M, Miao Y, Wang Z, Fu Q and Zheng J: Bortezomib improves adoptive carbonic anhydrase IX-specific chimeric antigen receptor-modified NK92 cell therapy in mouse models of human renal cell carcinoma. Oncol Rep 40: 3714-3724, 2018.
- 131. Li H, Ding J, Lu M, Liu H, Miao Y, Li L, Wang G, Zheng J, Pei D and Zhang Q: CAIX-specific CAR-T cells and sunitinib show synergistic effects against metastatic renal cancer models. J Immunother 43: 16-28, 2020.
- 132. Lau J, Liu Z, Lin KS, Pan J, Zhang Z, Vullo D, Supuran CT, Perrin DM and Bénard F: Trimeric radiofluorinated sulfonamide derivatives to achieve in vivo selectivity for carbonic anhydrase IX-targeted PET imaging. J Nucl Med 56: 1434-1440, 2015.
- 133. Dubois LJ, Niemans R, van Kuijk SJ, Panth KM, Parvathaneni NK, Peeters SG, Zegers CM, Rekers NH, van Gisbergen MW, Biemans R, et al: New ways to image and target tumour hypoxia and its molecular responses. Radiother Oncol 116: 352-357, 2015.
- 134. Justus CR, Dong L and Yang LV: Acidic tumor microenvironment and pH-sensing G protein-coupled receptors. Front Physiol 4: 354, 2013.
- 135. Liu JP, Nakakura T, Tomura H, Tobo M, Mogi C, Wang JQ, He XD, Takano M, Damirin A, Komachi M, *et al*: Each one of certain histidine residues in G-protein-coupled receptor GPR4 is critical for extracellular proton-induced stimulation of multiple G-protein-signaling pathways. Pharmacol Res 61: 499-505, 2010.
- 136. Ludwig MG, Vanek M, Guerini D, Gasser JA, Jones CE, Junker U, Hofstetter H, Wolf RM and Seuwen K: Proton-sensing G-protein-coupled receptors. Nature 425: 93-98, 2003.
 137. Damaghi M, Wojtkowiak JW and Gillies RJ: pH sensing and
- 137. Damaghi M, Wojtkowiak JW and Gillies RJ: pH sensing and regulation in cancer. Front Physiol 4: 370, 2013.
- 138. Pilon-Thomas S, Kodumudi KN, El-Kenawi AE, Russell S, Weber AM, Luddy K, Damaghi M, Wojtkowiak JW, Mulé JJ, Ibrahim-Hashim A and Gillies RJ: Neutralization of tumor acidity improves antitumor responses to immunotherapy. Cancer Res 76: 1381-1390, 2016.
- 139. Wiley SZ, Sriram K, Salmerón C and Insel PA: GPR68: An emerging drug target in cancer. Int J Mol Sci 20: 559, 2019.
- 140. Lardner A: The effects of extracellular pH on immune function. J Leukoc Biol 69: 522-530, 2001.
- 141. de Vallière C, Wang Y, Eloranta JJ, Vidal S, Clay I, Spalinger MR, Tcymbarevich I, Terhalle A, Ludwig MG, Suply T, *et al*: G Protein-coupled pH-sensing receptor OGR1 is a regulator of intestinal inflammation. Inflamm Bowel Dis 21: 1269-1281, 2015.
- 142. Wang Y, de Vallière C, Imenez Silva PH, Leonardi I, Gruber S, Gerstgrasser A, Melhem H, Weber A, Leucht K, Wolfram L, *et al*: The proton-activated receptor GPR4 modulates intestinal inflammation. J Crohn's Colitis 12: 355-368, 2018.

- 143. Frasch SC, McNamee EN, Kominsky D, Jedlicka P, Jakubzick C, Zemski Berry K, Mack M, Furuta GT, Lee JJ, Henson PM, *et al*: G2A signaling dampens colitic inflammation via production of IFN-γ. J Immunol 197: 1425-1434, 2016.
- 144. Li HM, Jang JH, Jung JS, Shin J, Park CO, Kim YJ, Ahn WG, Nam JS, Hong CW, Lee J, *et al*: G2A protects mice against sepsis by modulating kupffer cell activation: Cooperativity with adenosine receptor 2b. J Immunol 202: 527-538, 2019.
- 145. Su YS, Huang YF, Wong J, Lee CW, Hsieh WS and Sun WH: G2A as a Threshold regulator of inflammatory hyperalgesia modulates chronic hyperalgesia. J Mol Neurosci 64: 39-50, 2018.
- 146. Osthues T, Zimmer B, Rimola V, Klann K, Schilling K, Mathoor P, Angioni C, Weigert A, Geisslinger G, Münch C, *et al*: The lipid receptor G2A (GPR132) mediates macrophage migration in nerve injury-induced neuropathic pain. Cells 9: 1740, 2020.
- 147. Kern K, Schäfer SMG, Cohnen J, Pierre S, Osthues T, Tarighi N, Hohmann S, Ferreiros N, Brüne B, Weigert A, *et al*: The G2A receptor controls polarization of macrophage by determining their localization within the inflamed tissue. Front Immunol 9: 2261, 2018.
- 148. Kung CC, Dai SP, Chiang H, Huang HS and Sun WH: Temporal expression patterns of distinct cytokines and M1/M2 macrophage polarization regulate rheumatoid arthritis progression. Mol Biol Rep 47: 3423-3437, 2020.
- 149. Dai SP, Hsieh WS, Chen CH, Lu YH, Huang HS, Chang DM, Huang SL and Sun WH: TDAG8 deficiency reduces satellite glial number and pro-inflammatory macrophage number to relieve rheumatoid arthritis disease severity and chronic pain. J Neuroinflammation 17: 170, 2020.
- 150. Tsurumaki H, Mogi C, Aoki-Saito H, Tobo M, Kamide Y, Yatomi M, Sato K, Dobashi K, Ishizuka T, Hisada T, *et al*: Protective role of proton-sensing TDAG8 in lipopolysaccharide-induced acute lung injury. Int J Mol Sci 16: 28931-28942, 2015.
- 151. Tcymbarevich I, Richards SM, Russo G, Kühn-Georgijevic J, Cosin-Roger J, Baebler K, Lang S, Bengs S, Atrott K, Bettoni C, et al: Lack of the pH-sensing receptor TDAG8 [GPR65] in macrophages plays a detrimental role in murine models of inflammatory bowel disease. J Crohns Colitis 13: 245-258, 2019.
- 152. Chen P, Zuo H, Xiong H, Kolar MJ, Chu Q, Saghatelian A, Siegwart DJ and Wan Y: Gpr132 sensing of lactate mediates tumor-macrophage interplay to promote breast cancer metastasis. Proc Natl Acad Sci USA 114: 580-585, 2017.
- 153. Yan L, Singh LS, Zhang L and Xu Y: Role of OGR1 in myeloid-derived cells in prostate cancer. Oncogene 33: 157-164, 2014.
- 154. Holgate ST: Innate and adaptive immune responses in asthma. Nat Med 18: 673-683, 2012.
- 155. Aoki H, Mogi C, Hisada T, Nakakura T, Kamide Y, Ichimonji I, Tomura H, Tobo M, Sato K, Tsurumaki H, *et al*: Proton-sensing ovarian cancer G protein-coupled receptor 1 on dendritic cells is required for airway responses in a murine asthma model. PLoS One 8: e79985, 2013.
- 156. Kottyan LC, Collier AR, Cao KH, Niese KA, Hedgebeth M, Radu CG, Witte ON, Khurana Hershey GK, Rothenberg ME and Zimmermann N: Eosinophil viability is increased by acidic pH in a cAMP- and GPR65-dependent manner. Blood 114: 2774-2782, 2009.
- 157. Sanderlin EJ, Marie M, Velcicky J, Loetscher P and Yang LV: Pharmacological inhibition of GPR4 remediates intestinal inflammation in a mouse colitis model. Eur J Pharmacol 852: 218-230, 2019.
- 158. Velcicky J, Miltz W, Oberhauser B, Orain D, Vaupel A, Weigand K, Dawson King J, Littlewood-Evans A, Nash M, Feifel R and Loetscher P: Development of selective, orally active GPR4 antagonists with modulatory effects on nociception, inflammation, and angiogenesis. J Med Chem 60: 3672-3683, 2017.
- 159. Miltz W, Velcicky J, Dawson J, Littlewood-Evans A, Ludwig MG, Seuwen K, Feifel R, Oberhauser B, Meyer A, Gabriel D, et al: Design and synthesis of potent and orally active GPR4 antagonists with modulatory effects on nociception, inflammation, and angiogenesis. Bioorg Med Chem 25: 4512-4525, 2017.

- 160. Fukuda H, Ito S, Watari K, Mogi C, Arisawa M, Okajima F, Kurose H and Shuto S: Identification of a potent and selective GPR4 antagonist as a drug lead for the treatment of myocardial infarction. ACS Med Chem Lett 7: 493-497, 2016.
- 161. Lengacher S, Nehiri-Sitayeb T, Steiner N, Carneiro L, Favrod C, Preitner F, Thorens B, Stehle JC, Dix L, Pralong F, et al: Resistance to diet-induced obesity and associated metabolic perturbations in haploinsufficient monocarboxylate transporter 1 mice. PLoS One 8: e82505, 2013.
- 162. Draoui N, Schicke O, Fernandes A, Drozak X, Nahra F, Dumont A, Douxfils J, Hermans E, Dogné JM, Corbau R, *et al*: Synthesis and pharmacological evaluation of carboxycoumarins as a new antitumor treatment targeting lactate transport in cancer cells. Bioorg Med Chem 21: 7107-7117, 2013.
- 163. Reshkin SJ, Cardone RA and Harguindey S: Na⁺-H⁺ exchanger, pH regulation and cancer. Recent Patents Anticancer Drug Discov 8: 85-99, 2013.
- 164. Ferrari S, Perut F, Fagioli F, Brach Del Prever A, Meazza C, Parafioriti A, Picci P, Gambarotti M, Avnet S, Baldini N and Fais S: Proton pump inhibitor chemosensitization in human osteosarcoma: From the bench to the patients' bed. J Transl Med 11: 268, 2013.
- 165. Mentzer RM Jr, Bartels C, Bolli R, Boyce S, Buckberg GD, Chaitman B, Haverich A, Knight J, Menasché P, Myers ML, et al: Sodium-hydrogen exchange inhibition by cariporide to reduce the risk of ischemic cardiac events in patients undergoing coronary artery bypass grafting: Results of the EXPEDITION study. Ann Thorac Surg 85: 1261-1270, 2008.
- 166. Zeymer U, Suryapranata H, Monassier JP, Opolski G, Davies J, Rasmanis G, Linssen G, Tebbe U, Schröder R, Tiemann R, *et al*: The Na(+)/H(+) exchange inhibitor eniporide as an adjunct to early reperfusion therapy for acute myocardial infarction. Results of the evaluation of the safety and cardioprotective effects of eniporide in acute myocardial infarction (ESCAMI) trial. J Am Coll Cardiol 38: 1644-1650, 2001.
- 167. Théroux P, Chaitman BR, Danchin N, Erhardt L, Meinertz T, Schroeder JS, Tognoni G, White HD, Willerson JT and Jessel A: Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations. Main results of the GUARDIAN trial. Guard during ischemia against necrosis (GUARDIAN) Investigators. Circulation 102: 3032-3038, 2000.
- 168. Atwal KS, O'Neil SV, Ahmad S, Doweyko L, Kirby M, Dorso CR, Chandrasena G, Chen BC, Zhao R and Zahler R: Synthesis and biological activity of 5-aryl-4-(4-(5-methyl-1H-imidazol-4-yl) piperidin-1-yl)pyrimidine analogs as potent, highly selective, and orally bioavailable NHE-1 inhibitors. Bioorg Med Chem Lett 16: 4796-4799, 2006.
- 169. Harguindey S, Arranz JL, Polo Orozco JD, Rauch C, Fais S, Cardone RA and Reshkin SJ: Cariporide and other new and powerful NHE1 inhibitors as potentially selective anticancer drugs-an integral molecular/biochemical/metabolic/clinical approach after one hundred years of cancer research. J Transl Med 11: 282, 2013.
- 170. Huber V, De Milito A, Harguindey S, Reshkin SJ, Wahl ML, Rauch C, Chiesi A, Pouysségur J, Gatenby RA, Rivoltini L and Fais S: Proton dynamics in cancer. J Transl Med 8: 57, 2010.
- 171. Spugnini EP, Sonveaux P, Stock C, Perez-Sayans M, De Milito A, Avnet S, Garcia AG, Harguindey S and Fais S: Proton channels and exchangers in cancer. Biochim Biophys Acta 1848: 2715-2726, 2015.
- 172. Chiche J, İlc K, Laferrière J, Trottier E, Dayan F, Mazure NM, Brahimi-Horn MC and Pouysségur J: Hypoxia-inducible carbonic anhydrase IX and XII promote tumor cell growth by counteracting acidosis through the regulation of the intracellular pH. Cancer Res 69: 358-368, 2009.
- 173. Salmon H, Remark R, Gnjatic S and Merad M: Host tissue determinants of tumour immunity. Nat Rev Cancer 19: 215-227, 2019.