Abstract: Cancer metastasis is the second leading cause of death in the United States. Despite its morbidity, metastasis is an inefficient process that few cells can survive. However, cancer cells can overcome these metastatic barriers via cellular responses to microenvironmental cues, such as through mechanotransduction. This review focuses on the mechanosensitive ion channels TRPV4 and P2X7, and their roles in metastasis, as both channels have been shown to significantly affect tumor cell dissemination. Upon activation, these channels help form tumor neovasculature, promote transendothelial migration, and increase cell motility. Conversely, they have also been linked to forms of cancer cell death dependent upon levels of activation, implying the complex functionality of mechanosensitive ion channels. Understanding the roles of TRPV4, P2X7 and other mechanosensitive ion channels in these processes may reveal new possible drug targets that modify channel function to reduce a tumor’s metastatic potential.

Key Words: P2X7, TRPV4, cancer, metastasis, mechanotransduction, ion channels, mechanosensitive

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Cancer metastasis is associated with approximately 90% of cancer-related deaths. Metastasis can be divided into multiple steps. First, the cancer cells must become motile and invade local tissue. Subsequently, the cells intravasate into lymphatic or blood circulation from tumor-induced neovasculature. The cells are transported by flow to distant organs and must bind to the vessel for extravasation to occur. Finally, the cells colonize a secondary tumor through proliferation in the distant tumor site. Due to the complexity and many physiological checkpoints involved in this process, less than 0.1% of circulating tumor cells (CTCs) form distal metastases, meaning successful colonization of a distant site is contingent upon several factors. Recently, mechanical stimuli such as shear stress have received more attention in their effects on cancer progression (Fig. 1). For instance, studies have shown that shear stress has been associated with enhanced metastasis and cancer cell death. In our laboratory, we demonstrated the synergistic effect of shear stress on tumor necrosis factor-related apoptosis inducing ligand (TRAIL)-induced apoptosis of CTCs (Fig. 1C). These mechanical cues can be translated into biochemical responses in cells through the process of mechanotransduction. One form of cellular mechanotransduction occurs via the opening of mechanosensitive ion channels in response to mechanical stimuli. These ion channels transduce mechanical forces by facilitating the transport of second-messenger ions such as calcium, an ion that has been heavily implicated in cancer metastasis and apoptosis. Because of the many roles of calcium and other cations in cells, mechanosensitive ion channels represent an important area of study that could lead to novel antimetastatic cancer therapy approaches. For example, the mechanosensitive ion channels TRPC1, TRPC3, TRPC6, TRPM4, TRPM7, TRPV4, and P2X7 have all been implicated in cancer metastasis. This review focuses on the mechanosensitive ion channels TRPV4 and P2X7, however, as these two ion channels have been more extensively studied with respect to cancer metastasis than the other channels mentioned, whereas the functions of other ion channels are only just beginning to emerge. Furthermore, both channels have been specifically linked to the progression of multiple steps in cancer metastasis and also in cancer cell death, illustrating the diverse roles these ion channels can play in cancer progression (Table 1).

MECHANISMS OF TRPV4 AND P2X7

TRPV4 Activation

TRPV4 is a homotetrameric ion channel protein. TRPV4 contains 6 transmembrane regions, and 1 pore-forming subunit between transmembrane regions 5 and 6 that transports cations with a preference for calcium. This ion channel protein also contains multiple protein kinase C and protein kinase A phosphorylation sites, and a calmodulin binding site required for calcium-dependent activation (Fig. 2). TRPV4 has 2 primary nonindependent mechanical activation pathways. One pathway of mechanical activation is by phospholipase A2. Phospholipase A2 can access and cleave sn2-ester bonds of cellular phospholipids when cell membranes are stretched by mechanical strains, such as osmotic swelling or shear stress. This cleavage results in arachidonic acid (AA), which is metabolized by cytochrome p450 to epoxyeicosatrienoic acid. This epoxyeicosatrienoic acid is an agonist for TRPV4, causing the channel to open.

P2X7 Activation

P2X7 is a trimeric purinergic receptor consisting of 2 transmembrane domains, an extracellular ATP ligand-binding domain, and an integral ion channel. The P2X7 is also important in the activation of TRPV4. Phospholipase C (PLC) can be activated by the GPCR angiotensin II type 1 receptor (A2TR1) in response to mechanical stimulation. When A2TR1 is exposed to shear stress, an activating conformational shift of the receptor occurs, causing downstream activation of phospholipase C. This downstream cleaves phosphatidylinositol 4,5-bisphosphate into diacylglycerol and inositol triphosphate (IP3). Diacylglycerol activates protein kinase C, which phosphorylates TRPV4, sensitizing the ion channel to epoxyeicosatrienoic acid. The IP3 also assists in the activation of TRPV4 through TRPV4’s calcium-dependent calmodulin binding site. The IP3 binds to IP3 receptors on the endoplasmic reticulum causing calcium to be released into the cytoplasm. The calcium then causes the calmodulin to bind to TRPV4, allowing for enhanced potentiation of the ion channel. Some Phospholipase A2 isoforms require calcium binding to their C2 domain for activation, suggesting IP3 induced calcium release may be important for sustained activation of TRPV4 as well.
there are 3 total ATP binding domains (Fig. 3). Within milliseconds of ATP binding, its integral membrane pore opens, allowing for a small conductance of cations, such as calcium. When the P2X7 receptor has sustained activation of tens of seconds by ATP, a larger pore is formed that increases cation conductance, causing the pore to be permeable to 900-da proteins.

![FIGURE 1. Changes in cell functionality in response to shear stress. A, Circulating tumor cell (CTC) exposed to shear stress in blood flow (B). Tumor cell within its extracellular matrix, exposed to interstitial flows (C). When highly metastatic colon cancer cells COLO 205 were exposed to shear stress, the cells were sensitized to TRAIL-induced apoptosis with a significant decrease in cell viability of approximately 30%. *P < 0.05. †P < 0.01. NS: nonsignificant. Reprinted from Mitchell and King, New J. Phys, 2013.]
The time-dependent cation conductance of P2X7 can be explained by 2 contrasting mechanisms: (1) ATP binds to P2X7, causing a conformational change that dilates the initially small P2X7 cation pore; (2) sustained P2X7 activation causes the opening of distinct protein pores via direct signaling or second-messengers. A reconciling mechanism is that when a single ATP molecule is bound to P2X7, the ligand-binding domain undergoes a conformational shift, making it more difficult for other ATPs to bind. After a second ATP is bound, the small cation pore opens, while simultaneously causing another conformational change, increasing steric hindrance for the binding of the third and final ATP. Once the third ATP binds, the P2X7 pore dilates allowing for large molecules and increased amounts of cations to enter the cell. The influx of cations can then activate other pores that are able to transport large proteins in addition to the dilated pore of P2X7. This also explains the time dependence of P2X7’s pore dilation because more time is required to overcome the increasing difficulty of binding 3 ATPs.

P2X7’s method of mechanotransduction is indirect. Rather than a mechanical stimulus directly activating P2X7, another channel is altered and signals P2X7’s activation downstream. Panx-1 is a mechanosensitive channel that releases ATP into the extracellular fluid via shear stress and osmotic pressure. The Panx-1 released ATP then binds to P2X7’s binding domain causing the opening of the P2X7 and other associated pores.

**ROLE OF TRPV4 AND P2X7 IN CANCER METASTASIS**

**Angiogenesis**

Angiogenesis is the growth of tumor neovasculature that develops in response to an increased nutrient and oxygen demand for the growing malignancy. In addition to providing nutrients, the tumor neovascularization promotes dissemination by creating a route of escape for cancer cells to enter the hematogenous circulation. The loose endothelial gap junctions within tumor vasculature allow for easier cancer cell intravasation compared to normal vessels.

**TRPV4**

Arachidonic acid–based calcium entry has been linked to angiogenesis in breast-derived tumor endothelial cells, leading to the suspicion that TRPV4 may play a significant role in angiogenesis. In another study, TRPV4 was up-regulated in breast and renal tumor derived endothelial cells relative to healthy endothelial cells. TRPV4 stimulation by AA and subsequent calcium conductance was correlated with increased endothelial cell migration for breast tumor–derived endothelial cells. Migration of healthy endothelial cells, however, was not observed. Endothelial cell migration is important in angiogenesis, as it allows endothelial cells to organize and form new blood vessels. In addition, calcium and AA inhibition eliminated tumor-derived endothelial cell migration, further supporting TRPV4’s role in angiogenesis.

Conversely, another study by Adapala et al showed that TRPV4 expression was found to down-regulate angiogenesis. In this study, TRPV4 expression was found to be down-regulated in tumor endothelial cells with reduced calcium influx compared to healthy endothelial cells. When TRPV4 was activated by the small molecule agonist GSK1016790A, tumor vasculature was normalized. In a mouse angiogenesis study where mice were subcutaneously injected with Lewis lung carcinoma cells, TRPV4 knockout mice showed increased amounts of neovascularization with malformed blood vessels and large vessel diameters, in comparison to wild type mice. In this mouse study, it was suggested that TRPV4 reduces angiogenesis by inhibiting Rho activity because a Rho kinase inhibitor in mice lacking TRPV4 was used to normalize tumor vasculature. TRPV4’s regulation of Rho activity is significant as Rho activity is known to enhance angiogenesis by altering the cellular cytoskeleton, allowing for increased motility of tumor endothelial cells.

The disparate results may be explained by the different methods of studies used to study TRPV4. TRPV4 activation was correlated

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**FIGURE 2.** TRPV4 contains 6 transmembrane regions with a pore between transmembrane regions 5 and 6. The TRPV4 protein also has multiple protein kinase A, protein kinase C, and calmodulin binding sites that effect channel functionality.

**FIGURE 3.** P2X7 is homotrimeric structure with 3 extracellular ATP binding domains. As P2X7 binds ATP, its inner pore region opens, allowing for the flux of ions.
with enhanced migration for breast tumor derived endothelial cells with the use of a TRPV4 agonist. The TRPV4 mouse model study showed that TRPV4 deletion caused an increase in Rho activity, leading to increased angiogenesis. This suggests that basal levels of TRPV4 activation, as is the case with the mouse studies, may inhibit angiogenesis, whereas overactivation of TRPV4 by an agonist or mechanical stimulation may increase angiogenesis.

### P2X7

Tumor microenvironments are known to have high concentrations of ATP, whereas healthy tissues have negligible amounts of ATP in the extracellular fluid. This high extracellular concentration of ATP is due to increased secretion by cancer cells in response to mechanical stimuli, such as shear stress, cell swelling and stretching, resulting in P2X7 stimulation.

P2X7 activation in B16 mouse melanoma cells, CT26 mouse colon cancer cells and ACN human neuroblastoma cells resulted in the secretion of vascular endothelial growth factor (VEGF). The VEGF is important for the dissemination of cancer cells as it causes endothelial cells to proliferate and migrate to create neovascularure. This effect of P2X7 activation has made P2X7 a target for anti-cancer therapies. Mice injected with B16 cells were treated with benzoyl ATP, an agonist of P2X7 or a selective inhibitor of P2X7, AZ10606120. Mice treated with AZ10606120 had significantly reduced VEGF secretion and vessel formation compared to mice treated with benzoyl ATP. Oxidized ATP, an inhibitor of P2X7, was used to block the development of a CT26 mouse model engineered to express P2X7. This inhibition resulted in the reduction of tumor size and VEGF secretion. The study was also successful in using shRNA against P2X7 to block tumor growth and VEGF secretion. Another study injected neuroblastoma ACN cells into immunocompromised mice and saw significant reduction in VEGF secretion and tumor growth using a P2X7 inhibitor. These studies indicate that P2X7 activation plays a prominent role in tumor angiogenesis by controlling the secretion of VEGF.

### INTRAVASATION

Efficient dissemination is contingent upon tumor cells’ ability to enter the vasculature. A major barrier in tumor cell intravasation is the basement membrane (BM), which acts as a barrier between the epithelium and the stroma which surrounds subjacent blood vessels. The BM is normally tightly controlled in healthy tissue; however, cancer cells have been shown to release proteases with enhanced migration for breast tumor derived endothelial cells with the use of a TRPV4 agonist. The TRPV4 mouse model study showed that TRPV4 deletion caused an increase in Rho activity, leading to increased angiogenesis. This suggests that basal levels of TRPV4 activation, as is the case with the mouse studies, may inhibit angiogenesis, whereas overactivation of TRPV4 by an agonist or mechanical stimulation may increase angiogenesis.

### P2X7

Increased expression and activation of P2X7 has been linked to a variety of invasive cancer lineages, from lung cancer to thyroid cancer, lymphoma, pancreatic cancer, prostate cancer, and breast cancer. P2X7 activation correlates with increased activity of the specific metalloproteases (MMPs) 3, 9, and 13 in 4T07, MDA-MB-435, and leukocytes. Metalloproteases are proteolytic enzymes that play a key role in cancer metastasis as they can degrade virtually all types of ECM protein, assisting cancer cells in exiting the tumor and intravasating into the vasculature. The increased activity of MMP-9 is particularly important for intravasation, as shown by Kim et al., in which only cancer cells that expressed MMP-9 (i.e. HEp3, HT1080, MDA-MB-231, and PC3) were capable of intravasating. That is, the cancer cells lacking MMP-9 expression, such as the MCF-7 cell line, exhibited negligible intravasation. One mechanism by which P2X7 regulates MMP activity is through the release of cysteine cathepsin B. Cathepsin B is a proteolytic enzyme that cleaves tissue inhibitors of metalloproteases (TIMP), rendering the TIMPs nonfunctional. The inactivation of TIMPs reduces the inhibition of MMPs, allowing for greater ECM degradation. This implies that P2X7 may play a major role in cancer invasion by decreasing MMP inhibition to promote ECM degradation. P2X7 also aids in intravasation by promoting EMT. Like TRPV4, P2X7 activation has been linked to E-cadherin loss. The decrease in E-cadherin by P2X7 activation could be caused by multiple mechanisms, particularly through MMP-3 secretion.
as shown in MDA-MB-435 cells, and enhanced Akt signaling in T47D breast cancer cells. Metalloprotease-3 secretion by P2X7 is presumed to be significant to EMT because of the ability of MMP-3 to cleave E-cadherin.

Extracellular ATP has been shown to promote actin remodeling, implying that P2X7 may have a role in this process as well. Actin remodeling is another behavior associated with EMT and has been shown to increase cell motility through the formation of cellular protrusions, such as filipodia and lamellipodia. The proposed role of P2X7 in actin remodeling varies by study, but is not necessarily mutually exclusive between roles. In a breast cancer model of MDA-MB-435 cells, P2X7 caused a calcium influx that activates the calcium-dependent potassium channel SK3. SK3 channels have been previously shown to form cellular protrusions by actin remodeling and promote cell motility when intracellular calcium concentration is increased. When either P2X7 or SK3 activity was inhibited, cell protrusion formation was blocked. Another study in PC-9 human lung cancer cells supports the possible role of P2X7 in directing migrating cancer cell morphology for extravasation by decreasing cell motility. When P2X7 expression was inhibited, the cells created lamellipodia and filopodia around the periphery of the cell membrane, causing directionless migration. This suggests that P2X7 may play a role in regulating lamellipodia formation by localizing it to SK3 channels.

**EXTRAVASATION**

The steps of extravasation mirror the steps of invasion. After arrest of CTCs at a distal site, a microcolony may form that causes the vasculature to rupture, exposing the CTCs to the surrounding tissue. Alternatively, cells can penetrate the endothelial wall by increasing hyperpermeability through the secretion of vascular permeability factors, such as VEGF. Once entering the tissue parenchyma, a mesenchymal to epithelial transition occurs, now promoting cell polarity and cadherin junctions to decrease motility. The notion that certain organs are predisposed to dissemination has been investigated for decades, starting with the “seed and soil” hypothesis proposed by Stephen Paget. This widely adopted theory recognizes that certain tumors preferentially extravasate and colonize distant organs with hospitable milieus. Just as a seed requires the appropriate soil to grow, metastatic cancer cell survival is contingent upon the organ microenvironment where extravasation occurs.

**TRPV4**

TRPV4 expression has been shown to correlate with a cell’s metastatic potential to extravasate. This has been demonstrated by showing that TRPV4 is upregulated in metastatic breast cancer cell lines 4T07 and 4T1 that have undergone extravasation, compared to basal levels expressed in primary tumors that have unsuccessfully extravasated to distant organs. In addition, expression of phosphorylated proteins in breast cancer cells expressing TRPV4 show similarities in protein phosphorylation to that of leukocytes during immune-modulated extravasation. These phosphorylates include paxillin, β-catenin, and ezrin, all key proteins in promoting actin cytoskeleton deforming, implying that TRPV4 may be essential for the phosphorylation of these proteins that contribute to successful extravasation.

It is evident that this channel upregulation plays a key role in priming migrating cancer cell morphology for extravasation by increasing cell deformability as seen in 4T07 and MDA-MB-468 cells. Similar to intravasation, this TRPV4 activation initiates actin depolymerization in the cell cortex, reducing cell shear modulus and stiffness. This morphological softening allows more motile cancer cells that are capable of exiting the vasculature by passing through loose endothelial junctions. This increased cell motility is also attributed to E-cadherin and integrin down-regulation. Holistically, the up-regulation of TRPV4 consequently leads to cancer cells with a more aggressive phenotype that are able to overcome physiological barriers in surviving hematogenous transit and initial arrest in distant tissues. This notion is supported by clinical samples of patient tumors, as TRPV4 up-regulated tumors correlate with poor prognosis and an increase in metastatic lesions.

**P2X7**

The role of P2X7 in extravasation is more complex than that of TRPV4. P2X7 can act as a growth-promoting or death-inducing receptor, contingent upon the levels of stimulation by extracellular ATP. Under normal conditions, P2X7 up-regulation increases ATP production and induces cell proliferation in the absence of serum. This cellular response is likely to play a key role in promoting metastatic cell survival in foreign environments, enhancing survival and growth in the absence of microenvironmental nutrients present in the primary tumor niche. As previously mentioned, P2X7 activation has been linked to an increase in metalloprotease activity in the breast cancer lines 4T07 and MDA-MB-435. This MMP activation plays a major role in ECM degradation, making P2X7 an indirect protagonist in the promotion of extravasation. During the initial stages of transendothelial extravasation, membrane-type MMP’s localize to the leading edge of tumor cells, thereby anchoring the cells to endothelial junctions while promoting MMP activity to degrade the surrounding matrix. This MMP localization and production aids in proteolysis of the BM, promoting tumor growth and migration. Indeed, a study by Voura et al. found that for both MDA-MB-231 and WM239 cancer cells, extravasation can be reduced by over 35% through MMP inhibition alone, motivating further research into inhibitory MMP drug candidates to reduce metastatic potential, for which P2X7 may be a promising target.

**CELL DEATH**

Although TRPV4 and P2X7 have been implicated extensively in the progression of metastasis, both ion channels have been shown to induce cancer cell death. Induced apoptosis via the intrinsic pathway, permeabilizing the mitochondrial membrane to initiate the release of apoptotic enzymes. The apoptotic enzymes released consist of cytochrome c, apoptosis inducing factor (AIF), Smac/DIABLO, Omi/HtrA2 and endonuclease G. Cytochrome c is of particular importance because upon entering the cytosol, it forms the apoptosome with Apaf-1 and caspase 9. The formation of the apoptosome activates the effector caspases, caspase 3 and caspase 7. Caspases 3 and 7 are proteolytic enzymes that cleave proteins leading to cell death.

TRAIL-induced apoptosis also results in caspase activity causing cell death. Imposing that mechanosensitive ion channels may sensitize colon cancer cells to TRAIL-induced apoptosis by further increasing caspase activity through a mechanotransductive mechanism (Fig. 1). TRPV4 has also been linked to the induction of cancer cell oncosis. P2X7 has yet to be linked to oncosis in cancer cells, but P2X7-induced oncosis has been shown in murine leukocytes. Oncosis is a form of cell death associated with cell swelling, organelle swelling, blebbing and increased membrane permeability caused by a critical depletion in ATP.

**TRPV4**

Upon activation, TRPV4 induced apoptosis in the breast cancer cell line MDA-MB-468 by causing a major influx of calcium,
resulting in a critical increase of cytosolic calcium. 23,132 When TRPV4 activation raises the cytosolic calcium concentration sufficiently high, calcium is transported into the inner mitochondrial matrix where calcium can open the mitochondrial permeability transition pore. 133 The opening of the permeability transition pore then causes the mitochondria to osmotically swell, rupturing the outer mitochondrial membrane. 134,133 The rupturing of the outer membrane releases apoptotic enzymes, such as cytochrome c, which is stored in the space between the mitochondria’s outer membrane and inner membrane. 136 TRPV4 activation also induces oncosis in MDA-MB-468 cells by promoting calcium and sodium influx. To remove the excess amounts of these ions from inside the cell, Na+/K+ ATPase and Ca2+ ATPase channels experience sustained activation, causing ATP depletion. 23

P2X7

P2X7 also causes apoptosis in cancer cells by activating caspases 3 and 7. 7,21,120,137 These apoptotic cancer cells were also associated with a significantly increased calcium influx, 21 implying that P2X7 mediates apoptosis via the intrinsic pathway. Using pancreatic ductal adenocarcinoma cells including PANC1 and CAPAN1, it was found that the concentration of extracellular ATP determines if P2X7 will induce apoptosis or initiate the pro-metastatic changes described previously. When less ATP was present, P2X7 promoted cancer metastasis and growth. However, when more ATP was present, P2X7 caused cell death. 21 This contradiction based on extracellular ATP concentration is most likely due to P2X7’s differing responses to sustained activation. 138 When P2X7 does not have sufficient ATP available it only allows limited influx of calcium, causing pro-metastatic changes in cancer cells. However when more ATP is present, the calcium influx becomes toxic.

Oncosis is presumably caused by P2X7 in a similar manner to TRPV4. P2X7 creates a high cytosolic calcium concentration that must then be equilibrated using Ca2+ ATPases, depleting intracellular ATP content. P2X7 may further contribute to oncocytic cell death through the opening and dilation of downstream pores that are capable of transporting ATP out of the cell, further lowering cellular ATP.

CLINICAL TRIALS

TRPV4 and P2X7 have yet to be explored as therapeutic targets for cancer treatment in clinical trials. However, antagonists for both ion channels are receiving attention with respect to other diseases. The P2X7 antagonists AZD9056 and CE-224,535 have both been tested in phase II clinical trials as rheumatoid arthritis treatments. 140,141 Neither drug was deemed efficacious in treating rheumatoid arthritis, but both demonstrated acceptable safety, implying the potential of these drugs as safe anti-metastatic cancer therapies. The TRPV4 antagonist GSK2798745 was tested in a clinical trial for the treatment of congestive heart failure (https://clinicaltrials.gov indicator: NCT02497937). Unfortunately, the results and safety of this trial are yet to be reported at the time of publication of this article. To our knowledge, TRPV4 and P2X7 agonists have not yet been studied in clinical trials for any disease type. The lack of clinical trials using P2X7 and TRPV4 antagonists and agonists for cancer treatment leaves much room for the innovation of novel therapies based on these ion channels.

CONCLUSIONS

TRPV4 and P2X7 exemplify the complex roles that mechanosensitive ion channels have in cancer cell dissemination, and motivate the need for further study of these types of channels. Due to the transport of calcium, TRPV4 and P2X7 are host to a variety of prometastatic pathways, making the channels appear to be attractive targets for anti-metastatic cancer therapies. However, these channels have also been implicated in causing cancer cell death. This presents a challenge for the targeting of mechanosensitive ion channels for cancer therapies, as a tight therapeutic window is needed to maintain the beneficial effects of the ion channels, while preventing adverse behaviors. Determining the proper levels of activation for TRPV4, P2X7 and other mechanosensitive ion channels to leverage the apoptotic effects of these channels, while preventing the stimulation of pro-metastatic pathways, could lead to novel cancer therapies.

REFERENCES


