REVIEW ARTICLE

Cell volume regulation in cancer cell migration driven by osmotic water flow

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Abstract

Cancer metastasis is the most frequent cause of death for patients with cancer. The main current treatment for cancer metastasis is chemotherapy targeting cancer cells' ability to proliferate. However, some types of cancer cells show resistance to chemotherapy. Recently, cancer cell migration has become the subject of interest as a novel target of cancer therapy. Cell migration requires many factors, such as the cytoskeleton, cell-matrix adhesion and cell volume regulation. Here, we focus on cell volume regulation and the role of ion/water transport systems in cell migration. Transport proteins, such as ion channels, ion carriers, and aquaporins, are indispensable for cell volume regulation under steady-state conditions and during exposure to osmotic stress. Studies from the last ~25 years have revealed that cell volume regulation also plays an important role in the process of cell migration. Water flow in accordance with localized osmotic gradients generated by ion transport contributes to the driving force for cell migration. Moreover, it has been reported that metastatic cancer cells have higher expression of these transport proteins than nonmetastatic cancer cells. Thus, ion/water transport proteins involved in cell volume regulation and cell migration could be novel therapeutic targets for cancer metastasis. In this review, after presenting the importance of ion/water transport systems in cell volume regulation, we discuss the roles of transport proteins in a pathophysiological context, especially in the context of cancer cell migration.

KEYWORDS
cancer metastasis, cell migration, cell volume regulation, osmotic water flow, transporter

1 | INTRODUCTION

Cancer metastasis, the dissemination of cancer cells from the primary tumor to distant organs, is a major cause of cancer mortality. Metastasis is achieved through a multistep process including detachment from the primary tumor, travel around the body through the circulation, settlement at different organs and growth into additional tumor tissues. Despite these many steps, most of the current treatments for cancer metastasis target the cancer cells' ability to proliferate. However, it has been suggested that this type of chemotherapy is not effective for some cancer cells, especially those with low proliferation rates, such as cancer cells in dormancy or migration. Therefore, we must develop alternative strategies for cancer chemotherapies, and one possible target is cell migration.[1] In fact, cancer cell migration and invasion are critical steps of cancer metastasis; moreover, it has been reported that invasive cancer cells show increased expression of genes involved in

cell motility compared to noninvasive cancer cells.[2] Thus, cell migration could be a novel therapeutic target for cancer metastasis.

With regards to the mechanism of cell migration, the cytoskeleton has long been proposed to generate the driving force. Recently, however, it has been suggested that ion/water transport proteins are indispensable for cell migration, and that water flow due to the osmotic gradients generated by localized ion transport across the plasma membrane can also be the driving forces. Moreover, the osmotic gradient of the extracellular space influences cell migration by regulating ion/water transport proteins.[3] Thus, cell migration has begun to be studied from the point of view of cell volume regulation.[4]

2 | ION HOMEOSTASIS IN CELL VOLUME MAINTENANCE

The plasma membrane has low permeability to negatively charged macromolecules that abound inside cells, whereas it is highly permeable to water because of the presence of aquaporins (AQPs). Thus, even under steady-state conditions, cells are threatened by osmotic swelling due to the entrance of ions and water. However, cells are practically impermeable to sodium ions (Na^+) because of the low permeability of the membrane to Na^+ and because of active outward transport of Na^+ through Na^+-K^+-ATPase. In addition, potassium ions (K^+) leak outwardly through K^+ channels in accordance with the chemical potential gradient, which generates a negative charge inside cells that is followed by efflux of chloride ions (Cl^-). These ion transport proteins enable cells to keep intracellular ion concentrations lower than extracellular ion concentrations and to avoid osmotic cell swelling.[5] Thus, ion homeostasis achieved by the regulation of ion channels and transporters is crucial for cell volume regulation, even under steady-state conditions (Figure 1). Furthermore, these proteins have been proposed to be involved in many types of cellular activities accompanied by cell volume changes such as migration, inflammation, proliferation, and cell death.[5]

3 | VOLUME REGULATION IN CELL MIGRATION

3.1 | General mechanisms of cell migration

The initial step of cell migration is polarization along the axis of movement. Migration is achieved through a repeated cycle of protrusion of the leading edge and retraction of the rear part of the cell.[4] As a driving force of migration, the cytoskeleton has long drawn attention. In the process of cell migration, actin polymerization with the production of motile force for protrusion occurs predominantly at the leading edge, whereas myosin II associates with existing actin filaments to generate the force for rear retraction.[6] In fact, it has been suggested that the suppression of cancer cell migration by inhibition of actin polymerization could be an anticancer therapeutic target.[1]

3.2 | Role of ion/water transport systems in cell migration

Work from recent years provides evidence that cell volume regulation by ion/water transport is indispensable for efficient cell migration. Before giving an overview of the transport proteins involved in cancer cell migration, we will present the mechanisms of cell migration driven by water flow inside cells in this section.

3.2.1 | Cell migration by osmotic water flow

The relationship between osmotic volume change and cell motility was proposed in the 1980s. Oster and Perelson proposed that not actin polymerization but hydrostatic pressure generated by osmotic water flow is the driving force of the elongation of the acrosomal process in the sperm of *Thyone*, a sea cucumber.[7] Despite the implications of osmotic/hydrostatic pressure in cell motility, few studies have focused on it. In 1994, however, it was reported that cell migration is attenuated by extracellular hypertonicity in mammalian cells; hypertonic cell shrinkage was proposed to inhibit local volume gain,
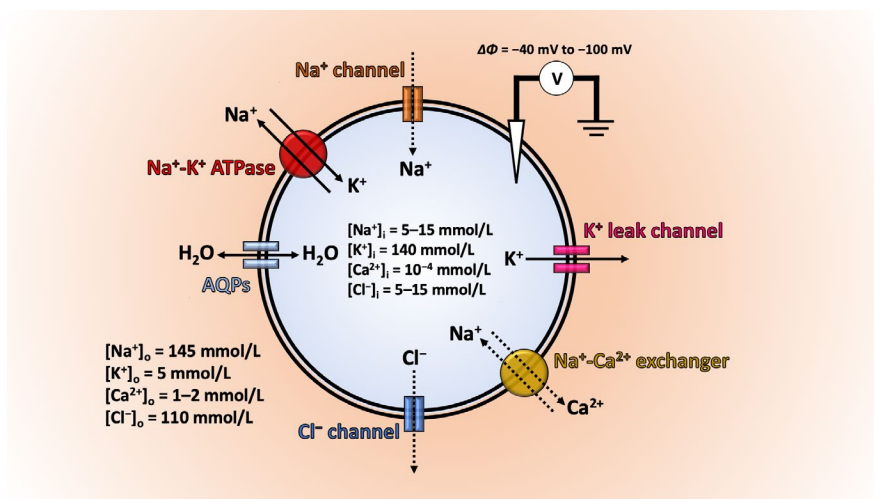

FIGURE 1 Cell volume regulation under steady-state conditions. Na^+-K^+ ATPase mediates Na^+ efflux and K^+ influx, which leads to the accumulation of K^+ inside cells. This K^+ accumulation leads to K^+ leakage through K^+ channels, which generates a negative membrane potential ($\Delta\Phi$ = −40 to −100 mV).[5] The solid line indicates the major flow of ions, and the dotted line indicates the minor flow of ions. AQP, aquaporin

which would facilitate cell migration.[8] Moreover, it was reported that cells move to regions with lower solute concentrations, which means that the extracellular solute gradient itself could be a driving force of cell migration.[9] From around this time, the relationship between water flow and cell migration has become the subject of much interest. Localized volume gain by water influx has been reported, and it has been suggested that the water influx is mediated by AQPs, which localize to the leading edges of migrating cells.[10]

The importance of cell volume regulation is evident, especially in osmotic stress responses. Osmotic stress results from the difference between intracellular and extracellular osmolality and causes the compulsive water influx/efflux followed by cell swelling/shrinkage. Under osmotic stress-induced cell volume perturbation, cells undergo volume recovery processes called regulatory volume decrease (RVD) and regulatory volume increase (RVI) by actively transporting ions.[5] In the process of cell migration, it has been suggested that RVI facilitates protrusion of the leading edge and that RVD contributes to rear retraction. In fact, ion/water transport proteins involved in RVD or RVI show different distributions during migration, which could generate localized osmotic gradients and subsequent volume changes for protrusion of the leading edge and retraction of the rear end.[4] Thus, osmotic water flow itself generated by ion transport could assist cell migration (Figure 2).

Recently, this kind of cell migration has been studied from the point of view of cancer metastasis. Interestingly, metastatic breast cancer cells can migrate in a confined tube even under conditions of actin polymerization suppression, whereas the inhibition of Na^+-H^+ exchanger 1 (NHE1) or AQP5 suppresses this type of cancer cell migration; moreover, changes in the extracellular osmolality affects the localization of NHE1, which eventually changes the direction of migration.[3] These observations suggest that osmotic water flow itself could be a driving force for cell migration, and the transport proteins concerned could be affected by changes in extracellular osmolality.

3.2.2 | Regulation of ion transport proteins under osmotic stress

As shown above, osmotic stress could change the localization or activity of ion/water transport proteins. It is important to elucidate the upstream regulation mechanisms of ion/water transport proteins to confirm the involvement of not only ion/water transport itself but also volume regulation systems in cell migration.

There are 2 main possible mechanisms for the regulation of ion/water transport proteins by osmotic stress. One involves the direct recognition of osmotic stress by ion transport proteins, and the other involves signal transduction inside the cells. Some ion channels have been reported to recognize osmotic stress by themselves. Leucine rich repeat containing 8 subunit A (LRRC8A), recently identified as a volume-regulated anion channel (VRAC),[11,12] is activated by hypoosmotic stress, and it has been proposed that the LRRC8 protein directly senses decreases in intracellular ionic strength after hypotonicity-induced water influx.[13] Transient receptor potential channels (TRPs) are polymodal sensors of a variety of chemical and physical stimuli, and some of them have been proposed to be activated under osmotic stress by recognizing membrane tension.[14,15] We will show in the next section how the ion channels mentioned in this section are involved in cell migration.
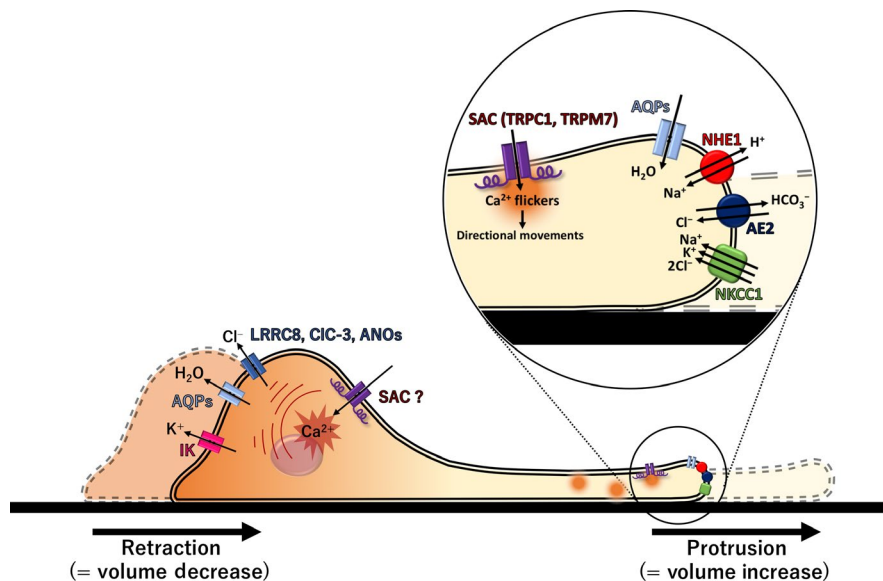

FIGURE 2 Cell volume regulation during cell migration. Net NaCl uptake occurs at the leading edge, which contributes to volume gain, whereas net KCl efflux leads to volume loss in rear retraction. The associated ion transporters are possibly regulated by the intracellular Ca^{2+} gradient during cell migration, which is highest at the rear part and lowest at the front. Directional movement is also regulated by very localized Ca^{2+} elevations called "Ca^{2+} flickers". These Ca^{2+} flickers have been proposed to be generated by stretch-activated Ca^{2+} channels (SACs), such as transient receptor potential channels (TRP)C1 and TRPM7.[4,5,64] The orange-to-pale yellow gradient corresponds to the high-to-low subcellular concentrations of Ca^{2+}. AE2, anion exchanger 2; ANO, anoctamin; AQP, aquaporin; ClC-3, voltage-gated Cl^- channel 3; NHE1, Na^+-H^+ exchanger 1; NKCC1, Na^+-K^+-$2Cl^-$ cotransporter 1

The other mechanism for the regulation of ion/water transport proteins under osmotic stress is kinase-dependent signal transduction, such as that through the stress-induced mitogen-activated protein kinase (MAPK) pathway and the with-no-lysine kinase (WNK)-STE20/SPS1-related proline/alanine-rich kinase (SPAK)/oxidative stress-responsive kinase 1 (OSR1) pathway (WNK-SPAK/OSR1 pathway), which change the activity or localization of ion transport proteins.[5,16] The MAPK pathway is activated by a wide variety of biological, chemical, and physical stimuli, including osmotic stress, and induces physiological processes, such as proliferation, survival, migration, and cell death. Mitogen-activated protein kinase signaling is composed of 3-layered kinase cascades including MAP3Ks, MAP2Ks, and MAPKs from upstream to downstream. Among MAPKs, ERK1/2, p38 MAPK, and JNK have been well investigated in the context of osmotic stress responses. These 3 MAPKs change their activity under osmotic stress, and play multiple roles in volume recovery.[16] These MAPKs have already been suggested to be involved in cell migration through the cytoskeleton and adhesion.[17] It is possible that these MAPK pathways regulate ion/water transport proteins in the process of cell migration. In fact, NHE1, which is crucial for cell motility, is regulated by p38 MAPK or JNK in some species.[4,16] WNK-SPAK/OSR1 is another signaling pathway for the regulation of ion transport proteins. With-no-lysine kinases and their downstream kinases, STE20/SPAK and OSR1, regulate K^+-Cl^- cotransporters (KCCs) and Na^+-K^+-$2Cl^-$ cotransporters (NKCCs), both of which are crucial for volume recovery under osmotic stress. It has been suggested that this WNK-SPAK/OSR1-NKCC pathway contributes to cell migration.[18] In fact, WNK1 is necessary for the homing of T cells because it activates migration.[19] Furthermore, glioma cells show higher WNK1, OSR1, and NKCC1 activity than other types of cells, which likely facilitates their migration.[20] As a common regulator of these kinases, apoptosis signal-regulating kinase 3 (ASK3), one of the stress-responsive MAP3Ks, plays an important role in osmotic stress responses.[21,22] It uniquely responds to osmotic stress in rapid, bidirectional, and reversible manners, and proper changes in its activity are necessary for RVD and RVI.[22,23] It is possible that ASK3 contributes to cancer cell migration through volume regulation. In fact, metastatic osteosarcoma cells show high expression of ASK3 compared to nonmetastatic ones,[24] and the overexpression of ASK3 in prostate cancer cells promotes metastasis.[25] Furthermore, metastatic melanoma cells shows high expression of ASK3 compared to nonmetastatic melanoma cells, and patients with high expression of ASK3 in tumor tissues have high mortality (Figure 3).

Thus, the regulation of cell volume by the MAPK and WNK-SPAK/OSR1 pathways and upstream molecules such as ASK3 could be novel therapeutic targets for cancer metastasis.

4 | DYSREGULATION OF ION TRANSPORT IN METASTATIC CANCER CELLS

With regard to volume regulation, net NaCl uptake and net KCl efflux lead to water flow across the plasma membrane during RVI and RVD, respectively.[5] These types of volume regulation locally occur during cell migration.[4] Here, we summarize them, focusing on how they are dysregulated in the volume regulatory systems of metastatic cancer cells.

4.1 | Aquaporins

Aquaporins are members of a family of water channels that contains 15 members identified in mammals (AQP0-AQP14). Their main function is to transport water across the membrane in accordance with the osmotic gradient. They play diverse physiological roles, including roles in cell migration, and they have been proposed to also be involved in cancer cell invasion and metastasis.[26,27]

The involvement of AQPs in physiological migration was first reported in 2005. AQP1 knockout mice show impaired angiogenesis because of the low motility of their endothelial cells, and thereby show resistance to tumor growth.[28] Since then, numerous studies have focused on the involvement of AQPs in cell migration, and AQP1, AQP3, AQP4, AQP5, AQP7, and AQP9 have been implicated in physiologically functional cell migration.[4] Furthermore, AQP1, AQP4, AQP5, and AQP9 have been reported to localize to the leading edge during migration.[3,10,28,29] This distribution of AQPs would enable localized water influx and subsequent volume gain, contributing to the protrusion of the leading edge. Among AQPs, AQP1 is the most intensively studied for its role in cancer cell migration. It has been reported to be highly expressed in many types of cancer cells. Notably, AQP1 shows an increase in its expression in a stage-dependent manner in astrocytoma cells and vasculature.[30] Furthermore, overexpression of AQP1 enhances the migratory and metastatic phenotype of mouse melanoma cells.[31] Thus, AQPs could be responsible for cancer metastasis.

4.2 | Ion carriers

Ion carriers, which are symporters or antiporters of ions with energy from chemical potential gradients, contribute to migration mainly through uptake of osmotically active ions such as Na^+, K^+, and Cl^- at the leading edge. Although Ca^{2+} transporters are also important for cell migration, Ca^{2+} contributes to the activation of other channels and carriers rather than to water influx itself because its physiological concentrations are much lower than those of Na^+, K^+, and Cl^-. We summarize the transporters of these three ions here and summarize Ca^{2+} transport proteins later in Section 4.6.

4.2.1 | Na^+-H^+ exchangers

Na^+-H^+ exchangers belong to the SLC9A family and carry out a 1:1 exchange of Na^+ and H^+ using a chemical gradient of Na^+. Here, we focus only on NHE1 because its function has been the most intensively studied among the NHEs (NHE1-NHE9). Na^+-H^+ exchanger 1 is a ubiquitously expressed protein involved in pH maintenance, volume regulation, and epithelial absorption.[5] It has been proposed to facilitate cell migration through cell volume regulation, actin polymerization, collagen-integrin adhesion, and degradation of the ECM.[32]

With regards to cell volume regulation, RVI after hypertonic shrinkage requires NHE1, which performs net NaCl uptake in co-operation with anion exchanger 2 (AE2).[5] Additionally, NHE1 contributes to solute uptake for protrusion during cell migration. In fact, NHE1 localizes to the leading edge of the cell in the process of protrusion.[33] Moreover, NHE1 is necessary for actin-independent cell migration, which is not attenuated by inhibition of actin polymerization but is suppressed by inhibition of NHE1; interestingly, osmotic shock around cells changes the localization of NHE1 and the direction of migration in this type of cell migration.[3]

Na^+-H^+ exchanger 1 is upregulated and enhances metastatic phenotypes in several types of cancer cells. The human breast cancer cell lines MCF-7 and MDA-MB-435 have higher NHE1 activity than normal human breast cells under serum deprivation conditions, a common environment in tumor tissue.[34] Moloney sarcoma virus (MSV)-transformed MDCK cells with an invasive phenotype have higher expression of NHE1 than nontransformed MDCK cells.[35] Notably, NHE1 in MSV-MDCK cells is more sensitive to an NHE1 inhibitor, ethyl-isopropyl amiloride (EIPA), than that in MDCK cells, and the migration of MSV-MDCK cells is indeed suppressed by EIPA.[35] Therefore, NHE1 is expected to be a novel therapeutic target for cancer metastasis.
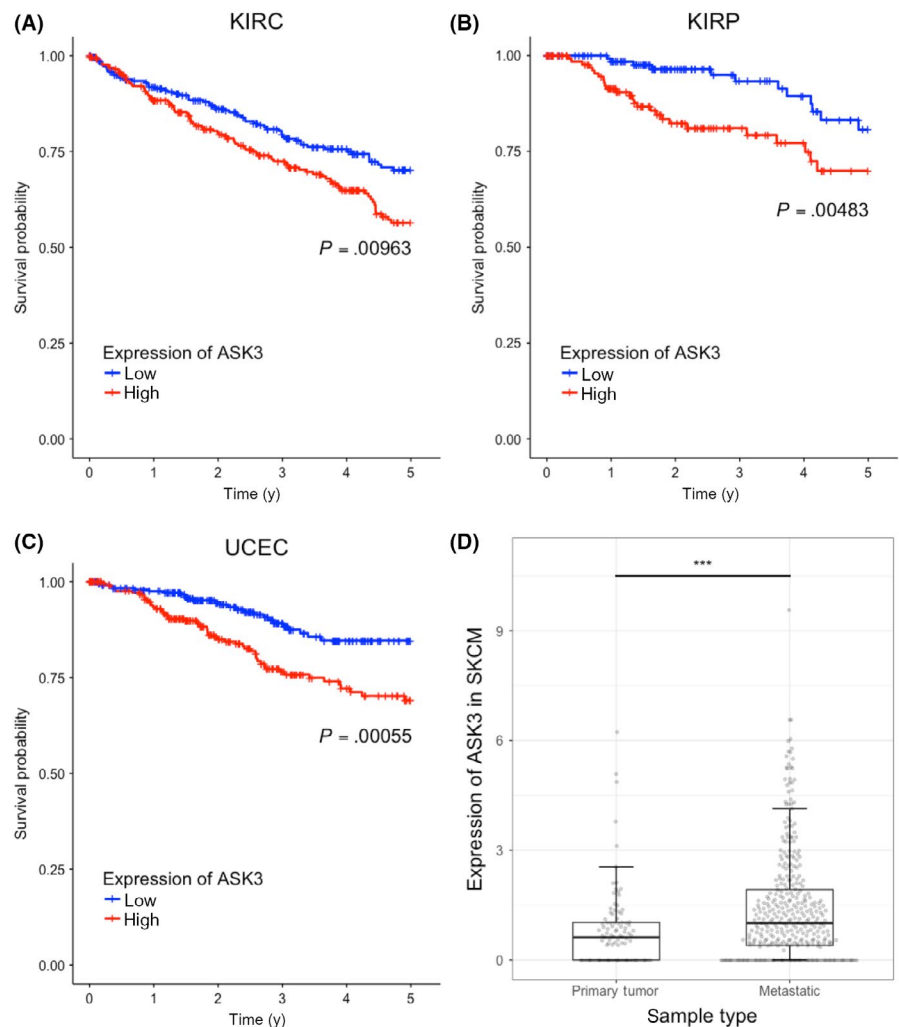
4.2.2 | Anion exchangers

Anion exchangers belong to the SLC4 family of transporters. They carry out a 1:1 exchange of Cl^- and HCO_3^- across the membrane. The direction of ion transport is determined by the chemical gradient of Cl^-. Among the 4 AEs, AE2 plays an important role in cell volume regulation. Anion exchanger 2 is widely distributed and is expressed at the basolateral membrane in most epithelial cells. Under conditions of hypertonic cell shrinkage, AE2 mediates net uptake of NaCl in cooperation with NHE1, which evokes subsequent water influx.[5] Anion exchanger 2 localizes to the leading edges of cells during migration, and facilitates protrusion.[33] Moreover, the expression of AE2 in thyroid cancer cells or breast cancer cells is higher than in normal cells. In addition, AE2 expression tends to increase in a stage-dependent manner (Figure 4A,B). Therefore, it is possible that AE2 is responsible for the metastatic phenotype of cancer cells.

4.2.3 | Na^+-K^+-$2Cl^-$ cotransporters

Na^+-K^+-$2Cl^-$ cotransporters belong to the SLC12A family, which is composed of cation-chloride cotransporters. Two NKCCs have been

FIGURE 3 Expression of apoptosis signal-regulating kinase 3 (ASK3) in cancer cells. A-C, Kaplan-Meier plots of the overall survival rates of patients with different kinds of cancer. The red line indicates the group with high expression of ASK3 in primary tumors, and blue indicates low expression. A, Kidney renal clear cell carcinoma (KIRC; n = 533). B, Kidney renal papillary cell carcinoma (KIRP; n = 289). C, Uterine corpus endometrial carcinoma (UCEC; n = 531). *P* values were calculated with the log-rank test in R. D, Boxplot of the expression of ASK3 in skin cutaneous melanoma (SKCM). Each dot indicates an individual value (Primary tumor, n = 103; Metastatic, n = 368). ***P < .005 by Wilcoxon rank sum test in R. Note that we excluded "Solid tissue normal" in this figure because there was only 1 available sample of SKCM. Datasets were extracted from The Cancer Genome Atlas[80]
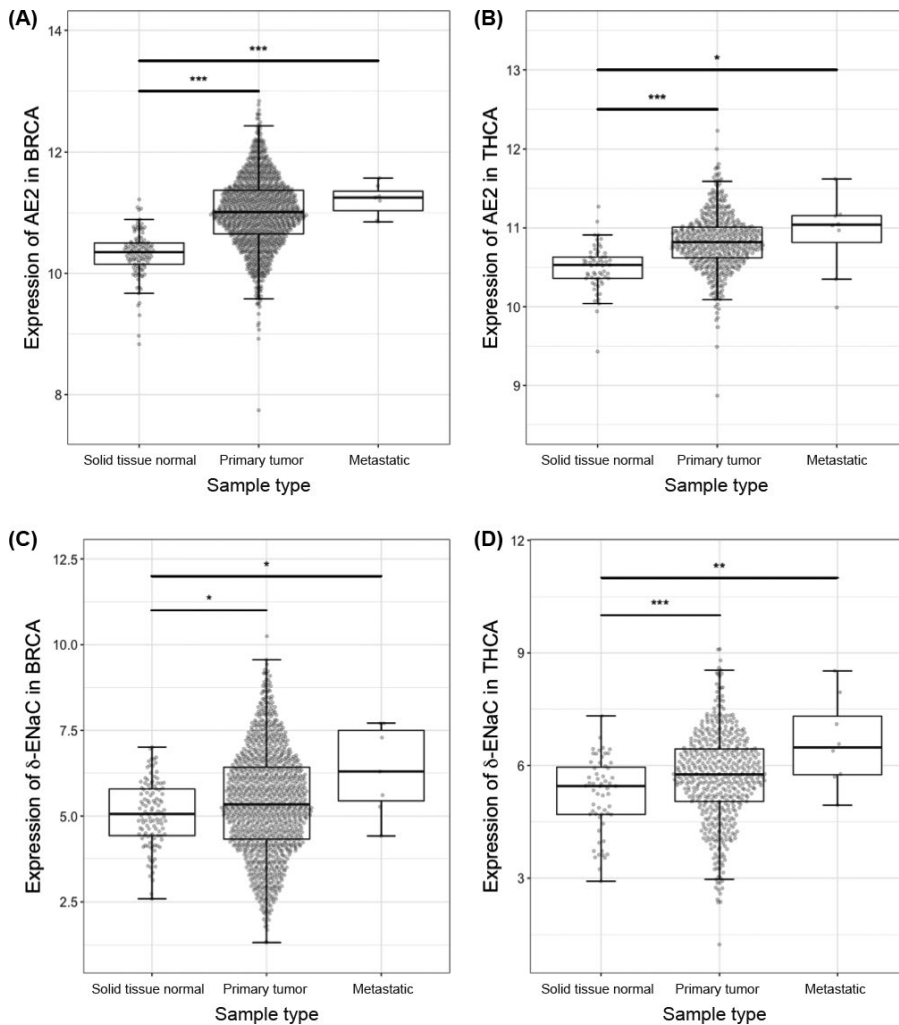
FIGURE 4 Enhancement of the expression of ion transport proteins in migratory cancer cells. A,B, Boxplots of the expression of anion exchanger 2 (AE2) in (A) breast invasive carcinoma (BRCA) and (B) thyroid carcinoma (THCA). C,D, Boxplots of the expression of epithelial Na$^+$ channel (δ-ENaC) in (C) BRCA and (D) THCA. Each dot indicates an individual value (BRCA: n = 113 for Solid tissue normal, n = 1095 for Primary tumor, and n = 7 for Metastatic; THCA: n = 59 for Solid tissue normal, n = 505 for Primary tumor, and n = 8 for Metastatic). *P < .05, **P < .01, and ***P < .005 by Steel-Dwass test in R. Datasets were extracted from The Cancer Genome Atlas[80]

identified so far, the ubiquitously expressed NKCC1 and the kidney-specific NKCC2, both of which carry out inward 1:1:2 transport of Na$^+$, K$^+$, and Cl$^-$ across the membrane. Na$^+$-K$^+$-2Cl$^-$ cotransporters are activated after hypertonic shrinkage and mediate ion influx followed by osmotic water influx (RVI).[5] Under hyperosmotic stress, the WNK1-SPAK/OSR1 pathway regulates NKCCs through direct phosphorylation.[18]

Because of its ability to increase cell volume, NKCC1 is also involved in cell migration. Initially, it was observed that the NKCC blockers furosemide and bumetanide suppress cell migration in mammals.[36] Afterward, it was revealed that NKCC1 localizes to the leading edges of protrusions under growth factor stimulation.[37]

With regards to the roles of NKCC1 in cancer cell migration, glioma cells, which are primary brain cancer cells and have a diffusely invasive phenotype, show ~10-fold higher concentrations of intracellular Cl$^-$ than noncancer cells, and this Cl$^-$ accumulation could be attributable to NKCC1.[38] Furthermore, NKCC1 depletion by shRNA and NKCC inhibition by bumetanide suppress the migration of glioma cells.[39]

4.3 | K$^+$ channels

In most cases, opening of K$^+$ channels leads to K$^+$ efflux in accordance with its chemical potential gradient. With regards to volume

regulation, K$^+$ channels mediate net KCl efflux in cooperation with Cl$^-$ channels and contribute to RVD.[5] Wide varieties of K$^+$ channels have been reported to be involved in cell migration so far. Although voltage-dependent K$^+$ channels and inwardly rectifying K$^+$ channels are both necessary for cell migration, they contribute to adhesion rather than volume regulation. Here, we focus on Ca^{2+}-sensitive K$^+$ channels (K$_{Ca}$ channels), which play an important role in rear retraction during cell migration.

The role of K$_{Ca}$ channels in cell migration was first determined in 1994. Inhibition of K$_{Ca}$ channels, especially K$_{Ca}$ channels at the rear ends of the cells, with charybdotoxin, suppresses the migration of MDCK-F cells.[36,40] Moreover, K$_{Ca}$ channels have been suggested to be necessary for rear retraction based on measurements of localized cell volume.[41] Since these discoveries, the molecular identity of the responsible channel has been intensively studied.

K$_{Ca}$ channels are classified into 3 types, BK, SK, and IK channels, in accordance with their conductance. Among the 3 types, the IK channel (K$_{Ca}$3.1) has been the most extensively studied in cell migration. K$_{Ca}$3.1 is necessary for cell migration[42] and is locally activated at the rear of migrating MDCK-F cells, possibly because of the Ca^{2+} gradient, as shown below.[40] Interestingly, K$_{Ca}$3.1 shows a stage-dependent enhancement of its expression in endometrial cancer cells,

and this enhancement could be responsible for the progressive or invasive phenotype of the cells.[43]

4.4 | Na⁺ channels

Na⁺ channels, such as voltage-dependent Na⁺ channels (Na$_v$s), epithelial Na⁺ channel (ENaC) and acid-sensing ion channels, play important roles in cell migration. Among them, however, only ENaC has been reported to contribute to cell migration through volume regulation. The ENaC is normally composed of 3 subunits, α- (or δ-), β-, and γ-ENaC. Knockdown of α-, β-, or γ-ENaC subunit impairs RVI after hyperosmotic stress-induced cell shrinkage.[44] The role of ENaC in cell migration is often shown by wound healing assays. Pharmacological inhibition of ENaC or knockdown of ENaC subunits leads to impaired wound healing after scratching.[45] In addition, ENaC is abundant at wound edges, which is consistent with the depolarization there.[46] Thus, ENaC could facilitate cell migration by volume gain at the cell front.[4]

It is possible that ENaC contributes to cancer cell metastasis. Adenomatous polyposis coli-mutated mice that develop multiple intestinal neoplasias show high expression of β- and γ-ENaC.[47] Furthermore, the expression of δ-ENaC tends to increase in a stage-dependent manner in thyroid cancer cells and breast cancer cells (Figure 4C,D).

4.5 | Cl⁻ channels

Although K⁺ efflux is an essential component of RVD and cell migration, it must be accompanied by Cl⁻ efflux to maintain electroneutrality. Thus, Cl⁻ channels also play important roles both in RVD after hypotonicity and in rear retraction during cell migration.[4,5] So far, there is substantial evidence that 3 kinds of Cl⁻ channels, VRACs, voltage-gated Cl⁻ channel 3 (ClC-3), and transmembrane protein 16s (TMEM16s), participate in cell migration through volume regulation. These channels have been reported to show high expression in invasive cancer cells, and in fact, Cl⁻ channels have attracted attention as therapeutic targets for cancer metastasis.[48]

4.5.1 | Volume-regulated anion channels/LRRC8

The existence of VRACs has long been proposed based on observations of Cl⁻ efflux followed by hypoosmotic cell swelling.[5] Through pharmacological inhibition, VRACs have been implicated in cell migration.[49] In addition, VRACs might be locally activated at the rear parts of the cells during cell migration by cholesterol, which actually shows a heterogenous distribution during migration.[50,51] Although the molecular identity of VRACs has long remained elusive, 2 groups reported in 2014 that the LRRC8 family, especially LRRC8A, is the responsible protein for hypotonicity-induced Cl⁻ currents and RVD.[11,12] Soon after the identification of its molecular identity, it was reported that LRRC8 senses hypotonicity by sensing intracellular ionic strength.[13]

Although there have been few reports about the involvement of LRRC8 in cell migration or cancer metastasis, its involvement is becoming the subject of intense study. Quite recently, it has been reported that knockdown of LRRC8A impairs migration of human colon cancer cells; furthermore, colon cancer tissue shows elevated expression of LRRC8A, and patients with high expression of LRRC8A have higher mortality than those with lower expression.[52] Thus, VRACs could be novel therapeutic targets for cancer metastasis.

4.5.2 | ClC-3

Although ClC-3 has been reported to be a VRAC,[53] this remains a matter of dispute.[5] However, the necessity of ClC-3 in glioma cell migration has been suggested in some reports showing that knockdown or pharmacological inhibition of ClC-3 suppresses glioma cell migration.[54,55] Moreover, the expression of ClC-3 in glioma tissue is enhanced in a stage-dependent manner. Thus, ClC-3 has been proposed to be responsible for invasive phenotypes of glioma cells.[54] It could be suggested that ClC-3 contributes to glioma cell migration through volume regulation because invasion through the extracellular space in the brain, which is too narrow for cells to migrate through, requires glioma cells to change their shape and volume by net KCl efflux.[56] Although whether volume decreases mediated by ClC-3 occur in the process of rear retraction remains elusive, it is possible that ClC-3 is locally activated through CaMKII by the intracellular Ca^{2+} gradient, which is the lowest at the leading edges and highest at the rear parts of the cells during cell migration, as shown below.[54]

4.5.3 | Transmembrane protein 16s

Transmembrane protein 16s, also known as anoctamins, are Ca^{2+}-activated chloride channels, of which 10 members have been identified (TMEM16A-TMEM16H, TMEM16J, and TMEM16K). Although whether TMEM16s form VRAC is controversial,[57] TMEM16s are necessary for RVD and apoptotic volume decreases.[58] Among the TMEM16s, TMEM16A has been suggested to contribute to cell volume decreases during rear retraction in directional migration.[59] Furthermore, TMEM16A is responsible for the metastatic phenotype of head and neck squamous cell carcinoma (HNSCC).[60,61] The expression of TMEM16A positively correlates with the migration of HNSCC. In fact, HNSCC patients with high expression of TMEM16A show lower overall survival rates than those with low expression.

4.6 | Ca²⁺ channels

Ca^{2+} influx itself contributes little to osmotic water influx because the physiological concentrations of Ca^{2+} are much lower than those of Na⁺, K⁺, and Cl⁻. However, intracellular Ca^{2+} regulation plays a critical role in cell migration.[4] During cell migration, the intracellular Ca^{2+} concentration is the lowest at the leading edge and highest at the rear part.[62] This Ca^{2+} gradient enables localized activation of Ca^{2+}-dependent K⁺ or Cl⁻ channels, which contribute to rear retraction.[4]

In fact, intracellular Ca^{2+} elevation is observed before rear retraction.[63] In addition, very localized and short-lived intracellular Ca^{2+} elevations called "Ca^{2+} flickers" are observed at the leading edge.[64] Although it has been suggested that Ca^{2+} flickers contribute to the maintenance of the leading edge structure, the precise role of Ca^{2+} flickers remains elusive.

It has been proposed that the Ca^{2+} gradient is due to the subcellular localization of Ca^{2+} stores in the cell body compared to the lamellipodia with few organelles, which enables localized Ca^{2+} release at the rear parts of migrating cells.[65] In addition, this Ca^{2+} heterogeneity has also been attributed to Ca^{2+} transport proteins. In particular, stretch-activated Ca^{2+} channels (SACs) have been proposed to be important for generating this Ca^{2+} heterogeneity. Stretch-activated Ca^{2+} channels have long been implicated in the hypoosmotic stress response; SACs are activated by hypotonic cell swelling and contribute to RVD.[5,66] The involvement of SACs in cell migration was first reported in 1999.[63] Rear retraction and transient Ca^{2+} elevation are inhibited by gadolinium (Gd^{3+}), a SAC blocker. Although the molecular identities of SACs in cell migration are still a matter of dispute, they have been proposed to belong to the group of TRP channels.[4] Here, we present 3 TRP channels, TRPM7, TRPC1, and TRPV4, which are major candidate SACs in cell migration.

4.6.1 | Transient receptor potential M7

Transient receptor potential M7 is a TRP melastatin channel. It has been reported to be activated by osmotic cell swelling or membrane suction.[14] In addition, TRPM7 is necessary for volume recovery under hypoosmotic stress.[67] Thus, TRPM7 has also been proposed to be one of the SACs. There is substantial evidence that TRPM7 expression positively correlates with cell migration.[4] In particular, TRPM7 produces a very localized Ca^{2+} elevation, called a Ca^{2+} flicker, during cell migration.[64] Thus, TRPM7 has been suggested to contribute to the protrusion of the leading edge. The expression of TRPM7 is higher in migratory tumor cells than in nonmigratory tumor cells or nontumor cells, and the suppression of TRPM7 attenuates tumor cell migration.[68,69] Very recently, it was reported that silencing of TRPM7 in ovarian cancer cells decreases metastasis to the lung and prolongs the survival of tumor-bearing mice.[70] Therefore, TRPM7 could be a novel therapeutic target for migratory cancer.

4.6.2 | Transient receptor potential C1

Transient receptor potential C1, which belongs to the TRP canonical channel subfamily, is activated by direct suction of the membrane.[71] It is necessary for directional migration, such as chemotaxis, but is not necessary for basal migration.[72-74] During cell migration, TRPC1 localizes to the leading edges of cells, which is proposed to contribute to the local elevations in intracellular Ca^{2+} at the very front of cells.[72,74] It could be suggested that TRPC1 plays roles similar to those of TRPM7 in facilitating protrusion through Ca^{2+} flickers.[4] Thus, TRPC1 plays an important role in polarization during cell migration by aiding protrusion of the leading edge rather than by aiding rear retraction. Although few reports have implicated TRPC1 in cancer metastasis, some reports have indicated that TRPC1 is necessary for cell migration of invasive tumors, such as thyroid cancer and glioma.[73,74]

4.6.3 | Transient receptor potential V4

Transient receptor potential V4 is a member of the TRP vanilloid channels family and is the ortholog of the osmosensitive channel (OSM-9) in *Caenorhabditis elegans*.[75] Transient receptor potential V4 is activated under hypoosmotic stress and mediates Ca^{2+} influx from the extracellular space.[76,77] It has also been reported that TRPV4 shows sensitivity to mechanical stress caused by direct suction of the membrane.[15] This Ca^{2+} influx has been suggested to contribute to RVD because knockdown of TRPV4 leads to impairment of RVD.[78] Given these findings, TRPV4 has been proposed to be one of the SACs. In addition, TRPV4 could also be the SAC in the context of cell migration because cell migration is enhanced by activation of TRPV4, whereas it is suppressed by knockdown of TRPV4.[79] Despite the involvement of TRPV4 in cell migration, whether it contributes to the Ca^{2+} gradient or to Ca^{2+} flickers, remains elusive. Interestingly, TRPV4 has been implicated in the migration of metastatic cancer cells. The expression of TRPV4 is upregulated in metastatic breast cancer cells, and the migration of these cells depends on the activity and the expression of TRPV4.[79]

5 | CONCLUSIONS AND PERSPECTIVES

For decades, cell migration has been proposed to be driven mainly by the cytoskeletons. However, recent studies have found that osmotic water flow itself could be the driving force for cell migration. This osmotic water flow is carried out by ion/water transport proteins at the cell surface. In fact, ion/water transport proteins that are involved in cell volume regulation also contribute to cell migration. Cell migration is achieved through a repeated process of protrusion of the leading edge and retraction of the rear part. At the leading edge, net influx of NaCl through NHE1, NKCC1, AE2, and ENaC leads to water influx through AQPs and subsequent volume gain, which facilitates the protrusion. In contrast, net KCl efflux through the IK channel, VRACs, ClC-3, and TMEM16s leads to volume loss, which causes rear retraction (Figure 2). In addition, the intracellular Ca^{2+} gradient generated by mechanosensitive Ca^{2+} channels orchestrates the localized activity of ion transport proteins, although there is no consensus on the molecular identities of these channels in the context of cell migration.

These ion/water transport proteins often have enhanced activity or expression in metastatic cancer cells. In addition, inhibition of these transport proteins leads to impaired cancer cell migration. Thus, ion/water transport proteins have the potential to be novel therapeutic targets. In fact, the Cl^- channel inhibitor chlorotoxin has been the subject of much interest as an anticancer drug. Moreover,

regulation of upstream signaling pathways could also be a promising strategy because targeting only a single transport protein does not address the problem of redundancy.

Although recent studies have elucidated how volume regulation is involved in cell migration, there are still unresolved issues, including: (a) the molecular identity of the mechanosensitive Ca^{2+} channels involved in cell migration, (b) the mechanisms by which ion/water transport proteins are regulated by intracellular signaling pathways, and (c) the mechanisms by which cells sense extracellular osmotic changes and reflect these changes in the form of cell migration. A more thorough understanding of cell migration through cell volume regulation could shed a new light on strategies for cancer chemotherapy.

ACKNOWLEDGEMENTS

DISCLOSURE

The authors have no conflict of interest.

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