REVIEW

The Ca\(^{2+}\)-activated chloride channel ANO1/ TMEM16A: An emerging therapeutic target for epithelium-originated diseases?

Yani Liua,b,*, Zongtao Liuc, KeWei Wanga,b,*

a Department of Pharmacology, School of Pharmacy, Qingdao University Medical College, Qingdao 266073, China
b Institute of Innovative Drugs, Qingdao University, Qingdao 266021, China
c Department of Clinical Laboratory, Qingdao Third People’s Hospital, Qingdao 266041, China

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Abstract
Anoctamin 1 (ANO1) or TMEM16A gene encodes a member of Ca\(^{2+}\) activated Cl\(^{-}\) channels (CaCCs) that are critical for physiological functions, such as epithelial secretion, smooth muscle contraction and sensory signal transduction. The attraction and interest in ANO1/TMEM16A arise from a decade long investigations that abnormal expression or dysfunction of ANO1 is involved in many pathological phenotypes and diseases, including asthma, neuropathic pain, hypertension and cancer. However, the lack of specific modulators of ANO1 has impeded the efforts to validate ANO1 as a therapeutic target. This review focuses on the recent progress made in understanding of the pathophysiological functions of CaCC ANO1 and the current modulators used as pharmacological tools, hopefully illustrating a broad spectrum of ANO1 channelopathy and a path forward for this target validation.

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Abbreviations: Ang II, angiotensin II; ANO1, anoctamin-1; ASM, airway smooth muscle; BBB, blood–brain barrier; CaCCs, Ca\(^{2+}\) activated chloride channels; CAMK, Ca\(^{2+}\)/calmodulin-dependent protein kinase; CF, cystic fibrosis; CFTR, cystic fibrosis transmembrane conductance regulator; DRG, dorsal root ganglion; EGFR, epidermal growth factor receptor; ENaC, epithelial sodium channels; ER, endoplasmic reticulum; ESCC, esophageal squamous cell carcinoma; FRT, fisher rat thyroid; GL, gastrointestinal; GIST, gastrointestinal stromal tumor; GPCR, G-protein coupled receptor; HNSCC, head and neck squamous cell carcinoma; HTS, high-throughput screening; ICC, interstitial cells of Cajal; IPAH, idiopathic pulmonary arterial hypertension; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor κB; PAH, pulmonary arterial hypertension; PAR2, protease activated receptor 2; PASMC, pulmonary artery smooth muscle cells; PIP2, phosphatidylinositol 4,5-bisphosphate; PKD, polycystic kidney disease; TGF-β, transforming growth factor-β; VGCC, voltage gated calcium channel; VRAC, volume regulated anion channel; VSMC, vascular smooth muscle cells; YFP, yellow fluorescent protein.

*Corresponding authors.
E-mail addresses: liuyani@qdu.edu.cn (Yani Liu), wangkw@qdu.edu.cn (KeWei Wang).

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1. Introduction

Ca\(^{2+}\)-activated chloride channels (CaCCs) are a heterogeneous group of Cl\(^-\) channels that can be synergistically activated by intracellular calcium and voltage. The CaCCs are found in almost all species ranging from invertebrates to mammals, and the ubiquitous expression of CaCCs indicates a variety of functions important for physiology, including regulation of epithelial Cl\(^-\) secretion, excitability of neuronal and cardiac cells, smooth muscle contraction and nociception.

A subtype of CaCCs was first described in Xenopus oocytes nearly 40 years ago and its molecular identity was debated until in 2008 when three independent laboratories reported that anoctamin-1 (ANO1) or transmembrane protein 16A (TMEM16A) underlies the molecular basis of a subgroup of CaCCs. The Ano1/Tmem16a gene encodes a 986-amino-acid protein that belongs to anoctamin family consisting of 10 members (ANO1–ANO10) in mammals. ANO1, as a CaCC, is primarily expressed in epithelial cells, smooth muscle cells and sensory neurons. ANO2, also as a CaCC, is expressed in the olfactory sensory neurons, photoreceptor synaptic terminals, hippocampal pyramidal neurons, thalamocortical neurons, and inferior olive neurons in the brain. Other ANOs family members including ANO6 and ANO7 were onetime considered to be CaCCs, but evidence shows that ANOs3–7 neither generate Ca\(^{2+}\)-activated Cl\(^-\) currents nor traffic into membrane, indicating that they are endoplasmic reticulum proteins. More studies reveal that ANOs3–7 and ANO9 are linked to the Ca\(^{2+}\)-dependent membrane phospholipid scramblases that are responsible for translocation of phospholipids. It is generally accepted that ANO1 and ANO2 are two members of the CaCC subfamily, whereas ANO3–ANO10 have been debated for their functions as CaCCs or Ca\(^{2+}\)-dependent membrane phospholipid scramblases, or other physiological proteins.

ANO1 was initially thought to have eight transmembrane domains, possessing multiple protein isoforms generated by alternative splicing of four segments (a, b, c, and d) located at the C-terminus and the first intracellular loop. ANO1 splice variant lacking segment b, for instance, increases the calcium sensitivity, whereas deletion of the segment c decreases apparent Ca\(^{2+}\) sensitivity and increases voltage-dependent activity of ANO1. ANO1 splice variant lacking segment c and the first intracellular loop is predominantly expressed in retinal pigment epithelium, non-neuronal tissue, peripheral and central neurons.

ANO1 protein expression is regulated by multiple transcriptional and post-transcriptional mechanisms, including phosphorylation, ubiquitination, and proteasomal degradation. ANO1 activation and desensitization of ANO1 are important for physiology, including regulation of epithelial Cl\(^-\) secretion, excitability of neuronal and cardiac cells, smooth muscle contraction and nociception.

In this review, we will mainly focus on the aspect of channelopathies and pharmacological validation of ANO1 as an emerging therapeutic target.

2. Pharmacological modulation of ANO1 channel

Validating ANO1 as a therapeutic target requires specific modulators that can serve as essential tools for understanding the channel pharmacology. Up to now, many ANO1 modulators have been reported, and unfortunately most of them are lack of potency and efficacy. Therefore, there exists a great need for discovery of more selective and potent modulators, inhibitors in particular, which can be used for ANO1 target validation.

2.1. Inhibitors

Some broad-spectrum blockers, such as NFA, FFA, DIDS, NPPB and 9AC, have been used as tools for understanding functions of CaCCs. These small molecules can block endogenous CaCCs in Xenopus laevis oocytes and are also known to non-specifically modulate other channels, such as inhibition of volume regulated anion channel (VRAC) and Kv4 by NFA and DIDS and activation of Ca\(^{2+}\)-activated K\(^+\) channel by NFA and FFA or potentiation of TRPV1 channel by DIDS. CaCCinh-A01, a CaCCinh-A01 was identified to inhibit CaCC current in 2008 from screen of 50,000 compounds using human intestinal epithelial HT29 cell that highly express endogenous CaCCs without obvious effects on intracellular Ca\(^{2+}\), Ca\(^{2+}\)/calmodulin-dependent protein kinase (CAMK) or cystic fibrosis transmembrane conductance regulator (CFTR). CaCCinh-A01 as an ANO1 inhibitor has been widely used in many investigations for the role of ANO1 in interstitial cells of Cajal (ICC), cardiac fibroblast and rod bipolar cells of retina, and channelopathies, including cancer, hypertension, nociception, diarrhea and high glucose induced renal cyst growth, and channelopathies, including cancer, hypertension, nociception, diarrhea and high glucose induced renal cyst growth.

CaCCinh-A01 is shown to inhibit cancer cell proliferation through increasing the ubiquitination of ANO1 and facilitating ER-associated, proteasomal degradation of ANO1, while other ANO1 inhibitors, such as T16Ainh-A01 and digallic acid, are shown to have no effect on cancer cell proliferation. Similarly, CaCCinh-A01 inhibits proliferation of cardiac fibroblast, but not another ANO1 inhibitor T16Ainh-A01. CaCCinh-A01 reduces upregulation of ANO1 expression and attenuates brain infarct size and neurological deficits after ischemic stroke whereas T16Ainh-A01 shows no effect. That ANO1 protein expression is reduced by CaCCinh-A01 but not T16Ainh-A01 may partially explain why the two ANO1 inhibitors show different effects on cell proliferation and ischemic stroke. In addition, intrathecal...
injection of CaCCinh-A01 reduces tactile allodynia and thermal hyperalgesia and also decreases ANO1 upregulation after spinal nerve injury. Since the identification of CaCCinh-A01, several ANO1 inhibitors have been screened out using the iodide-sensitive yellow fluorescent protein (YFP)-based high throughput screening (HTS) assay. T16Ainh-A01 was reported to inhibit human ANO1 current expressed in Fisher rat thyroid (FRT) cells with an IC50 of 1 μmol/L and had no effect on CFTR current. However, our previous study showed that T16Ainh-A01 at 10 μmol/L only inhibits mouse ANO1 current expressed in CHO cells about 28% at +80 mV, and it may because that the efficacy of T16Ainh-A01 on ANO1 inhibition dependents on splice variants of ANO1 and intracellular calcium. Nevertheless, T16Ainh-A01 has been used as a pharmacological probe to investigate the role of ANO1 in pancreatic ductal adenocarcinoma cells, and also physiological and pathological functions of ANO1 in different tissues, including contraction of vesical smooth muscle, iodide release from thyrocyte, initial waveform modulation in retinal rod bipolar cell and melatonin secretion in pineal glands (Table 1). T16Ainh-A01 attenuates the interleukin-13 (IL-13) induced increase of ANO1 expression and secretion of mucin in human nasal polyp epithelial cells from chronic rhinosinusitis patients, cultured human bronchial epithelial cells and goblet cells in the guinea pig asthma model, suggesting that T16Ainh-A01 may be useful for treatment of hypersecretion in asthma and other inflammatory airway diseases. In addition, several studies show that ANO1 inhibitor T16Ainh-A01 exerts therapeutic effect on cancer, neuropathic pain and eosinophilic esophagitis.

Figure 1 The molecular structure of ANO1/TMEM16A channel. (A) The Ca2+ -bound structure of mANO1 channel in dimer (chains A and B), and 2 yellow filled circles for Ca2+ in each monomer containing 10 transmembrane α-helices. (B) Ca2+ binding sites formed by residues N650, N651, E654 from α6, E702, E705 from α7, and E734, D738 from α8. (C) Residues critical for ion selectivity including R515 from α3, N546, D554 from α4, N591, V599 from α5, K603, R621 from α5–6 linker, S639 from α6, and Q709, F716 from α7. (D) Putative binding sites, R515 from α3, K603, R621 from α5–6 linker, and R788 from α8, for ANO1 inhibitors NTTP and 1PBC, and N650 from α6, A697, E705 from α7, and L746 from α8 for ANO1 activator GRb1. The structure is regenerated based on the cryo-EM structure of ANO1 channel (PDB 5OYB). The residue number labeling is based on the sequence of mTMEM16A (ac) isoform (UniProt Q8BHY3.2).
Table 1 Pharmacology of CaCC ANO1/TMEM16A inhibitors.

| Inhibitor       | Structure | IC₅₀ (μmol/L) | Test assay       | Selectivity                | Effect                                      | Ref.     |
|-----------------|-----------|--------------|------------------|-----------------------------|---------------------------------------------|----------|
| NFA             | ![Structure](image1) | 7–37         | *Xenopus oocytes* mANO1-CHO | KCa channel (+)             | Anti-cancer, Anti-asthma                     | 38–42    |
| FFA             | ![Structure](image2) | 14–35        | *Xenopus oocytes* mANO1-CHO | KCa channel (+) | [Ca²⁺]i (+) | Anti-asthma                         | 38,39    |
| NPPB            | ![Structure](image3) | 22–68        | *Xenopus oocytes* mANO1-CHO | K⁺ channel (−) | [Ca²⁺]i (+) | Anti-cancer, Antinociception        | 38,39,43,44 |
| DIDS            | ![Structure](image4) | 11–550       | *Xenopus oocytes* mANO1-CHO | Vracer (−) | [Ca²⁺]i (+) | Anti-cancer, Antinociception        | 38,39,44–47 |
| Dichlorophen    | ![Structure](image5) | 5.5          | ANO1-HEK293       | ANO2 (−) | CFTR (l) | Anti-asthma                         | 48       |
| Benzobromarone  | ![Structure](image6) | 10           | ANO1-HEK293       | ANO2 (−) | CFTR (l) | Anti-asthma                         | 48       |
| CaCCinh-A01     | ![Structure](image7) | 1–7.8        | HT-29             | hBest1 (−, 7 μmol/L) | ANO2 (−) | ANO2 (−) | Anti-cancer, Anti-hypertension, Anti-diarrhea, Antinociception, Anti-high glucose induced renal cyst growth, Anti-ischemic stroke induced BBB intergrity | 38,47,49–56 |
| T16Ahinh-A01    | ![Structure](image8) | 1            | hANO1-FRT         | mANO1-CHO                        | ANO2 (−) | ANO2 (−) | Anti-Asthma, Anti-itch, Anti-polyfertilization | 38,47,50, 52,54, 57–60 |
| MONNA           | ![Structure](image9) | 1.27         | hANO1-HEK293      | xANO1 (0.08 μmol/L) | ANO2 (−) | ANO2 (−) | Anti-cancer, Anti-itch, Anti-hypertension, Anti-polyfertilization | 47,61–65 |

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| Inhibitor      | Structure | IC₅₀ (µmol/L) | Test assay     | Selectivity                                      | Effect                  | Ref.   |
|---------------|-----------|---------------|----------------|-------------------------------------------------|-------------------------|--------|
| Ani9          | ![Ani9 structure](image1) | 0.08          | hANO1-FRT      | hCFTR (l) xBest2a (l) ANO2 (l) CFTR (l) ENaC (l) VRAC (l) ANO6 (slower activation) xBest2a (l) | Anti-cancer, Anti-polyfertilization | 47,57,65,66 |
| 10bm          | ![10bm structure](image2) | 0.03          | hANO1-FRT      | ANO2 (~, 0.4 µmol/L) CFTR (l)                     | NR                      | 67     |
| Ani9-5f       | ![Ani9-5f structure](image3) | 0.02          | hANO1-FRT      | ANO2 (l) CFTR (l)                                | Anti-cancer             | 68     |
| Dimer trans-ε-viniferin (TV) | ![Dimer trans-ε-viniferin structure](image4) | 1.1           | HT-29          | CFTR (−)                                         | Anti-diarrhea           | 69     |
| Tetramer, γ-2-viniferin (RV) | ![Tetramer, γ-2-viniferin structure](image5) | 12.3          | HT-29          | CFTR (−)                                         | Anti-diarrhea           | 69     |
| Niclosamide   | ![Niclosamide structure](image6) | 0.1−2.4       | hANO1-HEK293T  | CFTR (l) ANO6 (−) [Ca²⁺] (−)                     | Anti-asthma             | 42,66,70 |
| Compound     | Time (h) | Cell Line/Model | Channel/Transporter | Activity/Effect                      | Reference(s) |
|--------------|----------|-----------------|---------------------|-------------------------------------|--------------|
| Tannic acid  | 6--25    | hANO1-FRT       | CFTR (l)            | Anti-nociception, Anti-cancer, Anti-asthma | 38,50,62,71,73 |
|              |          | mANO1-CHO       | ENaC (l), hBest1 (--, 15 μmol/L), ANO2 (--)|                        |              |
| Eugenol      | 150      | hANO1-FRT       | CFTR (l), [Ca\(^{2+}\)] (l), Na\(^{+}\)-K\(^{+}\)-ATPase (l) | Anti-diarrhea, Local analgesia      | 74--76       |
|              |          | T84 cell        | Nav1.8 (--), TRPV1 (--), Kv1.5 (--), VGCC (--)|                        |              |
| Galangin     | 4.5--9.7 | mANO1-CHO       | NR                  | Anti-cancer                         | 77,78        |
| Luteolin     | 9.37     | hANO1-FRT       | ANO2 (--, 120 μmol/L) | Anti-cancer                         | 77--79       |
|              |          | mANO1-CHO       | ANO2 (--), L-type Ca\(^{2+}\) channel (--)|                        |              |
| Quercetin    | 13.7     | mANO1-CHO       | NKCC1 (+), Na\(^{+}\)-K\(^{+}\)-ATPase (+), CFTR (+), K+ channel (+), TRPM7 (--)| Anti-cancer, Attenuation of GI tract motility, Increasement of intestinal Cl\(^{-}\) secretion | 78,80 |
| Idebenone    | 9.2      | hANO1-FRT       | ANO2 (--), CFTR (l), [Ca\(^{2+}\)] (l)| Anti-cancer, Anti-renal cyst       | 81,82        |
|              |          | CFTR (/), K+ channel (/), Na\(^{+}\)-K\(^{+}\)-ATPase (/)|                        |              |
| Plumbagin    | 12.46    | hANO1-FRT       | CFTR (--), K+ channel (/), Na\(^{+}\)-K\(^{+}\)-ATPase (/)| Anti-cancer, Anti-diarrhea         | 81,83        |

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MONNA, the most potent blocker of anthranilic acid derivatives, blocks xAN01 and hAN01 with IC50 of 0.08 and 1.27 μM, respectively, without any effects on CFTR, CLC2 and BEST1. Similar to AN01 inhibitors CaCCinh-A01 and T16Ainh-A01, MONNA concentration-dependently inhibits agonist-induced rodent vesical constriction through hyperpolarization of vesical smooth muscle cells. MONNA also inhibits chloroquine-induced action potential discharge at itch nerve terminals and bouts of scratching, suggesting a role of AN01 in pruritus. MONNA has also been used to investigate the role of AN01 in blocking poly-fertilization.

Another small molecule Ani9 was also identified to inhibit hAN01 expressed in FRT cells with an IC50 of 0.08 μM using YFP-HTS assay (Table 1). Ani9 belongs to acetamides and shows a relatively high selectivity on AN01 over AN02 without inhibitory effects on CFTR, VRAC, epithelial sodium channels (ENaC) and intracellular Ca2+ signaling at concentration of 30 μM, whereas Ani9 can inhibit inward current and slow the time-dependent activation of ANO6. The Ani9 derivative 5f shows more potent inhibitory effect on AN01 with an IC50 of 20 nmol/L without activating on ANO2. Ani9 as a novel potent and selective AN01 inhibitor has also been used in several investigations for the physiological and pathological functions of AN01, including hypertension, polysermy block and cancer. As one of the 2-acylamino-cycloalkylithiophene-3-carboxylic acid arylamides, 10bm shows a potent inhibition on AN01 current with an IC50 of 30 nmol/L and exhibits dose-dependent inhibition on isometric smooth muscle contraction. The 10bm compound also inhibits ANO2 with an IC50 of 0.4 μmol/L without effect on CFTR. At present, synthesized AN01 inhibitors are only used as tools for preclinical studies (Table 1).

Many natural products from diverse plants have been found to inhibit AN01. Tannic acid presented in the green tea and red wine was identified as a blocker for both AN01 and AN02 without effect on CFTR or ENaC, showing an inhibitory effect on arterial smooth muscle contraction and intestinal Cl channel secretion. Like the synthesized small molecule AN01 inhibitors, tannic acid has also been used as a pharmacological tool for investigations of the biophysical feature and physiological functions of AN01. Series of flavonoids were recently identified to inhibit AN01, exerting anticancer effects. Natural products or synthetic analogues of natural products, including idebenone with anticancer activity, plumbagin with anti-diarrhea and matrine with anti-lung adenocarcinoma activity have been shown to inhibit AN01.

Several clinical drugs are recently found to have inhibitory effect on AN01, including clarithromycin, benz bromaramone, niclosamid, nitazoxanide and avermectins. Clarithromycin is a clinical antibiotic that was reported to decrease IL-13 induced AN01 expression in goblet cells from asthma models. Benz bromaramone, a clinical drug for gout treatment, shows anti-asthmatic effect through inhibiting IL-13 induced mucin secretion and methacholine induced airway smooth muscle (ASM) contraction via the inhibition of AN01. Niclosamide and nitazoxamide are clinical anthelmintics that as potent AN01 inhibitors can block ASM depolarization and contraction. Avermectins are a type of macrocyclic lactones that are widely used as pharmaceuticals against roundworms in humans and animals and also for crop protection. Several avermectins, including avermectin B1, ivermectin, doramectin, selamectin and moxidectin, exhibit inhibitory effects on cancer cell proliferation.
Table 2  Pharmacology of CaCC ANO1/TMEM16A Activators.

| Activator | Structure | EC₅₀ (µmol/L) | Test assay | Selectivity | Effect                  | Ref.   |
|-----------|-----------|---------------|------------|-------------|------------------------|--------|
| Eact      | ![Structure](image1) | 3             | hANO1-FRT  | TRPV1 (+)   | Pain                   | 66,109 |
|           |           |               |            | TRPV4 (+)   | Smooth muscle contraction | 111    |
|           |           |               |            | [Ca²⁺]ᵢ (+) |                        |        |
|           |           |               |            | ANO6 (+)    |                        |        |
| Fact      | ![Structure](image2) | 6             | hANO1-FRT  | NR          | NR                     | 109    |
| INO-4995  | ![Structure](image3) | NR            | hANO1-HEK293 | NR          | NR                     | 112    |
| Resveratrol | ![Structure](image4) | 48            | mANO1-HEK293T | NR          | Smooth muscle contraction | 113    |
| GRb1      | ![Structure](image5) | 38.4          | mANO1-HEK293 | NR          | Smooth muscle contraction | 114    |
| Cinnamaldehyde | ![Structure](image6) | 9.7           | ANO1-HEK293 | NR          | Smooth muscle           | 66     |

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and migration through inhibition of ANO1. These drugs may have repurposing potential for dysfunctional ANO1-related diseases, such as asthma and hypertension.

It is noticeable that many compounds can act on ANO1 with limited selectivity, which imposes a big challenge for validation of ANO1 as a therapeutic target. The reasons for lack of identifying specific ANO1 modulators can be in multiple folds including chemical designs without guidance of target structure, use of an indirect YFP fluorescence-based HTS assay, and compound screens against cells expressing endogenous xANO1 in oocytes (for MONNA), hANO1 in HT29 cells (for CaCCinh-A01), or heterogeneous hANO1 in FRT cells (for T16Ainh-A01, Ani9 and 10bm). In addition, ANO1 and ANO2 subunits share 62% of sequence, which also presents the selectivity challenge. The recent solved cryo-EM structure of ANO1 may help understand the biophysical features and physiological functions of the channel and also can greatly assist to develop more specific ANO1 modulators.

A pharmacophore model based on three-dimensional quantitative structure-activity relationship (3D-QSAR) for predicting best target and compound interactions also appears to be a promising tool for virtual screening and enhancing rational design for novel potent and specific ANO1 modulators.

2.2. Activators

During the past almost 40 years since the identification of CaCC in 1982, only a few activators of ANO1 have been reported. Two ANO1 activators Eact and Fact are the first discovered using an HTS approach. Eact and Fact are two different classes of chemicals that activate ANO1 through different mechanisms (Table 2). Eact is an activator that induces ANO1 current in the absence of intracellular Ca2+ with an EC50 of 3 μmol/L. Several studies indicate that Eact might indirectly activate ANO1 through an intracellular Ca2+ increase. Fact is a potentiator that increases ANO1 current in a Ca2+-dependent manner with an EC50 of 6 μmol/L. INO-4995 is an 1-O-octyl-2-O-butyryl-myoinositol 3,4,5,6-tetrakisphosphate octakis(propionoxymethyl) ester directly activates overexpressed ANO1 current but without effect on endogenous ANO1 currents in Xenopus oocytes, human airways and colonic cells.

A small molecule ANO1 potentiator ETX001 is recently shown to increase ANO1 current with an EC50 of 116 nmol/L without effect on intracellular Ca2+ signaling (Table 2). ETX001 enhances fluid secretory response in human bronchial epithelial cells from cystic fibrosis (CF) patients, and increases airway mucus clearance in sheep CF models. Several compounds from traditional Chinese medicines, including resveratrol, ginsenoside Rb1 (GRb1), cinnamaldehyde, and chitosan oligosaccharides, were recently reported to activate ANO1 channel although their mechanisms of action remain elusive. Activators of ANO1 can be useful for validating ANO1 as a therapeutic target for treatment of disorders associated with ANO1 hypofunction, including Sjogren’s syndrome, cystic fibrosis lung disease, and dry eye syndrome.

It should be mentioned that the relatively wide distribution of ANO1 may impose a liability concern or a challenge in developing organ- or tissue-specific therapy. Nevertheless, development of targeted drug delivery systems such as tissue-specific or selective organ targeting nanoparticles and controlled release of therapeutic agents may circumvent potential ANO1-originated complications. In addition, target-related complications can also be minimized thought means of different drug formulations,
such as topical preparations, sublingual, buccal and rectal administrations.

3. Pathological functions and related diseases

Since ANO1 was identified as a member of CaCCs in 2008, its investigations have been focused on the distribution and expression in numerous organs and tissues, and its roles in pathological conditions and diseases (Fig. 2).

3.1. Epithelial diseases

Epithelial cells are a group of tightly compressed cells that line the inside of all organs and function as a barrier between the inside and outside of an organ. Chloride ion is important for trans-epithelial secretion, whereas intracellular Ca$^{2+}$ is one of two major signals to modulate Cl$^{-}$ secretion, indicating an important role of Ca$^{2+}$-activated Cl$^{-}$ channels in regulating epithelial secretion. ANO1 is observed in many epithelial tissues, including but not limited airway, bronchus, salivary glands, pancreatic ductal cells and intestinal epithelium. Numerous literatures not only identify native ANO1 expressions in multiple epithelia tissues but also demonstrate the pathological functions of ANO1 important for the process of fluid and electrolyte secretion. Here, we review several epithelial diseases involved in ANO1 dysfunction.

3.1.1. Asthma

Asthma is a prevalent inflammatory airway disease characterized by chronic inflammation, remodeling, and excessive constriction of the airway. The mechanism underlying asthma is complex and treatment for asthma is still challenging. Hypersecretion of airway epithelium and hypercontraction of airway smooth muscle are two major contributions to asthma. The Ca$^{2+}$-activated Cl$^{-}$ channel ANO1 as an anion channel plays a critical role in airway transmural secretory function. Mucus secreted by goblet cells is composed of water and mucus protein, which requires anion channel activity to instill Cl$^{-}$ and HCO$_3$ for ensurance of proper salination, hydration and pH of the mucus gel layers. ANO1 is found robustly expressed in epithelial goblet cells and involved in mediating native Ca$^{2+}$ dependant Cl$^{-}$ current and ASM contractile response. ANO1 is also highly permeable to HCO$_3$ at higher intracellular Ca$^{2+}$ levels, likely contributing to mucus release by providing a secretory pathway for HCO$_3$ that is an essential ingredient in mucus. Downregulation of ANO1 markedly decreases epithelial mucus secretion, suggesting ANO1 inhibition beneficial for asthma. ANO1 expression is upregulated by cytokines IL-4, IL-13 and T helper type 2 that are asthmatic biomarkers, and upregulated in animal models of asthma. Pharmacological inhibition or gene silencing of ANO1 reduces Cl$^{-}$ secretion induced by muscarinic agonist carbochol, suggesting an involvement of Ca$^{2+}$-activated Cl$^{-}$ channel ANO1 in pathology of asthma. There is an increasing evidence that ANO1 plays an important role in the regulation of intestinal epithelial secretion and the pathogenesis of diarrhea based on the following observations: 1) ANO1 is expressed in intestinal epithelia and ANO1 gene knockdown reduces Ca$^{2+}$- activated Cl$^{-}$ secretion induced by muscarinic agonist carbochol; 2) Ca$^{2+}$- dependent Cl$^{-}$ secretion increases through enhancement of ANO1 expression or activation of ANO1 current in the distal colon in response to the rotavirus nonstructural glycoprotein NSP4 that causes infantile gastroenteritis; and 3) ANO1 expression is upregulated in animal models of diarrhea.

The increased activity of gastrointestinal (GI) tract is another important cause for diarrhea. ICC are mesenchymal cells located within the muscle layers in the GI tract and myenteric ICC serve as a pacemaker generating the bioelectrical slow wave potential that leads to contraction of GI smooth muscles in the GI tract. Recent studies have found that ANO1 is also highly expressed in the ICC and mediates the slow wave current in the ICC. The intracellular Ca$^{2+}$ rise from intracellular stores and VGCC in the ICC causes the generation and propagation of pacemaker potential that is also amplified by activation of ANO1 channels, suggesting that inhibition of ANO1 may reduce intestinal motility for diarrhea. Indeed, pharmacological inhibition or gene silencing of ANO1 blocks slow wave in intestinal smooth muscle. It is noted that several ANO1 inhibitors including T16Ainh-A01 and CaCCinh-A01 exhibit different potency on blocking gastric and small intestinal slow waves. For instance, CaCCinh-A01 blocks slow waves in the murine stomach at 5 μmol/L and the small intestine at more than 30 μmol/L.
these different sensitivities is not entirely clear, but several possibilities including other channels contributions, different splice variants and the local \( \text{Ca}^{2+} \) concentrations may contribute to these differences\(^{138}\). Nevertheless, both GI epithelium and ICC are synergistically involved in the pathogenesis of diarrhea and downregulation of ANO1 in either type of the two cells may help mitigate diarrhea.

Rotavirus has been known to cause severe diarrhea in infants and young children, whereas red wine extracts with alcohol-free and \text{CaCCinh-A01} can prevent intestinal fluid loss in a neonatal mouse model of rotaviral diarrhea through inhibition of ANO1-mediated \( \text{Ca}^{2+} \)-activated \( \text{Cl}^{-} \)secretion\(^{53}\). However, red wine extracts and \text{CaCCinh-A01} show no obvious effect on cholera toxin-induced diarrhea or CFTR current in cultured cells or intestinal absorption, suggesting the important role of ANO1 in diarrhea\(^{138}\). A recent study demonstrates that glucose and \text{NSP4} synergistically increase the expression of ANO1 and \( \text{Ca}^{2+} \)-activated \( \text{Cl}^{-} \)secretory in the mouse model of diarrhea\(^{137}\). Suppressing ANO1 expression in apical membranes of colonic epithelium decreases \( \text{Ca}^{2+} \)-activated \( \text{Cl}^{-} \)secretion in chemical dextran sulfate sodium-induced chronic colitis in mice\(^{144}\). There are also observations that inhibition of ANO1 by non-specific ANO1 inhibitors including eugenol\(^{74}\), shikonin\(^{145}\), plumbagin\(^{83}\), resveratrol dimer \text{trans-\v{e}Viniferin (TV)} and tetramer \( \gamma-2\-\text{Viniferin (RV)}\(^{69}\) can reduce water content in stools, but those observations should be confirmed with specific ANO1 inhibitors.

3.1.3. Cystic fibrosis

CF is an inherited disease of airway obstruction caused by mucus hypersecretion, mucus plugging and bronchoconstriction\(^{146}\). The dysfunction of CFTR \( \text{Cl}^{-} \)channel is considered to be a major cause for CF as defective CFTR \( \text{Cl}^{-} \)channel mutations are identified in CF patients\(^{147}\). It has been shown that small molecules can rescue dysfunctional CFTR mutation through increasing the number or open probability of CFTR \( \text{Cl}^{-} \)channels. Unfortunately, these CFTR correctors or potentiators only exhibit limited efficacy for CF patients because of their multiple and different mutation sites of CFTR\(^{148}\). Therefore, an alternative strategy is CFTR-independent approach for treatment of CF by increasing the activity of ANO1 channel to promote mucus secretion\(^{149}\). This hypothesis is supported by observations that: 1) ANO1 is abundantly expressed in the airway goblet cells and upregulated in
inflammatory conditions; 2) overexpression of ANO1 in CF human bronchial epithelia suppresses proinflammatory cytokine IL-8 secretion; 3) specific knockdown of ANO1 in respiratory airways eliminates Ca\(^{2+}\)-and cAMP-activated Cl\(^{-}\) secretion; 4) Ano1 gene knockout mouse exhibits abnormal trachea morphology, and also mucus obstruction and defective mucociliary clearance that presenting with a CF-like lung phenotype.

Several studies demonstrate that ANO1 has a functional cross talk with CFTR through PSD-95/Dlg/AO-1 proteins. Such an interaction between ANO1-mediated Ca\(^{2+}\)-activated Cl\(^{-}\) secretion and CFTR-mediated cAMP-dependent Cl\(^{-}\) secretion in airway epithelial cells strongly overlaps through a cAMP sensor protein termed exchange protein directly activated by cAMP and Ca\(^{2+}\)-sensitive adenylate cyclase type 1. ANO1 expression and activity are deficient in CF patients and upregulation of ANO1 can improve mucus dynamics in CF mice. These lines of evidence support the notion that ANO1 may represent an alternative therapeutic target to circumvent CFTR dysfunction in the airway epithelia of CF patients.

Some CFTR-independent drug candidates have been developed for potential CF therapy. Denufosol, a P2Y2 receptor agonist, promotes airway epithelial chloride secretion through activating CaCCs with P2Y receptors. Unfortunately, denufosol failed in phase III trial because of its short half-life in vivo. Recently, an ANO1 activator, ET000516-A-2 from pre-clinical study is expected to be evaluated in CF patients. Another novel ANO1 potentiator ETX001 developed by Enterprise Therapeutics (Brighton, UK) was recently reported that it can enhance fluid secretion and improve mucociliary clearance in both primary CF bronchial epithelial cells and sheep model of CF-like airway disease. These two candidates may bring us an exciting prospect for potential development of alternative CF therapy in the clinic.

### 3.1.4 Other epithelial diseases

A recent study shows that ANO1 is overexpressed in mouse pancreatic tissue of acute pancreatitis model. ANO1 promotes the pathogenesis of acute pancreatitis through activating the IP\(_R/\)Ca\(^{2+}\)-NF-\(\kappa\)B/IL-6 pathway, and inhibition of ANO1 by T16Ainh-A01 reduces the pancreatic damage in acute pancreatitis mice. ANO1 is also likely involved in other epithelial diseases, such as polycystic kidney disease, diabetic nephropathy and pulmonary fibrosis. The PKD is characterized by multiple bilateral renal cysts that gradually enlarge and can lead to a decline in renal function. ANO1 is found in human kidney and renal epithelial cell lines, and its expression is upregulated in the forskolin induced renal cyst model, autosomal dominant PKD patients and high-fat diet/streptozotocin-induced diabetic nephropathy mice, indicating a role of ANO1 in kidney disease.

ANO1 promotes renal cyst growth via induction of Cl\(^{-}\) secretion and proliferation of cyst lining epithelium. Inhibition of ANO1 by pharmacological inhibitors or gene knockdown significantly decreases glucose dependent cyst growth, reduces nephron numbers and also causes albuminuria and tubular damage. Mechanistically, ANO1 drives the growth of renal cysts through enhancing Ca\(^{2+}\) release from IP\(_R/\)sensitive Ca\(^{2+}\) stores and lipid peroxidation also promotes renal cyst growth through activating ANO1. In diabetic nephropathy, ANO1 deletion alleviates renal injury in diabetic mice through increasing nphin expression, reducing the expression level of apoptosis related factors and also suppressing the activation of P38/JNK signaling pathway.

On the contrary, ANO1 activation aggravates renal injury by activating P38/JNK signaling pathway to promote podocyte apoptosis in diabetic nephropathy mice and exacerbates inflammation via activating the TGF-\(\beta\)-SMAD3 pathway. Downregulation of ANO1 by shRNA can inhibit apoptosis and promote the proliferation of lung fibroblasts in mouse model of idiopathic pulmonary fibrosis. These observations suggest that inhibition of ANO1 may hold therapeutic potential for kidney and other epithelium-originated diseases.

### 3.2 Cancers

Epithelial cancer is also known as carcinoma that arises from epithelial tissues. Prior to identification as a CaCC, ANO1 was known as TMEM16A first described in 2003. ANO1 was also named as DOG1 (gastrointestinal stromal tumor 1), TAOS2 (tumor amplified and overexpressed sequence 2) and ORAOV2 (oral cancer overexpressed 2) because of its overexpression in these cancers. Investigations from ours and others show that ANO1 is overexpressed and involved in the pathogenesis of cancers especially originated from epithelial cancers, such as gastrointestinal stromal tumor (GIST), head and neck squamous cell carcinoma (HNSCC), prostate cancer, lung cancer, colon cancer, ovarian cancer, breast cancer, liver cancer, gastric cancer, esophageal cancers, pancreatic adenocarcinoma, salivary gland carcinoma and glioblastoma (Table 3).

ANO1 upregulation in many kinds of cancers is related to its gene location at chromosome 11q13 that is frequently amplified in many malignant tumors and amplification of 11q13 is associated with the increase of ANO1 gene copy numbers. Overexpression of ANO1 promotes proliferation and migration in multiple cancer cell lines, and ANO1 upregulation is associated with lower overall survival in patients with breast cancer, pancreatic cancer or gastric cancer. ANO1 mRNA is also highly expressed in the blood of GIST patients and patients with epithelial ovarian cancer, and the expression level of ANO1 mRNA decreases after surgical removal of tumors, suggesting that detection of ANO1 gene in blood may serve as a biomarker for early diagnosis of cancer.

Multiple signaling pathways have been shown to be involved in ANO1 modulation in cancer development (Table 3). In breast cancer and HNSCC, downregulation of ANO1 by knockdown or pharmacological inhibition inhibits cancer cell proliferation, induces apoptosis and reduces tumor growth through reducing epidermal growth factor receptor (EGFR) cell signaling pathways. Studies have also shown that ANO1 can affect the progression of intestinal cancer, liver cancer, and pancreatic cancer through the EGFR pathway, as well as the modulation of gliala by NF-\(\kappa\)B signaling. In prostate cancer, ANO1 can regulate TNF-\(\alpha\) signaling to contribute to cell growth and apoptosis. In epithelial ovarian cancer, silencing ANO1 can suppress cancer cell proliferation, migration and invasion as well as the growth of xenograft tumors through inactivation of PI3K/AKT cell signaling pathway. In gastric cancer, ANO1 overexpression can promote tumor invasion and predict poor prognosis through affecting TGF-\(\beta\) signaling function, that is also the target for ANO1 regulating cell proliferation, migration and invasion in esophageal squamous cell carcinoma (ESCC). In glioblastoma, ANO1 expression is regulated by CaMKII-\(\beta\) and suppression of CaMKII-\(\beta\) inhibits ANO1 mediated glioblastoma development, and CaMKII also plays a role in ANO1-mediated...
| Cancer type | High expression | Cell line | Human tissue | Clinical implication | Cell assay | Inhibition Tool | Xenograft tumor | Signaling pathways | Ref. |
|-------------|-----------------|-----------|--------------|----------------------|-----------|----------------|----------------|-------------------|------|
| Breast cancer | ZR75-1, HCC1954, MDA-MB-415 | + | Poor Prognosis (+) | + | NR | shRNA/CaCC\textsubscript{inh}-A01 | Tumor growth (−) | 11q13 amplification, Cl\textsuperscript{−} channel activity, Apoptosis, EGFR, CAMKII, AKT, MAPK | 51 |
| | YMB-1 | NR | NR | + | NR | siRNA/NFA | NR | Epigenetic regulation | 40 |
| | YMB-1, MDA-MB-453 | NR | NR | NR | NR | siRNA/T16\textsubscript{inh}-A01 | NR | AKT, STAT3 | 173 |
| | SKBR3 | NR | Improved response to biological therapies (−) | + | NR | siRNA/T16A\textsubscript{inh}-A01 | NR | EGFR, HER2, STAT3 | 174 |
| | MCF-7, T47D | + | Shorter overall survival in ER + patients (+) | + | NR | shRNA/T16A\textsubscript{inh}-A01 | Tumor growth (−) | EGFR/STAT3 signaling | 175 |
| | UM-SCC1, T24 | + | Poor prognosis (+) | + | NR | shRNA/T16A\textsubscript{inh}-A01 | Tumor growth (−) | MAPK, Ki67 | 176 |
| HNSCC | HEP-2, SCC-25 | + | A marker for distal metastasis (+) | No effect | + | siRNA/NFA, DIDS, Fluoxetine shRNA | NR | 11q13 amplification | 166 |
| | UM-SCC1, T24 | High in primary tumor and low in metastatic tumor | A biomarker for metastasis (−) | NR | − | shRNA | Tumor growth (−), Metastatic development (+) | Promoter methylation, E-cadherin | 177 |
| | FaDu | NR | Poor prognosis (+) | + | NR | shRNA/CaCC\textsubscript{inh}-A01 | Tumor growth (−) | 11q13 amplification, Cl\textsuperscript{−} channel activity, Apoptosis, EGFR, CAMKII, AKT, MAPK | 51 |
| | Cal-33, OSC19, UM-SCC-1, FaDu | NR | Increased efficacy of biologic therapies (−) | OSC19 (+) | NR | siRNA/T16A\textsubscript{inh}-A01 | ANO1-overexpressing tumors were heavier than control tumors | EGFR, HER2, STAT3 | 174 |
| | OSC19, FaDu, UM-SCC-1 | Positively correlated with tumor size | Recurrence of cancer (+) | + | NR | shRNA | ANO1-overexpressing tumors were greater than control tumors | ERK, BIM, Apoptosis | 178 |
| Tumor Type | Cell Line(s) | Treatment | Response | Regulator | Pathway |
|------------|--------------|-----------|----------|-----------|---------|
| ESCC | KYSE30, KYSE510 | + Lymph node metastasis and advanced clinical stage (+) | + NR | siRNA | NR 11q13 amplification |
| Prostate cancer | KYSE410, KYSE30, PC-3, LNCap, RWPE1 | + Poor prognosis (+) | + + | shRNA | NR TGF-β signaling, cell cycle |
| | | + Advanced stage (+) | NR | siRNA/T16Ainh-A01, CaCCinh-A01, MONNA, tannic acid | Tumor growth (−) |
| | | NR | + NR | siRNA/NFA | NR Epigenetic regulation |
| | | + Clinical TNM stage (+) | + NR | shRNA/DIDS | Tumor growth (−) |
| | | NR | NR | shRNA | Tumor growth (−) |
| | | + Clinical TNM stage (+) | + NR | shRNA/CaCCinh-A01, T16Ainh-A01, Ani9 | Tumor growth (−) |
| Gastric cancer | AGS, BGC823 | + Poor overall survival (+) | No effect | + shRNA | NR TGF-β, E-cadherin |
| | AGS, BGC823 | Negatively related with miR381 | Poor prognosis (+) | No effect | + siRNA | NR Regulated by miR-381, TGF-β, E-cadherin |
| | AGS, SGC7901 | + TNM stage (+) | NR | + siRNA | Tumor metastasis (−) |
| HCC | SMMC7721 | + NR | + + | siRNA | Tumorigenicity (−) MAPK signaling, cell cycle |
| | HepG2, SMMC7721 | + Tumor grade (+) | + + | shRNA | Tumor growth (−) PI3K/AKT-MAPK signaling pathway, apoptosis, cell cycle |
| Glioma | U87MG | + Tumor grade (+) | + + | siRNA | NR NF-κB signaling |
| | U251, U87MG, GLC82, NCI-H520 | NR | NR | NR | + shRNA/T16Ainh-A01 | NR |
| | H1299 | NR | NR | + + | shRNA | Tumor growth (−) |
| Lung cancer | U87MG, U251, H1299 | + Tumor grade (+) | + + | siRNA | NR |
| | | NR | NR | NR | + shRNA | Tumor growth (−) |
| | | + NR | + NR | shRNA | Tumor growth (−) |
| | | NR | NR | shRNA/T16Ainh-A01 | Tumor growth (−) EGFR/MAPK signaling |

(continued on next page)
| Cancer type                  | High expression                        | Cell assay                  | Inhibition                     | Signaling pathways | Ref. |
|-----------------------------|----------------------------------------|-----------------------------|--------------------------------|--------------------|------|
|                             | Cell line | Human tissue | Clinical implication | Proliferation/ viability | Migration/ invasion | Tool | Xenograft tumor |
| Pancreatic adenocarcinoma    | BxPC-3, AsPC-1, Capan-1 AsPC-1 | NR | NR | No effect | + | siRNA/T16Ainh-A01, CaCCinh-A01, NS3728 | NR | NR | 93 |
| GIST                        | GIST-T1, GIST-882 NR | NR | NR | No effect | NR | shRNA | NR | NR | 171 |
| NR                          | Cancer and PBMCs | NR | + | Poor prognosis (+) | Biomarker (+) | NR | NR | 165 |
| Salivary gland carcinoma    | GIST-T1, GIST-882 NR | NR | + | NR | NR | T16Ainh-A01 CaCCinh-A01 | NR | Cell cycle | 91 |
| Ovarian cancer              | SKOV3 Cancer and PBMCs | NR | NR | NR | NR | NR | NR | 172 |
| Colorectal cancer           | SW620 Cancer and PBMCs | NR | NR | NR | NR | NR | NR | 185 |
| Liver metastasis cancer tissue | DLD-1, HCT116 | + | Poor prognosis (+) | Poor prognosis (+) | + | siRNA | NR | 186 |
| SW480 | + | Poor prognosis (+) | NR | + | siRNA | NR | 187 |

+, positive effect; −, negative effect; NR, no report. HCC, hepatocellular carcinoma; PBMCs, peripheral blood mononuclear cells; TNM, tumor, lymph nodes and metastasis.
tumorigenic properties of HNSCC and breast cancer. It appears that ANO1 can regulate different signaling pathways in the same type of tumor or the same signaling pathway in different types of tumor.

Inhibition of ANO1 by gene silencing or pharmacological means can suppress cancer cell proliferation and migration, invasion and tumor growth. Small molecules, such as CaCCinh-A01, T16Ainh-A01, MONNA and Ani9, as well as natural products including avermectins and flavonoids exhibit anti-cancer effects through inhibition of ANO1 activity, suggesting that ANO1 may serve as a potential drug target for cancer therapy.

### 3.3. Hypertension

Hypertension or high blood pressure is a common condition in which the long-term force of the blood against the blood vessels is high enough that it may increase the risk of heart diseases and brain stroke. ANO1 is expressed in various smooth muscle cells of arteries and veins, suggesting a role of ANO1 in regulation of vasoonstriction. Activation of Ca\(^{2+}\)-activated Cl\(^{-}\) current can lead to Cl\(^{-}\) efflux after Ca\(^{2+}\) influx and membrane depolarization, thus resulting in vasoonstriction and increase of blood pressure. Indeed, ANO1 expression and activity are upregulated in murine pulmonary arterial myocytes induced by chronic hypoxia, which contributes to pulmonary hypertension. Similar results were also observed in monocrotaline-induced pulmonary hypertension rats, spontaneously hypertension rats, and high-flow-induced pulmonary arterial hypertension (PAH) rats. Conversely, cell-specific knockout of ANO1 reduces blood pressure and attenuates hypertension in mice and spontaneously hypertensive rats.

A recent study shows that upregulation of ANO1 depolarizes pulmonary artery smooth muscle cells (PASMC) membrane potential, contributing to vasoconstriction and the increased pulmonary vascular resistance in PAH rats. Conversely, pharmacological inhibition or gene silencing of ANO1 reverse the membrane depolarizes of PASMC. For instance, a specific ANO1 inhibitor MONNA hyperpolarizes the rat coronary artery smooth muscle cell membrane potential and increases coronary flow. Vascular smooth muscle cells (VSMC) are the stromal cells of the vascular wall and are responsible for regulating arterial tone, and blood pressure. Overexpression of ANO1 in healthy donor PASMC promotes the cell proliferation and produces an idiopathic pulmonary arterial hypertension (IPAH)-like phenotype. Pharmacological inhibition of ANO1 may reverse vasoconstriction and remodeling of pulmonary arteries in IPAH. These investigations suggest the involvement of ANO1 in VSMC contraction and vascular remodeling for hypertension.

Circulating angiotensin II (Ang II) as a major contributor of the renin–angiotensin system is upregulated during hypertension, and Ang II is frequently used to establish hypertension models. It has been shown that Ang II significantly enhances ANO1 expression in human umbilical vein endothelial cells and endothelial-specific ANO1 knockout significantly reduces Ang II-induced hypertension through ROS signaling pathway, whereas endothelial-specific transgenic ANO1 shows the opposite effect. These studies suggest that inhibition of ANO1 function may be beneficial for hypertension, and ANO1 inhibitors, including T16Ainh-A01, MONNA, and Ani9 can inhibit agonist-induced vesicle constriction and cause vasorelaxation.

### 3.4. Nociception

Nociception is a perception in response to painful or harmful stimuli, such as heat, cold, mechanical and chemical stimulus in the environment. Dorsal root ganglion (DRG) is a cluster of sensory neurons in the dorsal root of spinal nerves responsible for pain signal transmission. ANO1 is mainly expressed in small diameter DRG neurons that are intimately involved in nociception, suggesting a modulatory role of ANO1 in pain sensation. It has been shown that an inflammatory mediator bradykinin, released from damaged tissues or applied exogenously can activate ANO1 through B2 receptors and PLC pathway, subsequently depolarizing membrane potential and markedly stimulating firings in DRG neurons, and pharmacological inhibition of ANO1 attenuates pain behaviors. ANO1 expression is upregulated in the spinal cord and DRG neurons after spinal nerve injury, suggesting the involvement of ANO1 in development of neuropathic pain. Protease activated receptor 2 (PAR2), also known as G-protein coupled receptor 11, has been identified to be involved in the pathogenesis of pain. PAR2 and ANO1 are co-localized in DRG neurons, and their expression are increased in rat pain model of chronic constriction injury.

ANO1 can be activated by temperatures over 44 °C, and silencing of ANO1 in DRG neurons significantly reduces nociceptive behavior in thermal pain model, inflammation and nerve-injury induced hyperalgesia or allodynia. Thus, downregulation of ANO1 activity may present a potential therapeutic strategy for neuropathic pain. ANO1 as a potential target for pain is evidenced by observations that inhibition of ANO1 by small molecules NPPB, NFA, T16Ainh-A01 or CaCCinh-A01 reduces capsaicin-induced inward current and action potential firing, and as well as pain-related behaviors. Again, these ANO1 inhibitors are not specific and a key question such as whether ANO1 directly affects the excitability of nociceptors should be addressed.

### 3.5. Others

A recent study shows that ANO1 is expressed in mouse brain endothelial cells where ANO1 expression is upregulated after ischemic stroke induced by the middle cerebral artery occlusion. Targeting ANO1 with inhibitor CaCCinh-A01 or silencing attenuates blood–brain barrier (BBB) breakdown after ischemic stroke through decreasing intracellular adhesion molecule-1 via NF-κB signaling pathway, suggesting that downregulation of ANO1 protects BBB disruption after ischemia stroke. It is supported by another study that ANO1 inhibitor T16Ainh-A01 or siRNA inhibits proliferation and migration of brain capillary endothelial cells that comprise BBB. It would be interesting to see more studies that are designed to validate ANO1 as a therapeutic target for brain stroke.

### 4. Summary and perspectives

The CaCC ANO1 channel is expressed in a wide variety of epithelial cells, smooth muscle cells and neurons. Although abnormal expression or dysfunction of ANO1 is involved in the
pathology of many diseases, validating ANO1 as a therapeutic target still presents a big challenge. While a significant progress has been made for the distribution, expression, structure and pathophysiological functions of ANO1, there still exists an urgent need for selective modulators of the channel for target validation. Current available ANO1 modulators are also in preclinical stage without any treatments ready for clinical utility, which highlights the much-needed efforts in understanding the channel pharmacology and validation of ANO1 as therapeutic target. The recent structure of ANO1 solved by single-particle cryo-electron microscopy can provide a valuable model for the design of more potent and selective ANO1 modulators that can be used to help validate this emerging target for therapeutic potential of diseases, including cancer, inflammatory epithelial diseases, neuropathic pain and hypertension and cystic fibrosis lung disease.

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Author contributions

Yani Liu, Zongtao Liu, and KeWei Wang wrote and edited the manuscript. Yani Liu and KeWei Wang contributed to manuscript revision and discussion of the content.

Conflicts of interest

The authors declare that there is no conflict of interest.

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