SPANDIDOS PUBLICATIONS

EXPERIMENTAL AND THERAPEUTIC MEDICINE 20: 1220-1229, 2020

Pathophysiology of hepatic Na^+/H^+ exchange (Review)

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Received September 21, 2019; Accepted May 15, 2020

DOI: 10.3892/etm.2020.8888

Abstract. Na^+/H^+ exchangers (NHEs) are a family of membrane proteins that contribute to exchanging one intracellular proton for one extracellular sodium. The family of NHEs consists of nine known members, NHE1-9. Each isoform represents a different gene product that has unique tissue expression, membrane localization, physiological effects, pathological regulation and sensitivity to drug inhibitors. NHE1 was the first to be discovered and is often referred to as the 'housekeeping' isoform of the NHE family. NHEs are not only involved in a variety of physiological processes, including the control of transepithelial Na^+ absorption, intracellular pH, cell volume, cell proliferation, migration and apoptosis, but also modulate complex pathological events. Currently, the vast majority of review articles have focused on the role of members of the NHE family in inflammatory bowel disease, intestinal infectious diarrhea and digestive system tumorigenesis, but only a few reviews have discussed the role of NHEs in liver disease. Therefore, the present review described the basic biology of NHEs and highlighted their physiological and pathological effects in the liver.

Contents

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Key words: Na^+/H^+ exchanger, liver physiology, non-alcoholic fatty liver disease, hepatic fibrosis, liver cancer

1. Introduction

Intracellular pH (pHi) is usually maintained between 6.9-7.2; it is important to maintain a normal pHi as a decrease in pHi may influence the normal functioning of proteins, ion channels and several other physiological processes involved in cell proliferation, division and differentiation (1). Some ion exchanger families regulate proton flux at the plasma membrane, such as Na^+/H^+ exchanger (NHE), Cl-/HCO_3- exchanger, Na^+/HCO3$^-$ cotransporter and Na^+-driven Cl^-/HCO3$^-$ exchanger (2). The present review focused on the NHE family. NHEs are pH-regulated membrane proteins that exchange extracellular Na^+ for intracellular H+ with a stoichiometry of one for one (2). The inward Na+ gradient produced by Na^+/K^+-ATPase provides a constant driving force for H^+ efflux (3). Moreover, NHEs are activated by decreases in pHi, and thus are likely to respond to an increase in proton load during acute acid stimulation (4).

NHEs are evolutionarily conserved membrane transporters; the solute carrier 9A (SLC9A) family contains the well-characterized plasma membrane and intracellular NHE isoforms (NHE1-9), and the SLC9B subgroup consists of Na^+/H^+ antiporter 1 and Na^+/H^+ antiporter 2 (5). Different NHE isoforms are positioned differently. NHE1 is the 'housekeeping' isoform in NHE family (6) and is nearly ubiquitous in the plasma membrane of almost all tissues (7), while the other isoforms have more restricted localization and function. The specific localization of NHE1 may vary depending on cell type. In fibroblasts, NHE1 is mainly localized in lamellae and participates in migration and anchoring (8). However, in epithelial cells, NHE1 is distributed in the basolateral membrane (9). NHE2-5 are also localized to the plasma membrane, but have more restricted tissue distributions (10). For example, NHE2 is an apical membrane protein found mainly in the stomach and intestines (11). Both NHE3 and NHE4 are highly expressed in the kidney and gastrointestinal tract (12). In the gastrointestinal tract, NHE3 is mainly expressed in the intestine, while NHE4 is expressed in the stomach (12). Moreover, NHE5 is expressed primarily in the brain (13). The isoforms NHE6-NHE9 exist in intracellular organelles, where they participate in the maintenance of pHi (14). NHE6 is expressed in early recycling endosomes and mitochondria, NHE7 is located in the trans-Golgi network, NHE8 is in the mid- to trans-Golgi and NHE9 is expressed in late recovered endosomes (15,16).

Various subtypes of NHEs are related to the pathogenesis of digestive diseases, such as Barrett's esophagus (17), gastric cancer (18), IBD (19), colon cancer (20) or liver diseases. Currently, the vast majority of review articles have focused on

the role of the NHE family members in IBD (21), intestinal infectious diarrhea (22) and digestive system tumorigenesis (23), and to the best of our knowledge, only a few have reported the role of NHEs in liver disease. The liver is the largest digestive gland in the human digestive system, and plays an important role in metabolism, deoxygenation, glycogen storage and secretory protein synthesis (24). Disease development in the liver seriously affects the normal function of the body (25). Thus, it is important to study the physiological and pathological regulation of the liver. However, the role of NHEs in liver function is not fully understood, although all NHEs except NHE5 have been detected in this organ (26).

Therefore, the present review details the physiology and pathology of NHEs in the liver, including the regulation of hepatocyte volume, hepatocyte growth, regeneration, proliferation, apoptosis, bile formation and other physiological activities. The pathologies discussed include non-alcoholic fatty liver disease (NAFLD), liver fibrosis, liver cancer and other liver diseases.

2. Structure and function of NHEs

The sequences of the nine subtypes of NHEs in the SLC9A subfamily are significantly different, with amino acid identities ranging between <12% (NHE1 vs. NHE9) and >70% (NHE6 vs. NHE7) (27). Despite these differences, silico-predicted transmembrane protein domains have suggested very similar structural arrangements for all nine isoforms, which have a high degree of similarity in the NH2-terminal hydrophobic domain, which contains multiple predicted membrane-spanning segments (27). However, it is important to note that NHE membrane topology has been most extensively studied in the NHE1 isoform (28). The complete membrane protein consists of 815 amino acids, and the first 500 amino acids of the protein are speculated to consist of 12 transmembrane hydrophobic domains (29). A C-terminal hydrophilic cytosolic domain of ~315 amino acids regulates the protein and mediates cytoskeletal interactions (30,31). Moreover, NHE2, 3, 4 and 5 have been reported to have 42, 39, 42 and 39% amino acid homology to NHE1, respectively (12). The NHE1 protein contains N- and O-glycosylated residues (32) and the N- and C- termini of NHE1 are found in the cytosol (6). Growth factors, hormones, integrins, osmotic stress and other signaling pathways regulate the activity of NHE1 via the mediation of the C-terminal domain, thus determining the pHi (33). In addition, there are some binding sites in the C-terminus, such as calmodulin (CaM), CaM homologous protein (CHP) and esrin/radixin/moesin (ERM) (4). When CaM binds to NHE1, it eliminates self-inhibition and activates NHE1. CHP AND ERM are bound to the cytosolic regulatory tail and also support the physiological activity of NHE1 (4) (Fig. 1).

3. NHEs in hepatic physiological regulation

NHEs are the most widely studied pHi regulators in various animal cells, including hepatocytes (34). Intracellular acid load produced by normal hepatocyte metabolism activates NHE proteins to catalyze the electroneutral exchange of one extracellular Na^+ with one intracellular H^+, thus constituting a key component that prevents cell acidosis (4). Furthermore, this exchange process depends on the inward-directed Na+ gradient produced by the Na^+/K^+-ATPase to excrete H^+ from the cytoplasm (9,35). NHEs are also involved in regulating the volume of hepatocytes (36), hepatocyte growth, regeneration, proliferation, apoptosis and bile formation, and a series of physiological activities, which are described below.

Regulation of cell volume is critical for liver function in healthy and disease states (37). Shrinkage or swelling of cells may result in disruption of the integrity of the cell membrane and cytoskeletal structure. To survive, ions must pass via certain ion transporters to avoid excessive changes in cell volume (38,39). When cells are exposed to hypertonic extracellular media, cell contraction triggers a regulated cell volume increase, which is largely accomplished by cellular ion uptake (40,41). Cellular contraction also stimulates $Na^+/K^+/2Cl^-$ cotransporters (NKCC) and/or NHEs in parallel with Cl^-/HCO_3^- exchangers (40). H^+ excretion via NHEs and HCO_3^- exiting via the Cl^-/HCO_3^- exchanger are replenished in the cell by H_2CO_3, which is readily produced by CO2; this process achieves NaCl entry (42). On the other hand, Na^+, which enters the cell via the NKCC and NHE, is pumped out by the Na^+/K^+-ATPase in exchange for K^+, which eventually causes KCl uptake by the cells (42) (Fig. 2). Moreover, the NKCC isoforms NKCC1 and NKCC2 (43) and the NHE isomers NHE1, 2 and 4 are activated by cell contraction, while NHE3 is inhibited by cell contraction (43).

The volume sensitivity of metabolism is an integral part of hormone signaling (44,45). The insulin involves changes in the volume of hepatocytes (42). In addition to activating NHEs and NKCC to expand hepatocyte volume and serving an antiproteolytic role, (42) insulin activates NHEs by binding to receptors linked to tyrosine kinases in hepatocytes and promotes the growth of hepatocytes (46). Similar to insulin, several growth factors, such as hepatocyte growth factor (HGF) (47,48), epidermal growth factor (EGF) (49) and transforming growth factor (TGF)-α (50), increase cell volume by stimulating NHEs, which is essential for stimulating cell proliferation (40). HGF is one of the most effective mitogens in hepatocytes and is often used to study the mechanism of hepatocyte proliferation (51). HGF, similar to insulin, activates NHEs in hepatocytes via a tyrosine kinase CaM-dependent pathway (51). EGF rapidly stimulates NHEs before increasing DNA synthesis, which not only promotes the proliferation of hepatocytes, but also participates in the regulation of liver regeneration (52). Dallenbach *et al* (53) measured pHi and homeostasis NHEs mRNA expression to compare the activity and regulation of NHEs in hepatocytes isolated after two-thirds partial hepatectomy or sham operation. These authors reported that during liver regeneration induced by partial hepatectomy in rats, NHEs in hepatocytes were activated at early, transient and posttranscriptional levels (53). The effect of liver regeneration on NHEs in hepatocytes is similar in qualitative and quantitative terms to that of hepatocytes exposed to EGF (54), suggesting that NHEs may participate in the regulation of liver regeneration as EGF in liver injury.

The effects of cell proliferation may be mediated by enhancing cell survival or inhibiting apoptosis (55). Intracellular acidification and cell volume reduction are markers of apoptosis, while NHE1 intracellular alkalization

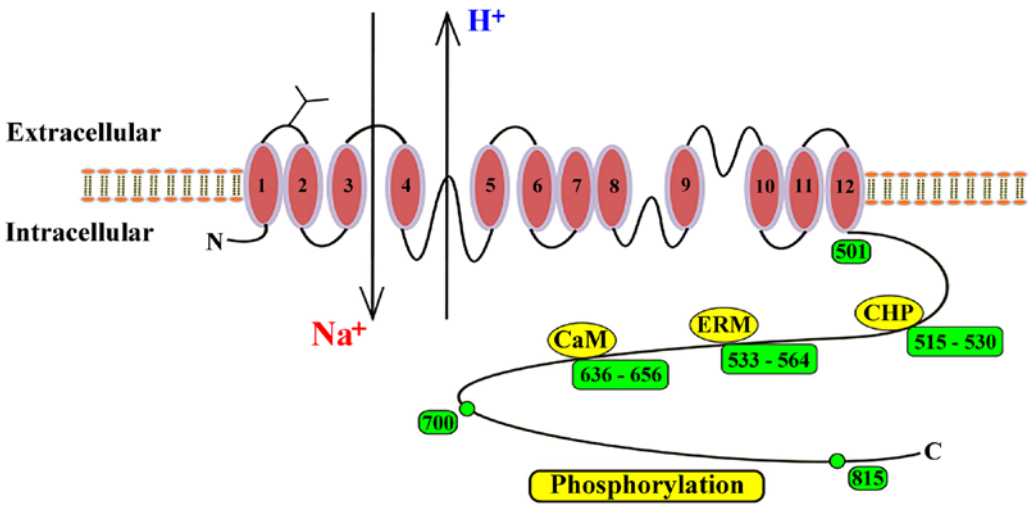

Figure 1. Structure of NHE1. Both the N- and C- termini of NHE1 are located in the cytosol. The first 500 amino acids of NHE1 consist of 12 transmembrane hydrophobic domains. The C-terminal hydrophilic cytoplasmic domain of 315 amino acids contains binding sites of various proteins, such as CaM, CHP and ERM and the region of the cytoplasmic domain involved in phosphorylation and activity regulation. CaM, calmodulin; CHP, calmodulin homologous protein; ERM, esrin/radixin/moesin; NHE, Na^+/H^+ exchangers.

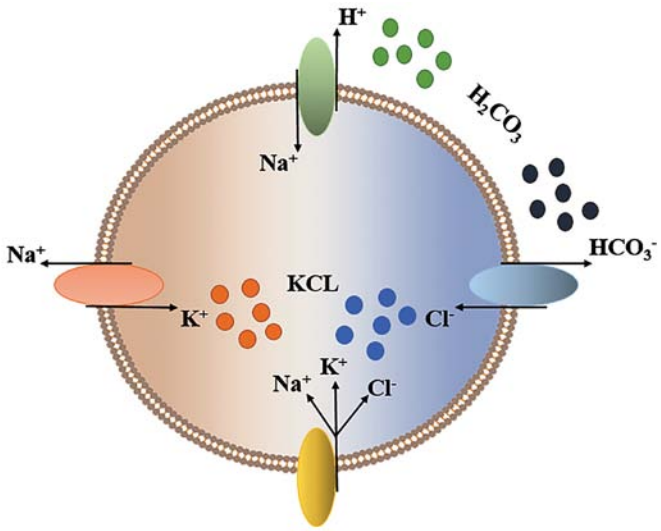

Figure 2. Regulation of various transporters on the membrane during cell contraction. Cell contraction, Cl^-/HCO_3^- exchanger, $Na^+/K^+/2Cl^-$ cotransporter and Na^+/H^+ exchanger coactivate to produce NaCl in cells. Then, K^+ is pumped by the Na^+/K^+-ATPase and is exchanged for intracellular Na^+, which eventually leads to the uptake of KCl by cells.

and the regulatory volume increases may be antiapoptotic signals (2,56). TGF-β, which induces apoptosis of hepatic parenchymal cells (57), has a significant inhibitory effect on NHE activity in short-term cultured rat hepatocytes, especially in cells isolated from perivenular regions, in which apoptosis is more frequently observed (58).

Hepatocytes and bile duct epithelial cells are involved in bile secretion and absorption (59). Bile duct epithelial cells also serve a role in the transport of water, electrolytes, sugar, bile and amino acids, and express several transport proteins to modify the primary production of hepatocyte bile (60). In biliary cells, four isoforms (NHE1-4) have been identified (61,62). Basolateral NHE1 is generally speculated to be involved in pHi, cell volume homeostasis and fluid and electrolyte transport, particularly secretin-induced bile secretion (63,64). In addition, NHE3 has been detected in cholangiocarcinoma cells in rats (65,66) and in gallbladder epithelial cells in calves (67) and prairie dogs (68). Targeted destruction of the NHE3 gene results in inhibition of fluid reabsorption in isolated bile duct units. For example, a study in mice found that decreased gallbladder absorption of bile may be the result of a decrease in NHE3 activity caused by an increased level of NHE3 phosphorylated at serine-552; this increase in phosphorylation is hypothesized to lead to a higher turnover of NHE3, which leads to a decrease in the gallbladder's concentrating function (69). Moreover, prairie dogs represent a good animal model for human gallstone formation, and their gallbladder epithelial cells exhibit H^+ gradient-dependent Na+ uptake via NHE1 (~6% of total intake), NHE2 (~66% of total intake) and NHE3 (~28% of total intake), indicating a significant contribution of NHEs to epithelial Na^+ absorption (68,70). Along with the findings showing increased absorption of Na^+ and liquid in the early stage of gallstones (71), it has been proposed that apical membrane NHEs may be involved in the pathogenesis of gallstones (69). In conclusion, the aforementioned results suggested that decreases in NHE activity affects the absorption capacity of bile duct cells.

NHE1 may also regulate cell differentiation, as the absence or inhibition of NHE1 impairs the differentiation pathway (72). Furthermore, NHE1 function is important in cytoskeletal tissue and cell migration (22). The cytoplasmic tail of NHE1 acts as an anchor for actin filaments via the binding of ezrin, Radixin and moesin proteins, and the destruction of these interactions or inhibition of NHE1 activity leads to the inhibition of cell migration and the formation of external adhesions (2).

4. NHEs in hepatic pathology

Role of NHEs in NAFLD. NAFLD is closely related to liposome imbalance, and hepatic steatosis is considered to be the first stage in the development of NAFLD (73). With the development of fibrosis and inflammation, NAFLD

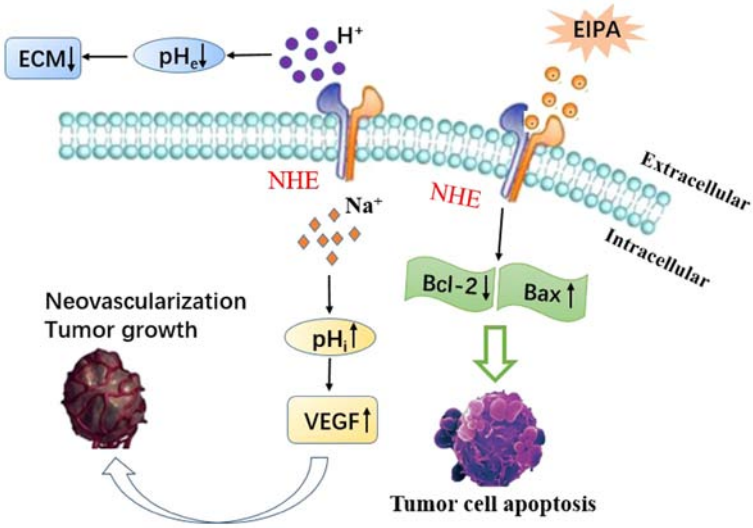

Figure 3. In hepatocellular carcinoma cells, activation of NHEs to produce acidic pHe promotes the degradation of ECM. Alkaline pHi promotes the expression of VEGF, thus inducing neovascularization and promoting tumor growth. On the other hand, the use of EIPA (an inhibitor of NHEs) downregulates the expression of Bcl-2, a proapoptotic gene, and the expression of the proapoptotic gene Bax is upregulated, which ultimately promotes apoptosis of hepatocellular carcinoma cells. ECM, extracellular matrix; VEGF, vascular endothelial growth factor; pHi, intracellular pH; pHe, extracellular pH; NHE, Na^+/H^+ exchangers.

can progress to non-alcoholic steatohepatitis (NASH) and eventually lead to liver fibrosis, cirrhosis and cancer (74,75). NHE activity is associated with steatosis in NAFLD. Previous studies (76) have compared the expression of NHE1 in the livers of normal diet mice and high-fat diet mice, and revealed that the expression of NHE1 in the livers of high-fat diet mice was nearly tripled and the long-term ablation of NHE1 activity in mice weakened high lipid diet-induced liver lipid accumulation, which suggests that NHE activity plays a role in the development of NAFLD (76). Farnesoid X receptor (FXR) is a nuclear hormone receptor. It has been reported that activation of FXR attenuates the development of hepatic steatosis (77,78), and FXR agonists reduce NASH-associated fibrosis (79,80). The prolipogenic liver X receptor (LXRa) is an important regulator of lipid metabolism. LXR activation promotes hepatic steatosis (81,82), and treatment with liver-specific LXR inhibitors reduced the development of hepatic steatosis (83). Prasad *et al* (76) revealed that in the livers of NHE1-null (KO) mice, increased expression of FXR and downregulation of LXRa expression were consistent with the results of long-term NHE1 deletion in reducing liver lipid accumulation induced by high-fat diet. In addition, the key regulators of adipogenesis, acetyl-CoA carboxylase α (Acc1) and Acc β (Acc2), are downregulated in NHE1-KO liver (76). Moreover, downregulation of Acc1 and Acc2 expression levels can reverse hepatic steatosis (84). Based on these findings, it was speculated that the loss of NHEs in the liver may lead to increased expression of FXR, downregulation of LXRa and downregulation of Acc1/Acc2 expression, which may reduce or reverse liver steatosis during the occurrence of fatty liver diseases such as NAFLD, which slows the development of NAFLD.

NHEs in liver fibrosis. Hepatic fibrosis is a common response to chronic liver injury of variable origins, such as viruses and metabolism (85). The mechanisms of liver fibrosis include activation of hepatic stellate cells (HSCs) and extracellular matrix (ECM) protein deposition, including various collagens and matrix glycoconjugates (86). During the development of fibrosis, HSCs proliferate and the activation process is characterized by the appearance of myofibroblast-like phenotypes that accumulate near necrotic areas (87). Activated HSCs are characterized by the expression of α-smooth muscle actin (SMA) (87), increased cell numbers, loss of retinoic acid (88,89) and increased expression of collagen fibrin (90). Currently, oxidative stress (91,92), paracrine stimulation of damaged hepatocytes, cytokines (93,94) and mitogens, such as platelet-derived growth factor (PDGF), TGF-β and insulin-like growth factor I, have been reported to promote the proliferation of HSCs and matrix synthesis. All of these factors activate NHEs, mostly likely the subtype 1, in the liver, and NHE activation is one of the earliest responses to mitogens and growth factors in most cell types (95). Previous studies have shown that NHE1 is the main pH regulator in HSCs, and its activity increases with the activation process of HSCs (96,97). When different growth factors and oxidative stress stimulate HSC proliferation and collagen type synthesis, the activity of NHE1 protein increases (96,98-100). Furthermore, the mechanism of HSCs proliferation is related to the increase in cell volume caused by NHE activation. For example, an increase in cell volume itself induces multiple changes in cellular function and gene expression by activating the osmosignaling pathway, and is a prerequisite for cell division and proliferation (38,101). It has also been reported that an increase in cell volume is parallel to the process via which fibroblasts transition from G1 to S phase (102).

Inhibition of NHEs selectively blocks ribonucleotide reductase, an enzyme that is critical for DNA synthesis (103). The antioxidant resveratrol also inhibits nucleoside reductase activity (104), thus suggesting the possible role of reactive oxygen species as a common mechanism of NHE activation in HSCs; the promotion of fibrosis caused by oxidative stress is also partly due to NHE activation in HSCs (99). Benedetti et al (105) used the NHE inhibitors amiloride and cariporide, and

revealed that these inhibited the proliferation and activation of NHE and HSCs by blocking PDGF and oxidative stress in vitro, suggesting that NHE inhibitors may reduce DNA synthesis in HSCs by inhibiting the activity of NHEs. Benedetti *et al* (105) also found that in vivo administration of amiloride and cariporide reduced the formation of α-SMA positive chains and the expression of type I procollagen in the local proliferation induced by two different liver injury models, dimethyl nitrosamine and bile duct ligation. Therefore, NHE appears to play a catalytic role in the pathogenesis of liver fibrosis (105). Collectively, it was speculated that the activity of NHEs may be closely related to the activation of HSC during liver fibrosis.

NHEs in liver cancer. Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in the world and has a poor prognosis (106). The occurrence and development of cancer are closely related to dysregulation of cell energy metabolism, which is known as the Warburg effect (107). As a supportive anticancer therapy, glucose restriction (GR) inhibits enhanced glycolytic activity in cancer cells via energy-dependent signaling pathways, including the insulin like growth factor-1/PI3K/Akt/mTOR pathway (108). Previous studies have reported that intracellular alkalization is a major transformation event for cancer cells. For instance, one study showed that glioblastoma (MG) is a highly glycolytic malignant tumor that has a strong dependence on pH, and NHE1 activation drives cytoplasmic alkalization (109). However, inhibition of NHE1 in MG can acidify tumor cells, while healthy astrocytes are not affected; this finding may facilitate the development of treatment for malignant tumors (109). Targeting proton dynamics associated with pHi gradients has been proposed as a potential cancer prevention strategy and treatment (110). Kim et al demonstrated that curcumin treatment or GR slightly inhibited NHE1, while the combined treatment of curcumin and GR further enhanced the inhibitory effect on NHE1 and reduced pHi (110). Since the activation of NHE1 depends on energy and Akt (111), GR enhances the ability of curcumin to synergistically inhibit NHE1. Therefore, the combined treatment of GR and curcumin may have an important role in the regulation of pH in human hepatoma cells.

The expression of NHE1 in tumor tissues is not only related to the tumor size in HCC, but also venous invasion and pathological TNM staging (112). Previous studies have reported that inhibition of NHE1 blocks the invasion and metastasis of SMMC-7721 and HepG2 liver cancer cells (113,114). Invasion and migration of malignant tumor cells require destruction of the basement membrane and ECM proteolysis (115). The acidic extracellular pH (pHe) of tumor cells is also crucial in the activation of extracellular proteinases and the degradation of ECM in tumors (116,117). In the early stages of cell migration, invasion and metastasis, ECM decomposition is mainly mediated by matrix metalloproteinases (MMP)-2, -3 and -9 (114,118). Moreover, alkaline pHi promotes the expression of vascular endothelial growth factor (VEGF), which plays an important role in inducing neovascularization and promoting tumor growth and metastatic potential (Fig. 3) (119-121). Hypoxia is also a common feature of the tumor microenvironment and has been shown to stimulate invasion and metastasis (122,123). Hypoxia activates ERK1/2, a family of mitogen-activated protein kinases that play a major role in signaling pathways that are involved in cell scatter, motion, invasion, proliferation and survival (124). ERK1/2 also regulates the expression of MMPs and VEGF (125,126). Yang *et al* (114) demonstrated that inhibition of NHE1 by ethyl-isopropyl-amiloride (EIPA) inhibited HepG2 cell invasion and metastasis, and that EIPA inhibition acts by downregulating MMP-2, MMP-9 and VEGF in an ERK1/2-dependent manner. Moreover, NHE1 not only affects the migration and invasion of tumor cells, but is also related to the apoptosis of tumor cells (127). Bcl-2 family members, such as Bcl-2, Bcl-xL and Bax, play a crucial role in controlling apoptosis (127). Previous studies have reported that the NHE1 inhibitor EIPA downregulates Bcl-2, and upregulates Bax expression in HepG2 cells, leading to tumor cell apoptosis (128) (Fig. 3).

Interleukin 6 (IL6) is a key cytokine involved in the development and progression of inflammation-associated HCC. For example. Xu *et al* (128) found that IL6 activates the functional activity of NHE1, induces the interaction of NHE1, Na^+/Ca^{2+} exchanger1 (NCX1) and calmodulin (CaM), and upregulates the expression of NHE1 in human hepatoma cells. Benzo[a]pyrene (B[a]P) is a prototype of polycyclic aromatic hydrocarbons, and is a human carcinogen (107). In addition to triggering apoptotic signals, B[a]P may induce survival signals and participate in the promotion of cancer (107). Previous studies have also reported that B[a]P induces metabolic reprogramming, which involves the activation of NHE1, and it leads to epithelial-mesenchymal transition (129-131). Ginsenoside Rg3, the main pharmacologically active compound extracted from Chinese ginseng, has been widely recognized as having antitumor properties in various cancer types (132,133), including inhibition of HCC cell proliferation, induction of apoptosis and inhibition of angiogenesis (134) and metastasis (135). Recently, it has been reported that Rg3 inhibits HCC cell proliferation and induces apoptosis by decreasing NHE1 expression and activity, and Rg3-mediated NHE1 inhibition is dependent on the EGF/EGFR/ERK1/2/hypoxia-inducible factor-1α signaling pathway (136). As a common ion transporter, NHEs are regulated by numerous substances. For example, IL6 and B[a]P can activate the activity of NHE1, while Rg3 can inhibit the activity of NHEs (107,128,132). Therefore, based on the aforementioned description of IL6, B[a]P and Rg3 in HCC, it was suggested that NHEs may be a potential therapeutic target in HCC. However, further studies are required to identify the potential mechanisms.

Cholangiocellular carcinoma (CCC) is the second most common primary liver cancer after HCC, with an increased incidence (137). Furthermore, chronic inflammation and oxidative stress play a key role in the development of CCC (138). NHEs form a potential link between controlling pHi and tumor development. Therefore, Elsing *et al* (62) determined the effect of oxidative stress on NHEs using tert-Butyl hydroperoxide (t-BOOH), a hydrogen peroxide, in the biliary epithelial cancer cell line Mz-Cha-1. These authors demonstrated that t-BOOH reduced NHE activity in a dose-dependent manner; at 4 mM t-BOOH, the NHE activity was almost absent and glutathione supplementation and intracellular Ca^{2+} chelation partially restored NHE activity. Moreover, in Mz-Cha-1 cells, inhibition of NHE by oxidative stress depends in part on the presence of intracellular Ca^{2+} and intracellular glutathione levels (62).

5. NHEs in other liver diseases

Hepatic failure (HF) is a life-threatening disease with a very high mortality rate (139), and hepatocyte apoptosis leading to HF is an important event in hepatocyte death. Tumor necrosis factor-α (TNF-α) is an inflammatory factor and an inducer of hepatocyte apoptosis (140). It has been reported that TNF-α induces NHE activity in hepatocytes in a time-dependent manner (141). Activation of NHEs increases intracellular Na^+, promotes Na^+/Ca^{2+} exchange and causes Ca^{2+} overload (142), which is considered to be the key factor in cell damage. Moreover, an increase in intracellular Ca^{2+} concentration automatically activates calpain, a calcium-dependent protease (143). The antiapoptotic family member Bcl-xL is a natural substrate for calpain, and NHE mediates TNF-α-induced hepatocyte apoptosis via Ca^{2+}/calpain-dependent degradation of Bcl-xL (141). However, the NHE inhibitor cariporide reverses the effects induced by TNF-α and has a protective effect on acute HF (141).

Endotoxin-mediated production of proinflammatory cytokines plays an important role in the pathogenesis of liver disease (144). Previous studies have reported that lipopolysaccharide (LPS) causes liver damage by increasing the release of TNF-α in a NASH model (145). Therefore, interfering with LPS-induced inflammatory responses may help to alleviate inflammation associated with liver disease. Heat shock proteins (Hsp70) play an important role in LPS-mediated inflammatory responses (144). NHE1 is considered to be a mediator of inflammatory responses in macrophages (146-148) and has been reported to interact with Hsp70 (149). Inhibition of Hsp70 substrate binding activity in vivo reduces induction of proinflammatory factors (144). Huang *et al* (144) treated macrophages and livers with LPS and revealed a significant increase in the association of NHE1-Hsp70, suggesting that the formation of the NHE1-Hsp70 complex is essential for the induction of proinflammatory factors. Therefore, LPS-induced liver damage may be prevented by disrupting the NHE1-Hsp70 interaction.

In addition, the beneficial effects of the NHE inhibitor EIPA in blocking NHEs in a partial hepatic ischemia rat model suggested a positive role for NHE1 in oxidative liver injury, and indicated that inhibition of NHEs is a potential strategy for preventing or reducing ischemic liver injury (27).

6. Conclusion

NHEs are ion transporters that are widely present in a variety of organisms and are important regulators at the cellular, tissue and systemic levels (150). To the best of our knowledge, the current review is the first detailed description of the physiology and pathology of NHEs in the liver. While NHEs are common targets for various inflammatory stimuli, the effect of selective targeted therapy of NHEs in the liver are inconclusive, and thus further studies are required. Numerous experimental models currently show that NHE inhibitors lack major toxic effects, and several, such as cariporide, have been used in preclinical and clinical trials (151). However, a large number of studies have only analyzed the effects of single factors, and have not considered that their function in physiological and pathological conditions may mainly be the result of the interaction of various transporters. Therefore, more comprehensive methods are required to alter the function, and regulate and target NHEs in liver-related pathology.

Acknowledgements

The authors would like to thank Professor Jiaxing An (Department of Gastroenterology, Affiliated Hospital to Zunyi Medical University) for their highly professional services.

Funding

This study was supported by grant from the Clinical Medical Research Center for Digestive Diseases,Guizhou province,China

Availability of data and materials

Not applicable.

Authors' contributions

TL wrote the manuscript and participated in information collection, analysis, organization and figure design. BT primarily revised and final editing. All authors 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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