Advances in research on the regulatory mechanism of NHE1 in tumors (Review)

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Abstract. Tumors pose a major threat to human health and present with difficulties that modern medicine has yet to overcome. It has been demonstrated that the acid-base balance of the tumor microenvironment is closely associated with the dynamic balance in the human body and that it regulates several processes, such as cell proliferation and differentiation, intracellular enzyme activity, and cytoskeletal assembly and depolymerization. It has been well established that the regulation of intra- and extracellular pH depends on a series of functional ion transporters and hydrogen ion channels, such as the Na^+/H^+ exchanger (NHE) protein and thee Cl/HCO_3^- exchange protein, among which the NHE1 member of the NHE family has been attracting increasing attention in recent years, particularly in studies on the correlation between pH regulation and tumors. NHE1 is a housekeeping gene encoding a protein that is widely expressed on the surface of all plasma membranes. Due to its functional domain, which determines the pHi at its N-terminus and C-terminus, NHE1 is involved in the regulation of the cellular pH microenvironment. It has been reported in the literature that NHE1 can regulate cell volume, participate in the transmembrane transport of intracellular and extracellular ions, affect cell proliferation and apoptosis, and regulate cell behavior and cell cycle progression; however, research on the role of NHE1 in tumorigenesis and tumor development in various systems is at its early stages. The aim of the present study was to review the current research on the correlation between the NHE family proteins and various systemic tumors, in order to indicate a new direction for antitumor drug development with the pH microenvironment as the target.

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Contents

1. Introduction

The human body can regulate the acid-base balance during normal metabolism. Under normal conditions, the body's pH will remain 7.35-7.45 over a long period of time. This balance is important for the maintenance of physiological processes in the body, including cell proliferation and apoptosis, the regulation of various enzyme activities, and assembly and depolymerization of the cytoskeleton, and it is closely associated with the precise regulation of pH (1,2). In recent years, with continued in-depth research, scholars have found that the acidic microenvironment of tumors participates in the regulation of various abnormal biological behaviors of tumor cells. The imbalance of intracellular and extracellular pH is an important factor in tumorigenesis and continued tumor development. Studies have reported that with the decrease in pH (acidification), the cells will undergo apoptosis and necrosis, and the acidic extracellular pH accelerates cell metabolism and enhances the abilities of tumor cell migration, invasion and metastasis. On the one hand, tumor cell invasion and metastasis largely depend on a series of proteases, such as metalloproteinases, thiol proteases, serine proteases and acid proteases, which can degrade a series of tissue barriers, and their activity can be enhanced in an acidic microenvironment, creating conditions favoring cancer cell invasion (3,4). On the other hand, the driving force for the migration and invasion of tumor cells is derived from the plate-like pseudopodia, which are primarily composed of actin and can protrude into the extracellular matrix (ECM) to assemble a microfilament mesh that

drives cytoskeletal formation; therefore, the acidic extracellular microenvironment of tumor cell \]may be an important factor in the early events of malignant transformation.

2. NHE family and NHE1

The formation of the extracellular acid-base milieu is mainly dependent on the efflux of excess intracellular hydrogen ions. A number of channels and genes are known to be involved in intra- and extracellular pH regulation, such as the Na^+/H^+ exchanger (NHE), Na^+-dependent and Na^+-independent Cl^-/HCO_3^--exchange protein, monocarboxylate transporter 1 (MCT1) and V-ATPase (5-7) (Fig. 1). The NHE1 subtype of the NHE family has been confirmed as an important hydrogen ion exchanger on the cell membrane, and it plays an important role in the regulatory mechanism of the pH balance inside and outside the cell.

The NHE family is a membrane-incorporated protein family that is widely expressed on eukaryotic cells, and it regulates the intra- and extracellular pH balance mainly through the 1:1 exchange of intracellular H^+ with extracellular Na^+ (8). Structurally, there are 12 α-helices at the N-terminus of the NHE, which play an important role in ion exchange. The C-terminus is a cytoplasmic regulatory domain, which is modified by various cell surface receptor-mediated extracellular signals that combine to regulate NHE transport activity (9). Nine subtypes of the NHE family have been identified and named NHE1-9 (10,11) according to the order of discovery, with each subtype exhibiting unique tissue distribution and functional characteristics. NHE1 is widely distributed in the plasma membrane of all tissues; NHE2 is mainly distributed in the kidney, intestine and adrenal gland; NHE3 is mainly distributed on the surface of the epithelial cell basement membrane (12) and is highly expressed in the small intestine and kidney; NHE4 is mainly expressed, albeit at low levels, in gastric epithelial cells and in cells of the small intestine and kidney (13); NHE5 is mainly expressed in the brain; and NHE6-NHE9 are distributed in intracellular compartments (14), namely the Golgi complex and Golgi postendosomal compartments in human cells.

The function of NHE1 is the most extensively investigated among all the subtypes. It is known that NHE1 consists of 815 amino acids, including a hydrophobic N-terminus consisting of 500 amino acids and a hydrophilic C-terminus consisting of 315 amino acids. The hydrophobic N-terminus mediates the transport of ions. Na^+/K^+ ATPase produces a Na^+ chemical gradient, driving one H^+ from the inside to the outside of the cell in exchange for one extracellular Na^+ that enters the cell (15). The Na^+/K^+ ATPase C-terminus participates in the regulation of NHE1 activity and function, mainly by mediating the following three functions: i) The intracellular H^+ level, which enhances the conformational change of the proton-binding site on the C-terminus, increases NHE1 activity and pumps H^+ into the extracellular space; ii) extracellular stimulation can phosphorylate some of the serine/threonine residues in the C-terminus, thereby activating NHE1 activity (16); and iii) C-terminus-specific protein binding sites that activate NHE1 upon binding of specific proteins, such as calmodulin MJ, calcineurin homologous protein I-J, carbonic anhydrase II-J and ERM protein 91 (Fig. 2).

Current domestic and foreign studies have revealed that NHE1 participates not only in the maintenance of the pH
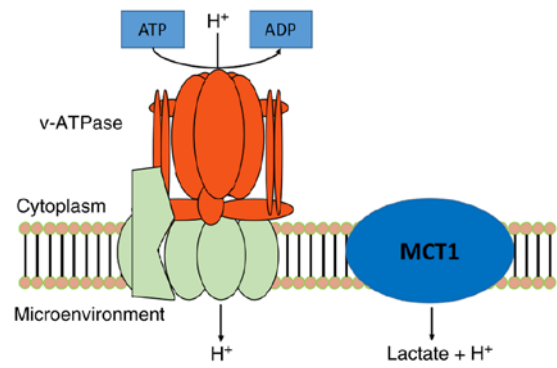

Figure 1. Structural characteristics and functions of V-ATPases and MCT1. V-ATPases are ATP-driven proton pumps, composed of a peripheral V (1) domain that hydrolyzes ATP and an integral V (0) domain that transports protons, that function within both intracellular compartments and the plasma membrane in a wide array of normal physiological and pathophysiological processes. MCTs facilitate lactic acid efflux from glycolytic cancer cells. In particular, MCT1 and MCT4 are important contributors to tumor aggressiveness. Therefore, they are important pH regulators. V-ATPase, vacuolar type ATPase; MCT, monocarboxylate transporter.

balance inside and outside the cell and the prevention of intracellular acidification, but also in several processes such as cell migration, proliferation control, adhesion and apoptosis, as well as expression and function changes (such as the occurrence and development of inflammation, cardiovascular disease and diabetes) that are closely associated with a number of diseases (17). Recent research reported that replication of subgroup J avian leukosis virus depends on a functional cellular receptor, the chicken Na^+/H^+ exchanger type 1, and editing the gene for a virus receptor may confer resistance to this virus and its associated diseases (18). More importantly, it has been reported that NHE is widely expressed in different cancer tissues, where both its activity and expression levels are apparently increased during processes closely associated with invasive growth and distant metastasis of tumor cells. NHE is a key transporter involved in the pH-dependent activation of cathepsin B, matrix metallopeptidase (MMP)-9, MMP-2 and membrane type 1 (MT1)-MMP (5,19). In MV3 cells, NHE1 overexpression causes rearrangement of F-actin at the cell cortex, which is associated with an increase in cortical stiffness. This rearrangement in F-actin likely relies on the pH-sensitive and, thus, NHE1-dependent interaction between cortactin and cofilin. Despite their higher cortical stiffness, NHE1-overexpressing cells are considerably more invasive in a collagen type I substrate, which is most likely due to increases in MMP-3 secretion and activity (20). In hypoxic cancer cells, NHE1 localizes to invasive podocytes, where it promotes the formation of invasive pseudopods by expressing and activating p90 ribosomal S6 kinase (p90RSK) (21). However, NHE1 can also affect invasive function by controlling the proteolytic activity of the cell cycle. In addition, activated NHE1 can also increase the expression of MMP-9 and MMP-2 and fuse with pseudopods via lysosomes and vesicles, where it targets the transport of proteases and promotes the hydrolysis of ECM proteins (22-26).

To date, there is no systematized literature to clarify the regulatory mechanism of NHE1 in various tumors or the progress of NHE1-targeted therapy. Therefore, the present study was undertaken to review the research advances on NHE1 expression and regulatory mechanisms in various tumors (Fig. 3),
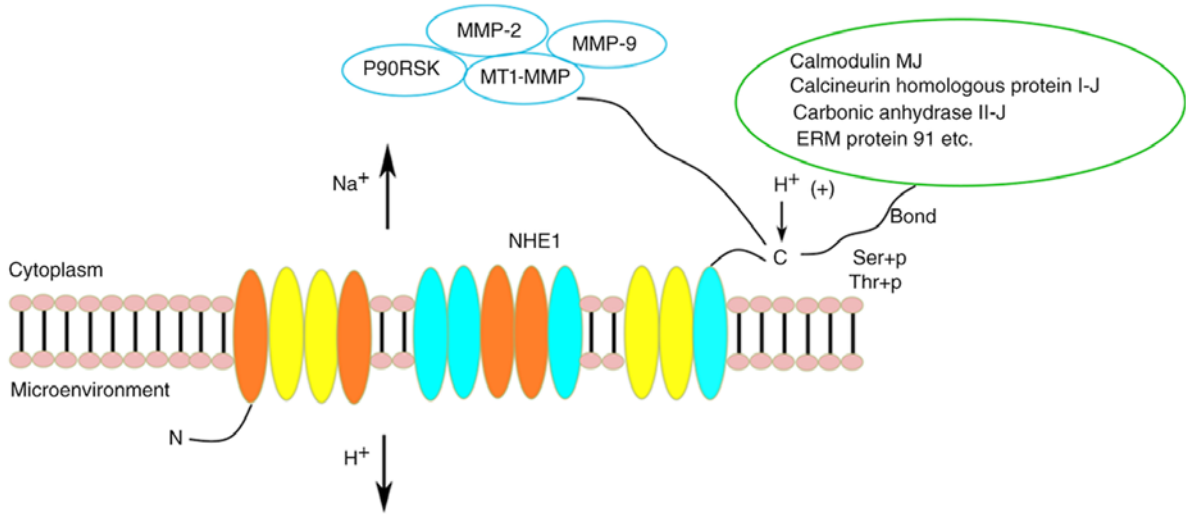
Figure 2. Structural characteristics, functions and activities of NHE1. The hydrophobic N-terminus of NHE1 mediates the transport of ions. The C-terminus participates in the regulation of NHE1 activity and function, mainly through three pathways. NHE1 maintains the pH and also participates in cellular processes such as migration, proliferation, adhesion and apoptosis. It serves as a key ion transporter. NHE1, Na$^+$/H$^+$ exchanger isoform 1; MMP, matrix metallopeptidase; MT1-MMP, membrane type 1-MMP; p90RSK, p90 ribosomal S6 kinase.

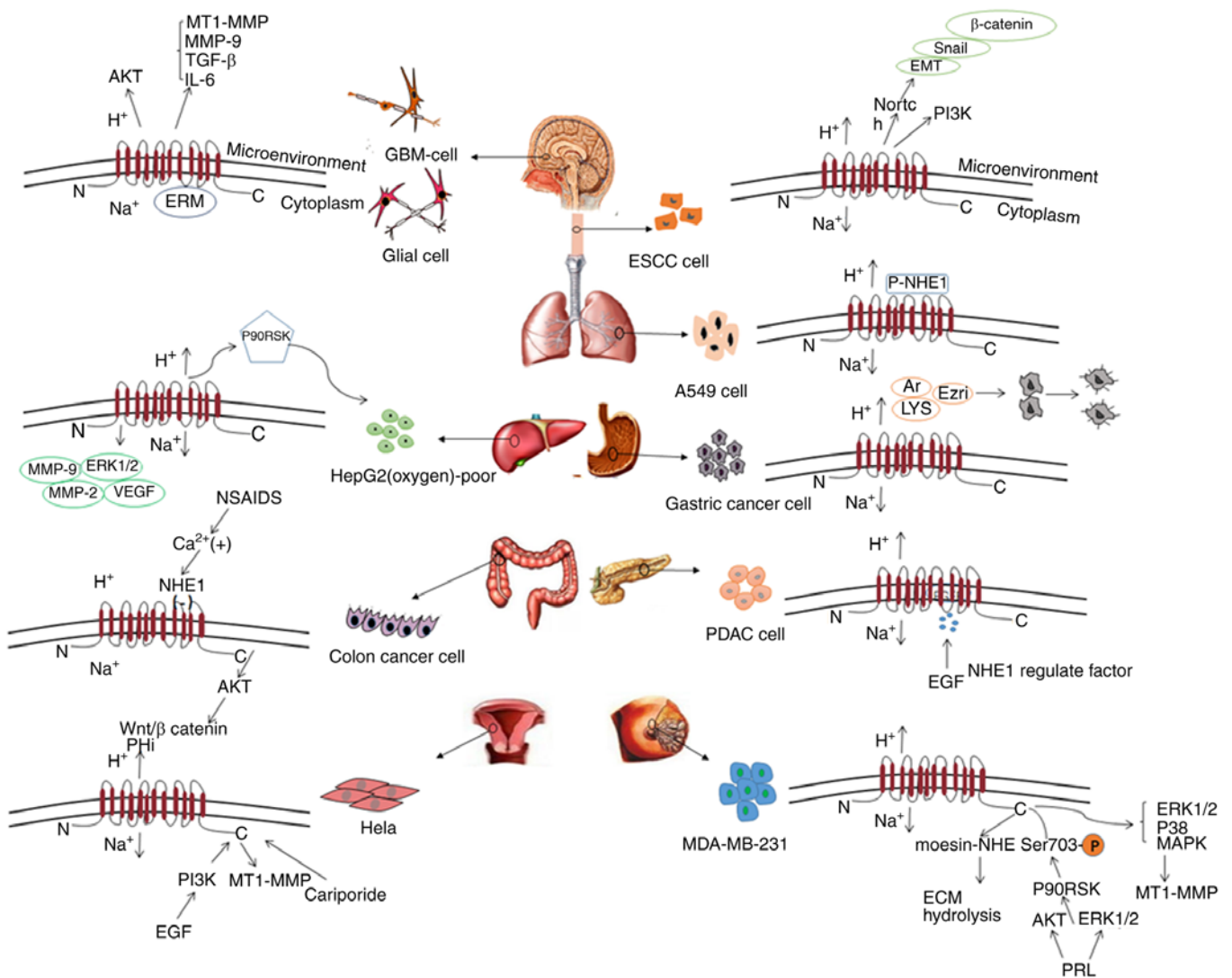Figure 3. Pathological regulatory mechanisms of NHE1 in tumors in various systems. NHE1 directly or indirectly regulates the proliferation, migration and invasion of tumor cells in various systems. The signaling pathways involved include the Notch, AKT, Wnt/β-catenin and PRL/PRLR-NHE1 signaling axes, whereas a number of regulatory factors and proteins are also involved. NHE1, Na$^+$/H$^+$ exchanger isoform 1.
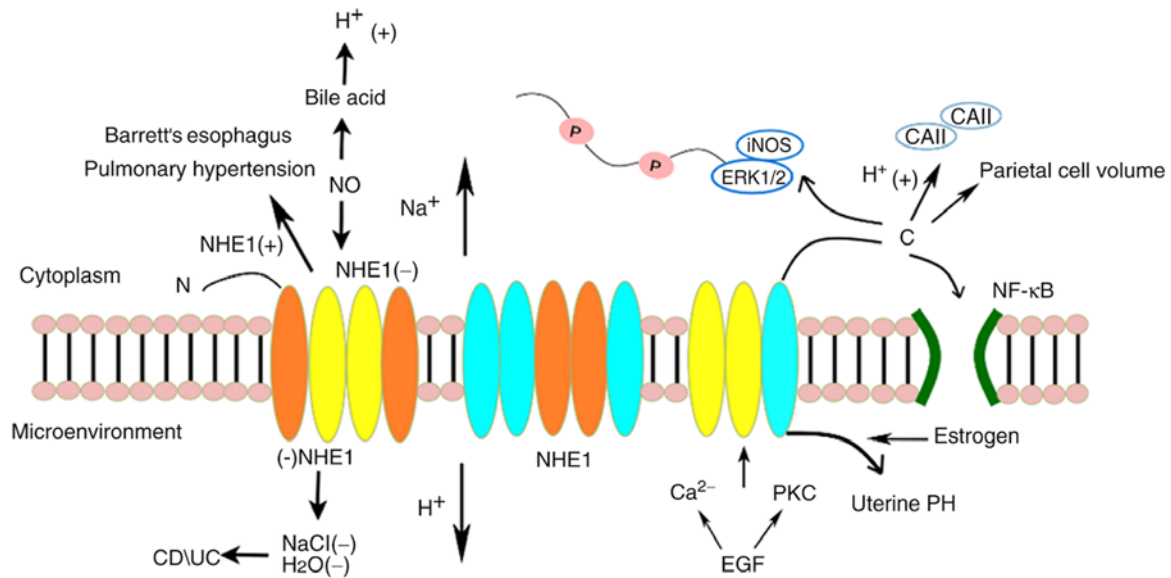
Figure 4. Effect and regulatory mechanisms of NHE1 in diseases other than cancer. NHE1 mediates the systemic inflammatory response and multiple organ damage and involves regulatory pathways such as NF-κB activation, neutrophil infiltration, increased expression of iNOS, and phosphorylation of ERK1/2. NHE1 is also involved in the regulation of ulcerative colitis, Crohn's disease, Barrett's esophagus, pulmonary hypertension, gastric acid secretion, etc. NHE1, Na⁺/H⁺ exchanger isoform 1; NF-κB, nuclear factor-κB; iNOS, inducible nitric oxide synthase; ERK, extracellular signal-regulated kinase; PKC, protein kinase C; EGF, epidermal growth factor.

with the aim of providing research directions for developing novel antitumor drugs.

3. NHE1 and gastric cancer

The incidence of gastric cancer China is high, and most cases are diagnosed at an advanced stage. Therefore, it is particularly important to seek prognostic indicators and therapeutic targets for gastric cancer. It is a well-known fact that cells undergoing progressive acidification can undergo apoptosis and necrosis. Tumor cells rely on NHE1 to maintain a neutral or even alkaline intracellular environment to prevent apoptosis and necrosis. Previous studies have confirmed that NHE1 is highly expressed in gastric mucous cells, which are integrated between the parietal cells and the surface mucous cells of the basolateral membrane. In both types of cells, intracellular acidification and hypertonicity can increase NHE1 activity, while carbonic anhydrase II is highly expressed on the surface of gastric mucous cells and cervical mucous cells (Fig. 4) expressing NHE1; therefore, it may be inferred that NHE1 is closely associated with gastric acid secretion (27,28). It has been confirmed that, in rabbit gastric parietal cells, NHE1 and NHE4 are involved in the regulation of the acid secretion phase, thereby regulating the volume of gastric acid secretion (29). Niu *et al* found that the NHE1 mRNA and protein expression levels were upregulated in gastric cancer tissues. In addition, downregulation of NHE1 gene expression induced by transfected genes led to intracellular acidification and the induction of apoptosis (30), which enabled the treatment of gastric cancer in experimental studies. The aforementioned results suggest that NHE1 may be involved in the initiation process of gastric cancer (31). In addition, Liu *et al* found that the carboxy-terminal proximal membrane region of NHE1 contains a large number of lysine and arginine residue sequences, which can interact with the coding gene of ezrin,

which belongs to the ERM family and is involved in mediating cell-cell interactions and the formation of a cell surface adhesion complex that promotes cell-cell and cell-ECM adhesion. If the surface of the tumor cells expresses a large number of ezrin proteins, the cell surface adhesion complex is diminished, and the adhesion between tumor cells decreases or disappears, thereby promoting tumor metastasis. The clinical data reported by Xie *et al* also demonstrated that, compared with normal gastric mucosal tissue, the mRNA and protein expression of NHE1 and ezrin were significantly increased in gastric cancer tissues (32). This increased expression was positively correlated with gastric cancer stage, depth of invasion and extent of lymph node metastasis (3), suggesting that NHE1 and ezrin may be important factors for gastric cancer metastasis and that NHE1 may be of value as a new target for the treatment of gastric cancer.

4. NHE1 and liver cancer

Liver cancer is the fifth most prevalent malignant tumor worldwide, and no definitive indicator is currently available for estimating the prognosis or as targeted therapy of liver cancer. NHE1 mediates systemic inflammatory response and the subsequent damage of multiple organs, including the heart, liver and kidney (33), through regulatory pathways that mediate NF-κB activation (Fig. 3), neutrophil infiltration, increased iNOS expression and ERK1/2 phosphorylation. It was previously demonstrated that the expression of NHE1 was significantly increased in hepatocellular carcinoma (HCC) tissues and cells (4), and this expression was closely associated with tumor size (34), extensive vascular invasion and advanced tumor stage (pTNM). In addition, the survival time of patients with high levels of NHE1 expression was significantly shortened. Other reports have confirmed that some inflammatory

factors, such as TNF-α and IL-6, can upregulate the expression of NHE1 in hepatocytes, thereby affecting the functional activity of HCC cells (35) and suggesting that NHE1 may be important for the diagnosis and treatment of patients with HCC. During the process of invasion and metastasis of HCC cells, NHE1 plays a key role in upregulating the expression and activity of MMP-2, MMP-9, ERK1/2 and VEGF (36-38). In HepG2 cells, hypoxia promotes the expression of NHE1, and extracellular acidification promotes the production of invasive pseudopods by inducing p90RSK (21). The formation of invasive pseudopods contributes to ECM degradation and promotes invasion and migration of cancer cells. In addition, NHE1 can also affect tumor cell apoptosis, as it has been reported that the NHE1 inhibitor EIPA downregulates Bcl-2 and upregulates Bax expression in HepG2 cells, leading to tumor cell apoptosis (35). As common ion transporters, NHEs are regulated by numerous substances. For example, IL6 and B[a]P can enhance the activity of NHE1, while Rg3 can inhibit the activity of NHEs (35,39,40). Kim et al demonstrated that curcumin treatment or glucose restriction (GR) slightly inhibited NHE1, while the combined treatment with curcumin and GR further enhanced the inhibitory effect on NHE1 and reduced pHi (41). Therefore, based on the aforementioned description of IL6, B[a]P and Rg3 in HCC, it was suggested that NHEs may represent a potential therapeutic target in HCC. However, further studies are required to identify the potential underlying mechanisms (42).

5. NHE1 and pancreatic cancer

Pancreatic cancer is a common malignant tumor of the digestive tract. In recent years, its incidence and mortality have continued to increase, severely affecting the health and quality of life of the patients. Pancreatic ductal carcinoma (PDAC) alone accounts for ~90% of pancreatic cancer cases, and new diagnostic and therapeutic options are urgently needed. However, little research has been conducted on the association between NHE and PDAC in recent years. NHE1 and NHE4 are distributed on the basolateral side of pancreatic acinar cells, and on the basal side of pancreatic ducts (43,44), while NHE2 and NHE3 are mainly distributed in the luminal membrane. In terms of physiological function, NHE1 has been shown to be a major regulator of pHi in pancreatic acinar cells that are stimulated at rest by muscarinic agonists (45). In a study on pancreatic diseases, the human pancreatic gene expression database revealed that the promoter of NHE1 transfer is associated with epidermal growth factor receptor (EGFR) in PDAC, which may drive three-dimensional growth and early invasion of the basal membrane and EGF stimulation via digestion of the ECM through invasive pseudopods to subsequently regulate pancreatic cancer development. EGF promotes the formation of an EGFR and NHE1 complex via the scaffold protein Na^+/H^+ exchange regulator 1, and controls the extent and duration of EGFR-mediated transduction of oncogenic signals in a negative regulatory state (46), in which NHE1 is specific for cell proliferation or invasion. The type of dimeric subtype complex that is separated by the transient signaling of the EGFR/Na^+/H^+ exchange regulator 1/NHE1 complex differs by lipid raft membrane domains. Laminin γ2 (LAMC2) was found to be responsible for generating the extracellular acidic conditions that mediate invasion of pancreatic cancer cells by activating AKT/NHE1 signaling. LAMC2 is a characteristic prognostic and therapeutic factor in PDCA (47).

6. NHE1 and esophageal cancer

Previous studies have demonstrated that NHE1 induction may be the basis of the pathogenesis of gastroesophageal reflux disease and may play an important role in the protection of esophageal epithelial cells from acid. Some studies have found that EGF can activate NHE1 through Ca^{2+}/calmodulin and protein kinase C pathways to protect the esophageal epithelium from acid (48,49). Barrett's esophagus is a precancerous lesion associated with an increased risk of esophageal adenocarcinoma and is a current hot research topic (50). Goldman et al found that the activity of NHE1 in Barrett's epithelium (Fig. 3) was significantly increased to accommodate cellular acidification, while bile acid-induced cellular acidification involved NO-mediated NHE inhibition, which resulted in increased intracellular acidification and DNA damage that may lead to mutations and cancer progression (51). Clinical data from a study of patients with esophageal cancer revealed that, compared with that in normal esophageal mucosa, the expression of NHE1 in primary esophageal squamous cell carcinoma (ESCC) was significantly increased, particularly in patients with highly differentiated tumors. In the low NHE1 expression group, the 5-year survival rate of patients was significantly better compared with that of the high NHE1 expression group, suggesting that NHE1 may be associated with poor prognosis in patients with ESCC. In vitro cell experiments also confirmed that NHE1 was highly expressed in primary ESCC cells and significantly promoted their proliferation, migration and invasion, whereas NHE1 inhibition led to increased apoptosis. The mechanism may be associated with NHE1 downregulation of PI3K-AKT and Notch signaling (52,53). Epithelial-to-mesenchymal transition (EMT)-related genes and proteins, including Snail, β-catenin, NF-κB, AKT and p21, are involved in the progression and metastasis of cancer and have prognostic value. It has been confirmed that NHE1 inhibits the Notch signaling pathway, whereas the upregulation of Snail, β-catenin and other EMT markers leads to EMT in esophageal cancer cells (54,55), thereby increasing cell migration and invasion. In general, NHE1 exerts a tumor-promoting effect on ESCC and represents a prognostic indicator for patients with ESCC and a promising new target for ESCC treatment.

7. NHE1 and breast cancer

NHE1 is widely distributed on the plasma membrane of all tissues, promoting the formation of the acidic tumor microenvironment. Extracellular acidification promotes the formation of new blood vessels in metastatic breast cancer (56), thus promoting tumor invasive ability. It has been demonstrated that the expression and activity of NHE were increased in breast microinvasive foci and adjacent ductal cells, which are associated with local infiltration and distant metastasis of cancer cells (57). In MDA-MB-231 breast cancer cells, NHE1 stimulated the expression of MT1-MMP by activating the ERK1/2 and p38 MAPK signaling pathways (23,58,59), thereby mediating the invasion and metastasis of MDA-MB-231 cells.

In addition, it has been found that cytoskeletal agonist-binding proteins are novel upstream regulators of the subcellular distribution and activity of moesin-NHE1 (60), which can promote invasive pseudopod maturation and mediate NHE1 regulation of tumor invasion. Specifically, the invasive pseudopod acts as a site for regulatory protease activity to induce ECM proteolysis under the stimulation of the acidic microenvironment produced by NHE1 (61). Elena Pedraz-Cuesta *et al* also found that the PRL/PRLR-NHE1 signaling axis is involved in breast cancer cell invasion, PRL-induced NHE1 activation and the resulting NHE1-dependent invasion, thus promoting the invasive behavior of human breast cancer cells. Among the signaling pathways involved in cancer cell invasion, PRL-mediated activation of AKT and ERK1/2 activates p90RSK, which leads to the phosphorylation of NHE1 Ser703, thereby increasing NHE1 activity, including NHE1-dependent cell migration (62). As a pHi regulatory protein, a previous study examined the role of negatively charged amino acids of extracellular loop 3 (EL3) in the activity of the NHE protein, and demonstrated that amino acids E217 and D226 form part of a negatively charged coordination sphere, which facilitates cation transport in the NHE1 protein (63). NHE7, a unique protein member of the NHE family, dynamically shuttles across the Golgi network between endosomes and the plasma membrane to regulate the intraluminal pH in these organelles. NHE7 overexpression in MDA-MB-231 breast cancer cells enhances the tumor cell coverage area, cell-cell adhesion and cell invasion, which is independent of tumor growth and tumor sphericity (64), according to the first report (63) indicating that NHE participates in tumor regulation at the organelle level. In studies on antitumor therapy, it was confirmed that the inhibition of NHE1 expression and activity may act synergistically with paclitaxel to induce cell apoptosis, and it has been confirmed that PKA and p38 are upstream regulatory points of NHE1 in the induction of paclitaxel-dependent apoptosis. It has been suggested that NHE1 may provide a new approach to improving the efficacy of paclitaxel chemotherapy. Studies have also found that pyrazinoylguanidine-type NHE1 inhibitors potently inhibit the growth and survival of cancer cell spheroids, and this effect is unrelated to NHE1 inhibition (65).

8. NHE1 and lung cancer

With the obvious increase in the incidence of lung cancer on a global scale, lung cancer is currently the most common cause of cancer-related mortality worldwide, as well as in China. However, the mechanism of NHE1 regulation in lung cancer remains unclear. Recent studies have demonstrated that NHE1 is expressed in all parts of the human airway, and regional differences in NHE1 mRNA levels suggest that differential expression may be associated with differences in airway absorption, electrolyte secretion and acid load (66,67). For example, in pulmonary artery smooth muscle cells under chronic hypoxia, NHE1 expression and activity are significantly increased (68), which may be associated with the mechanism of the development of pulmonary hypertension (Fig. 3). There is also evidence that NHE1 is involved in the regulation of malignant cytological behaviors, such as growth, proliferation and invasion of lung cancer cells (69). The investigators (70) transfected pNHE1 (NHE1 antisense expression vector) into A549 human lung cancer cells to induce the inhibition of NHE1 mRNA expression and related activity, which resulted in significantly decreased pHi, suppressed cell proliferation and increased apoptosis rate of tumor cells. In addition, in a study on the effect of the NHE1 antisense gene sequence on cell proliferation and apoptosis in drug-resistant small cell lung cancer (SCLC) (71), it was observed that the NHE1 antisense gene could induce acidification and apoptosis in H446/CDDP drug-resistant human SCLC cells, which suggests a potential new treatment for multidrug-resistant SCLC.

9. NHE1 and brain tumors

Malignant gliomas are the most common primary brain tumors, and options for their treatment are currently limited. The majority of the patients with clinically diagnosed glioblastoma (GBM) have a survival time of <1 year. Studies by Pizzonia *et al* have demonstrated the expression of NHE1 throughout the human brain (72), including the hippocampus, where it interacts with ERM proteins to promote glial cell survival, apoptosis resistance, migration and invasion by regulating cell proliferation (73), pHi, extracellular and tumor microenvironment pH. It has been reported in the literature that NHE1 activation may inhibit cell apoptosis by activating the AKT signaling pathway (74). Tumor cell migration and invasion may be associated with NHE1 regulation of cell volume, cytoskeletal stability (75-77) and the plasma membrane. Therefore, NHE1 has great potential for cancer treatment, including for GBM (78). Studies by Zhu *et al* found that NHE1 is highly expressed in glioma-associated microglia, and NHE1 in glial cells regulates microglia-derived factors (79), such as MT1-MMP, MMP-9, TGF-β and IL-6, thus promoting the proliferation and viability of glial cells. In addition, lactic acidosis is a common characteristic of ischemic brain tissue. Some scholars have found that NHE1 can regulate the pHi of glial cells, particularly under pathologically low pH conditions (73), enhancing the NHE1 regulatory role. Inhibition of NHE1 during oxygen deprivation contributes to the maintenance of the energy state of glial cells. In addition to NHE1, NHE5 is also highly expressed in brain tissue (80), and it may play a role in neuronal tissue. Recently, Kurata *et al* demonstrated NHE activity in brain tumors using C6 glioma cells which expressed both NHE1 and NHE5. NHE5 knockdown C6 glioma cells exhibited downregulation of MET and EGFR, resulting in loss of invasion and proliferation ability, while NHE1 knockdown did not exert the same effects (81).

10. NHE1 and colorectal cancer

The NHE family proteins are widely expressed in the intestine, but they exhibit certain differences in localization. For example, NHE1 is evenly distributed throughout the intestine. NHE2 is mainly distributed in the small intestine, distal colon and proximal colon. NHE3 is expressed, in descending order of prevalence, in the ileum, jejunum and colon (82). It is known that, in ulcerative colitis and Crohn's disease (Fig. 3), inhibition of NHE1 transcription can reduce NaCl and water uptake in the colonic lumen (83), resulting in diarrhea. In addition, NHE2 and NHE3 also appear to be involved in the absorption of Na^+ in the intestine (84). In a study of colon cancer, the acidic

tumor microenvironment and the Wnt/β-catenin activation pathway were identified as two key elements associated with the development and progression of cancer. It has been reported in the literature that T84 human colon cancer cells contain three isoforms of NHE1, NHE2 and NHE4 (85). A study of rat colon cancer cells also strongly supported the hypothesis that AKT activity may be associated with the NHE1 regulation of pHi and the Wnt/β-catenin signaling pathway (86). In colorectal cancer cells, β1 integrin-mediated adhesion to ECM proteins, such as collagen type I and adhesin, triggers an early and transient pHi alkalinization, from 6.7 to 7.2. The effect is caused by NHE1 activation and is modulated by the activity of the voltage-dependent K^+ channel KV11.1 (87). With respect to antitumor therapy, cisplatin is considered as a broad-spectrum antitumor drug. Some scholars have suggested that inhibition of NHE1 at the plasma membrane may promote the activation of acid sphingomyelinase and increase membrane fluidity. The aforementioned process plays an important role in the cytotoxicity mediated by cisplatin (88), suggesting that NHE1 is a new potential target for cisplatin antitumor therapy. In colorectal cancer, non-steroidal anti-inflammatory drugs (NSAIDs) appear to be promising for chemoprevention. It has been reported that certain NSAIDs, such as sulindac and celecoxib (89), prevent colorectal cancer through a mechanism that may be associated with the downregulation of NHE1, the increase in intracellular Ca^{2+} and the activation of the downstream calpain 9 signaling pathway.

11. NHE1 and cervical cancer

Studies of the correlation between cervical cancer and NHE are still in the early stages. It has been demonstrated that the NHE1-specific inhibitor cariporide can inhibit the migration and invasion of HeLa cervical cancer cells *in vitro* (90). Further mechanistic studies (90) have reported that NHE1 mediates the metastasis of HeLa cells by regulating the expression and localization of MT1-MMP. An experiment by Chiang *et al* demonstrated that EGF is involved in the regulation of NHE1 expression via the PI3K signaling pathway in cervical cancer (91), and NHE1 can also interact with the actin-related protein ezrin to reconstitute the cytoskeleton and stimulate the migration and invasion of cervical cancer cells. It was also reported that Andrographolide regulates the expression of apoptotic proteins to induce cells apoptosis, and concentration-dependently decreases pHi by decreasing the activity of NHE1/V-ATPase and the expression of NHE1 in HeLa cells; thus, Andrographolide may represent a promising novel agent in the treatment of cervical cancer in the clinical setting (92). In addition, NHE has also been found to be involved in the regulation of uterine fluid pH (Fig. 3) under the influence of estrogen (93). Studies by Ismail *et al* have confirmed that enhancement of NHE1, NHE2 and NHE4 protein and mRNA expression in the cervix can help restore cervical-vaginal fluid pH and prevent cervical and vaginal complications associated with menopause (94).

12. Outlook

NHE is widely expressed in human multisystem tumors. Recently, there have been several studies on the role and regulatory mechanism of NHE1 in tumors, but studies on subtypes other than NHE are rare. As an important regulator in tumors, NHE1 is associated with the regulation of tumor drug resistance, invasion, metastasis, apoptosis resistance and other malignant biological behaviors, contributing to poor prognosis. With continued in-depth research, NHE1 may be used as a new target for the development of novel anticancer drugs, in the hope of improving the treatment of tumors.

Availability of data and materials

Not applicable.

Authors' contributions

YH and JL drafted the initial manuscript. ZJ, XY, WS, YH, QD and QL performed the literature review. JX primarily revised and finalized the manuscript. RX revised the manuscript for clarity and style. All authors have read and approved the final manuscript.

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.

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