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Ion Channels in Cancer: Orchestrators of Electrical Signaling and Cellular Crosstalk

Jerry J. Fan and Xi Huang

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Abstract Ion channels are pore-forming transmembrane proteins that govern ion flux to regulate a myriad of biological processes in development, physiology, and disease. Across various types of cancer, ion channel expression and activity are often

J. J. Fan (✉) and X. Huang (✉)
Program in Developmental and Stem Cell Biology, The Hospital for Sick Children, Toronto, ON, Canada

Arthur and Sonia Labatt Brain Tumour Research Centre, The Hospital for Sick Children, Toronto, ON, Canada

Department of Molecular Genetics, University of Toronto, Toronto, ON, Canada
e-mail: jj.fan@mail.utoronto.ca; xi.huang@sickkids.ca

dysregulated. We review the contribution of ion channels to multiple stages of tumorigenesis based on data from in vivo model systems. As intertumoral and intratumoral heterogeneities are major obstacles in developing effective therapies, we provide perspectives on how ion channels in tumor cells and their microenvironment represent targetable vulnerabilities in the areas of tumor-stromal cell interactions, cancer neuroscience, and cancer mechanobiology.

Keywords Bioelectrical signaling · Cancer · Ion channels · Mechanobiology · Membrane potential · Metastasis · Tumor heterogeneity · Tumor initiation · Tumor microenvironment · Tumor progression

1 Introduction

1.1 Tumor Heterogeneity and the Multi-Step Tumorigenic Process Are Necessary Considerations for Personalized Cancer Medicine

Cancer is the second leading cause of death worldwide. Current standard-of-care is often non-specific and toxic to normal cells, causing side effects which reduce quality of life in survivors. Targeted therapeutic strategies have been developed through efforts in cancer genomics. Identification of oncogenic mutations, cellular mechanisms, and signaling networks have aided risk stratification and prediction of therapeutic response (The Cancer Genome Atlas Research Network 2008, 2011, 2012; The Cancer Genome Atlas Network 2012a, b). Tumorigenesis is a multi-step process which drives normal cells toward oncogenic transformation (initiation), stimulates tumor cells to survive and expand (progression), promotes dissemination and colonization of tumor cells at distant sites (metastasis), and confers therapeutic resistance (resistance and relapse). It is crucial to identify actionable targets in the exact step of tumorigenesis to develop effective therapeutic approaches.

Major obstacles remain in the fight against cancer. Malignant cells possess distinct phenotypic and functional characteristics between different tumors (intertumoral heterogeneity) and within individual tumors (intratumoral heterogeneity). Tumor heterogeneity is a vital consideration in validating therapeutic targets to eliminate tumor cell populations. Intertumoral heterogeneity hinders subtype-agnostic use of targeted therapies. Intratumoral heterogeneity creates therapy-resistant subclones that underlie disease relapse. Intratumoral heterogeneity can arise from several sources. First, it can arise from subclonal genetic mutations (different tumor cells and their progeny acquire different mutations). Second, functional heterogeneity exists in the form of quiescent cancer stem cells, fast-cycling tumor cells, post-mitotic tumor cells, and tumor stromal cells, each with unique molecular profiles and chemosensitivity. Third, tumor cells can possess distinct

biophysical heterogeneity, such as electrical and mechanical properties. Importantly, all cells regulate ionic homeostasis to maintain proper resting membrane potentials, and many cells within tumors are capable of generating electrical activity in response to cell extrinsic or intrinsic stimuli. Thus, tumor cells can form electrical networks with input from their local microenvironment. Recent work on electrical and chemical synapses in cancer has revealed that cancer is an electrically active entity and that these electrical networks can be therapeutically targeted. As central regulators of cellular electrical properties, ion channels are implicated in all steps of tumorigenesis.

1.2 Why Target Ion Channels in Cancer?

As pore-forming transmembrane proteins, ion channels are regulated by chemical and physical stimuli to mediate ion flux along electrochemical gradients (Hille 2001). The voltage-gated ion channel superfamily is encoded by over 140 genes in the human genome, making it the third largest group of signaling molecules after G-protein-coupled receptors and kinases (Alexander et al. 2019). Broadly, ion channels can be classified as permeating anions or cations. Anion channels conduct chloride and other less abundant anions, while cation channels can be classified into potassium, sodium, and calcium-permeating families, in addition to the non-selective cation channels which include transient receptor potential (TRP) channels. Ion channels are present in all cells and regulate a myriad of biological processes ranging from rapid electrical signaling in excitatory cells to slower processes such as proliferation, volume regulation, migration, apoptosis, and hormone secretion. For example, ion channels control cell excitability through action potential propagation and regulate membrane potential during cell cycle progression and proliferation (Lang et al. 2005). Coordinated ion channel activity mediates cell migration (Schwab et al. 2012) through differential subcellular channel distribution and local ion flux at leading or trailing edge membranes (Schwab et al. 1995; Schneider et al. 2000; Huang et al. 2015). In addition, ion channels relay information from the extracellular environment (e.g., ion concentration, osmolarity, voltage, mechanics) and integrate external cues into signaling cascades within tumor cells. Ion channels promote tumor growth and survival, render cancer cells resistant to apoptotic and anti-proliferative signals, or play tumor-suppressive roles to prevent aberrant proliferation or oncogenic transformation.

Many ion channels have well-studied pharmacology and frequently localize at the cell surface, making them accessible drug targets. Small molecule ion channel modulators have been identified by high-throughput screening for proof of concept preclinical studies. Medicinal chemistry of existing chemical structures or rational design of small molecules can be guided by information on ion channel protein structures, domains that confer ion selectivity, and electrophysiological characteristics. Therefore, as the molecular targets of approximately 15% of US FDA-approved drugs (Overington et al. 2006), ion channels represent prime candidates for drug

repurposing to treat cancer. Through use of classic methods (such as patch clamp) in conjunction with more recent approaches (such as automated patch clamp, genetically encoded voltage/ion indicators, optogenetics, and chemogenetics), ion channel function in tumorigenesis can now be dissected with unprecedented resolution.

1.3 Criteria for Selection of Studies in this Review

In this review we highlight ways in which the fields of ion channel and cancer biology intersect (Table 1). First, we review how ion channel expression and alterations have been implicated in multiple steps of tumorigenesis. Second, we highlight in vivo evidence of ion channel function in cancer. While many pioneering studies were performed using cancer cells lines, in vitro systems do not adequately model complex tumor cellular architecture, microenvironment, drug bioavailability, and organismal toxicity. Thus, we focus on in vivo findings primarily in the form of orthotopic xenograft tumor models or genetically engineered animal models. We discuss examples of pharmacological targeting of ion channels with emphasis on drug repurposing, medicinal chemistry, use of preclinical models, and consideration for side effects. Third, we discuss emergent areas of ion channel function in cancer. Ion channels may mediate tumor cell co-option of neuronal synapses to establish electrical networks in cancer. Cell non-autonomous interactions and propagation of electrical activity in cancer require ion channels and gap junctions. Furthermore, mechanosensitive ion channels can perceive and respond to the altered tissue mechanics in cancer to regulate malignant progression. We provide perspectives on these aspects as the field moves forward.

We refer readers to excellent complementary reviews of ion channels in cancer (Bates 2015; Prevarskaya et al. 2018) and their breakdown by ion type: potassium (Pardo and Stühmer 2014; Huang and Jan 2014), sodium (Djamgoz and Onkal 2012; Fraser et al. 2014; Roger et al. 2015), calcium (Yang et al. 2010; Monteith et al. 2017), TRP channels (Santoni and Farfariello 2011; Prevarskaya et al. 2011; Ouadid-Ahidouch et al. 2013), chloride (Cuddapah and Sontheimer 2011; Peretti et al. 2015), and bioelectrical signaling (Tuszynski et al. 2017; Payne et al. 2019). As we discuss ion channel classes, in each section we present them in the order of potassium, calcium, sodium, chloride, and non-selective channels.

2 Ion Channel Expression in Human Cancer

Molecular classification and gene expression analysis allow the distinction between cancers with favorable diagnosis, which can be managed with conservative treatment, from those associated with poor prognosis, which require more aggressive therapy. The expression of a single ion channel or group (gene signature) may offer value in stratifying risk and determining the treatment plan. Ion channel expression

Table 1 Ion channels that are implicated in cancer growth in vivo

Tumorigenic process	Ion channel	Nature of dysregulation	Cancer type	Model	Therapeutic relevance	Reference
Tumor initiation	CACNA1D	Mutation	Endocrine	Human sequencing	n.d.	(Scholl et al. 2013)
	KCNJ5	Mutation	Endocrine	Human sequencing	Macrolide antibiotics normalize mutant KCNJ5 currents in cancer cells	(Choi et al. 2011; Scholl et al. 2017)
	KCNQ1	Loss of function	Gastrointestinal	Mouse genetics	n.d.	(Starr et al. 2009; Than et al. 2014)
	CFTR	Loss of function	Gastrointestinal	Mouse genetics, Human patients	n.d.	(Starr et al. 2009; Than et al. 2016) (Neglia et al. 1995; Maisonneuve et al. 2013)
Tumor promoting	KCNA3	Expression	Leukemia, melanoma, pancreatic	Mouse xenograft	PAP-1 derivatives inhibit KCNA3 to elicit tumor-specific apoptosis	(Leanza et al. 2012, 2013, 2017)
	EAG1	Overexpression	Melanoma, pancreatic, prostate	Mouse xenograft	EAG1 antibody visualizes and targets tumor cells	(Pardo et al. 1999) (Hartung et al. 2011; Napp et al. 2016)
	EAG2/eag	Overexpression	Medulloblastoma	Mouse xenograft, Human patient, *Drosophila*	FDA-approved thioridazine inhibits EAG2 and displays anti-tumor efficacy in a medulloblastoma patient	(Huang et al. 2012, 2015)
	KCNT2	Overexpression	Medulloblastoma	Mouse xenograft	n.d.	(Huang et al. 2015)
	Paralytic	Expression	*Drosophila* brain neoplasia	*Drosophila*	n.d.	(Piggott et al. 2019)
	CLIC1/clic	Overexpression	Medulloblastoma	Mouse genetics, Mouse xenograft, *Drosophila*	n.d.	(Francisco et al. 2020)
	PIEZO1/piezo	Overexpression	Glioma	Mouse xenograft, *Drosophila*	n.d.	(Chen et al. 2018)
	TRPA1	Overexpression	Breast, lung	Mouse xenograft	TRPA1 inhibitor AM-0902 reduces tumor growth and confers chemosensitivity	(Takahashi et al. 2018)
	TRPML1	Overexpression	Melanoma, bladder, head and neck cancer	Mouse xenograft	n.d.	(Jung et al. 2019; Kasitinon et al. 2019)
	TRPM3	Overexpression	Clear cell renal cell carcinoma	Mouse xenograft	NSAID mefenamic acid inhibits TRPM3 and reduces *in vivo* tumor growth	(Mikhaylova et al. 2012; Hall et al. 2014)
Tumor suppressive	TRPV1	Overexpression	Glioma, gastrointestinal	Mouse genetics, Mouse xenograft	TRPV1 agonist arvanil prolongs survival of glioma-bearing mice	(Stock et al. 2012; de Jong et al. 2014)
Metastasis	EAG2	Overexpression	Medulloblastoma	Mouse xenograft, Human patient	FDA-approved thioridazine inhibits EAG2 and reduces metastatic burden in a medulloblastoma patient	(Huang et al. 2015)
	KCNN4	Overexpression	Glioma	Mouse xenograft	n.d.	(Turner et al. 2014)
	Orai1/STIM1	Expression	Breast	Mouse xenograft	SKF96365 inhibits store-operated Ca^{2+} entry and reduces breast-to-lung metastasis	(Yang et al. 2009)
	Orai1/KCNN3	Expression	Breast	Mouse xenograft	Ohmline disrupts KCNN3-Orai1 localization and impairs breast-to-bone metastasis	(Girault et al. 2011; Chantome et al. 2013)

The table lists ion channels implicated in specific stages of tumorigenesis and evidence on pharmacological targeting of ion channels. n.d. not determined

is frequently dysregulated in cancer, occurring through various mechanisms, such as amplification, copy number alterations, mutations, and overexpression.

2.1 Dysregulated Expression

In breast and prostate cancer, decreased expression of potassium channel KCNA3 correlates with increased tumor grading (Abdul and Hoosein 2006; Brevet et al. 2009; Comes et al. 2013). Potassium channel EAG1 (KCNH1) is overexpressed in a wide array of cancers, including breast, prostate, colon, lung, liver, and soft tissue sarcoma (Hemmerlein et al. 2006; Mello de Queiroz et al. 2006). In addition to solid tumors, EAG1 expression is also elevated in myelodysplastic syndrome and leukemia (AML and CML), where high *EAG1* expression in AML predicts poor outcome (Agarwal et al. 2010). In normal tissues, EAG1 is mainly expressed in the brain with restricted expression in the periphery (Hemmerlein et al. 2006). Broad EAG1 overexpression across tumor types offers great therapeutic potential. Through conjugation to fluorescent or apoptosis-inducing ligands, EAG1-targeting antibodies can be used to visualize (Napp et al. 2016) or kill EAG1-expressing tumor cells (Hartung et al. 2011).

Potassium channel HERG1 (KCNH2) is highly expressed in colorectal cancers, while HERG1 is not detected in the normal colonic mucosa. In agreement with its expression being further elevated in metastatic disease, HERG1 regulates colon cancer cell invasiveness in vitro (Lastraioli et al. 2004). In the pediatric brain tumor medulloblastoma, potassium channels EAG2 (KCNH5) and KCNT2 are upregulated in several molecular subgroups (Huang et al. 2012, 2015).

In non-small cell lung cancer (Bonnet et al. 2007) and glioma (Preußat et al. 2003), potassium channel KCNA5 expression is inversely correlated with higher tumor grade. Low expression of KCNQ1 is associated with poor patient prognosis in gastrointestinal cancer (Than et al. 2014) and colon cancer (den Uil et al. 2016). Elevated expression of small conductance potassium channel KCNN4 is found in glioma and clear cell renal carcinoma. KCNN4 overexpression is associated with poor survival and increased metastatic potential, with *KCNN4* mRNA expression being further elevated in metastatic tumors compared to non-metastatic renal carcinomas (Turner et al. 2014; Rabjerg et al. 2015). In non-small cell lung cancer, promoter hypomethylation and increased *KCNN4* expression are associated with poor progression-free survival and overall survival (Bulk et al. 2015).

Store-operated calcium channel *ORAI1* is overexpressed in gastrointestinal stromal tumors and correlates with high-risk grading. Loss of ORAI1-mediated store-operated calcium suppresses tumor cell proliferation and induces apoptosis in vitro (Wang et al. 2017b). Mechanosensitive cation channel PIEZO1 is overexpressed in glioma, glioblastoma, breast cancer, and gastric cancer and is associated with poor prognosis (Li et al. 2015; Zhang et al. 2018; Chen et al. 2018). Transient receptor potential channel *TRPA1* is overexpressed in a variety of cancer types, including breast, kidney, lung, and malignant peripheral nerve sheath tumors. In breast and

lung cancer, *TRPA1* overexpression promotes oxidative stress tolerance and chemoresistance and is associated with worse patient survival (Takahashi et al. 2018). TRP channel TRPM3 is overexpressed in clear cell renal carcinoma relative to normal kidney. In particular, TRMP3 expression is elevated in VHL-mutant tumors relative to VHL-wild type tumors (Hall et al. 2014). The vanilloid receptor TRPV1 is highly expressed in high-grade astrocytomas compared to non-tumor brain, and *TRPV1* expression positively correlates with tumor grading (Stock et al. 2012). Elevated expression of TRPML1 is associated with a worse prognosis in melanoma (Kasitinon et al. 2019) and in HRAS-driven bladder and head and neck cancers (Jung et al. 2019). Increased expression of TRPML2 is found in glioma of higher pathological grades (Morelli et al. 2016).

Chloride intracellular channel CLIC1 is overexpressed in multiple brain cancer types, including medulloblastoma, glioma, ependymoma, atypical teratoid rhabdoid, and primitive neuroectodermal tumors (Francisco et al. 2020). Additionally, CLIC1 is overexpressed in glioblastoma, pancreatic, lung, and gallbladder cancer, and elevated CLIC1 expression correlates with worse overall survival (Wang et al. 2011; Setti et al. 2013; Ding et al. 2015; Lu et al. 2015; Jia et al. 2016). CLIC1 overexpression in gastric cancer correlates with increased metastasis, invasion, and poor prognosis (Chen et al. 2007; Li et al. 2018). Calcium-activated chloride channel TMEM16A is upregulated in over 75% of pancreatic cancers, and high TMEM16A expression is associated with poor patient survival (Crottès et al. 2019).

In a cohort of low-grade to high-grade gliomas, an 18-ion channel gene signature predicts survival. Downregulation of 16 out of 18 ion channel genes is associated with high-grade gliomas and shorter survival (Wang et al. 2015). Among these, high *KCNB1* and *KCNJ10* expression correlate with favorable prognosis, while high *CLIC1* and *CLIC4* expression correlate with worse survival. In a separate cohort, *KCNB1* expression inversely correlates with glioma prognosis, and *KCNB1* overexpression induces autophagy and reduces tumor cell growth (Wang et al. 2017a). In a comparison between glioblastoma stem cells and normal neural cell types, KCNB1 is among four GSC-enriched ion channels associated with survival. shRNA-mediated *KCNB1* knockdown reduces GSC viability in vitro (Pollak et al. 2017). Analysis of different patient cohorts may underlie these inconsistent findings, and further analysis is required to determine whether KCNB1 has oncogenic or tumor suppressive functions. Alternatively KCNB1 may play opposing roles in GSCs compared to more differentiated tumor cell types, a hypothesis that has not been definitively tested. Given the wide array of overexpressed ion channels, gene regulatory pathways may be leveraged as a cancer-specific vulnerability. For example, oncogenic mutations in chromatin remodeling factors may promote ion channel gene transcription and oncogenic transcription factors may bind regulatory elements of ion channel genes. Functional validation is crucial in determining whether ion channel overexpression is causal or correlative in cancer.

2.2 Structural Variations and Copy Number Alterations

Structural variations can lead to oncogene amplification, tumor suppressor deletion, or ectopic fusion genes with hypermorphic, hypomorphic, or neomorphic properties. *KCNK9* is overexpressed or amplified in breast, lung, ovarian, and colorectal cancers (Mu et al. 2003; Kim et al. 2004; Innamaa et al. 2013). Functionally, KCNK9 overexpression confers resistance to hypoxia and serum deprivation, resulting in enhanced growth of human breast cancer cells in vitro (Mu et al. 2003). *TRPA1* is amplified in a subset of breast cancers and malignant peripheral nerve sheath tumors (Takahashi et al. 2018). The impact of copy number alterations on ion channels in cancer has not been fully characterized. Integration of copy number and gene expression data should reveal additional cases of amplification or deletion-dependent ion channel dysregulation.

Rare *PIEZO1-RSPO2* fusions are present in traditional serrated adenomas (TSA) (Hashimoto et al. 2019), although the functional consequence remains unknown. The fusion spans exon 1 of *PIEZO1* and exon 3 of *RSPO2*, comprising only 21 N-terminal amino acids of PIEZO1 while retaining critical functional domains of RSPO2. The relatively minor contribution of PIEZO1 amino acids suggest that *PIEZO1* promoter, rather than ion conductance, contributes to the fusion product to promote *RSPO2*-dependent WNT activation. Colon cancers harbor other recurrent fusions comprising *RSPO* genes (Seshagiri et al. 2012), highlighting a convergence on aberrant RSPO function in tumorigenesis. Many oncogenic fusions involving receptor tyrosine kinases display constitutive kinase activity due to truncation of regulatory domains. In contrast, there has been a paucity of fusion products comprising ion channel genes in cancer. One possible reason may be that ion channel function requires stereotyped membrane topology, and truncations abolishing gating or pore-forming domains may not be beneficial to the cancer cell.

2.3 Mutations

A subset of endocrine tumors (aldosterone-producing adenomas) harbor recurrent somatic and germline mutations in *KCNJ5*, an inwardly rectifying potassium channel, and *CACNA1D*, a voltage-gated calcium channel. *KCNJ5* mutations occur near the potassium selectivity filter and reduce potassium while increasing sodium conductance (Choi et al. 2011). *CACNA1D* mutations reside in the S6 pore-lining segment, which increases calcium influx (Scholl et al. 2013). Glioblastomas harbor sodium, calcium, and potassium channel mutations associated with poor prognosis (Joshi et al. 2011).

Metastatic urothelial carcinomas contain somatic missense mutations in ion channel genes including *CACNA1S*, *KCNK9*, and *SCN8A* (Sharma et al. 2019). As this data comes from the case report of a single patient, the prevalence and functional consequence of these ion channel mutations is unknown. There exist few reports of

highly recurrent ion channel mutations spanning multiple cancer types, suggesting that ion channels may not simply be categorized as oncogenes or tumor suppressors. Rather, ion channel mutations in cancer are context and cell-type dependent.

2.4 Ion Channel Expression and Tumor Heterogeneity

To date, whether ion channel expression displays heterogeneity in cancer has been poorly explored. Expression heterogeneity can occur between patients (intertumoral), within different regions of the tumor (intratumoral), or change with disease progression (temporal). As an example of intertumoral heterogeneity, Group 4 medulloblastomas, which comprise one third of all medulloblastomas, display overexpression of potassium channel KCNA1. Immunohistochemical detection of KCNA1 is routinely used for molecular subgrouping (Northcott et al. 2011; Remke et al. 2011; Taylor et al. 2012), although KCNA1 function in medulloblastoma remains undetermined. Interestingly, integrated DNA methylation and gene expression analysis reveal enrichment of ion channel genes in a subset of SHH-activated medulloblastoma (Cavalli et al. 2017). In addition to being overexpressed in multiple tumor types, *TRPA1* is enriched in breast cancer with the exception of the normal-like subtype (Takahashi et al. 2018). Intratumor heterogeneity implies that distinct cells within the same tumor are dependent on different oncogenic events and that monotherapy will be insufficient. Likewise, temporal heterogeneity indicates that genetic alterations identified at diagnosis evolve as new mutations arise following treatment. Ion channel dysregulation in spatial or temporal tumor heterogeneity remains to be explored. We note that such studies are of great importance and will elucidate whether specific ion channels represent stable or dynamic therapeutic targets in cancer.

It is also important to note that genomic and proteomic analyses cannot entirely predict ionic events. Post-transcriptional and post-translational modifications regulate ion channel folding, stability, and trafficking to membrane domains. Various modes of stimulation govern the “on” or “off” state of ion channels. Furthermore, electrogenic proteins, including ion channels and transporters, work in concert to maintain ionic homeostasis and cellular resting membrane potential (Vmem), which can exert critical influence on cancer cell behavior (Yang and Brackenbury 2013; Payne et al. 2019). Therefore, it is crucial to consider how electrical signals propagate through tumors and holistically investigate ion channels at the genomic, proteomic, and physiological levels.

3 Ion Channel Functions in Tumorigenesis

3.1 Ion Channels in Tumor Initiation

A DNA transposon-based forward genetic screen implicates potassium channel *Kcnq1* and chloride channel *Cftr* loss-of-function in the initiation of murine gastrointestinal cancer (Starr et al. 2009). Intestinal-specific knockout of *Cftr* or global knockout of *Kcnq1* in the sensitized Apc^{min} mouse model of intestinal cancer increases tumor incidence (Than et al. 2014, 2016). These two channels are proposed to functionally interact in healthy intestinal epithelium, where Kcnq1-mediated basolateral potassium export provides the electrochemical drive for apical export of chloride by Cftr. Transcriptomic analysis of Kcnq1-or Cftr-deficient tumors displays enrichment for dysregulation of immune response and lipid metabolism. However, the mechanism by which loss of *Kcnq1* or *Cftr* initiates gastrointestinal cancer remains to be fully defined. Interestingly, *CFTR* mutations are causal for cystic fibrosis, and patients with cystic fibrosis have increased risk of digestive tract cancers (Neglia et al. 1995; Maisonneuve et al. 2013), providing relevance for the mouse data to human disease.

Somatic and germline gain-of-function mutations in calcium and potassium channel genes are suggested to initiate human endocrine tumors (Choi et al. 2011; Scholl et al. 2013). In approximately 40% of adrenal aldosterone-producing adenomas, recurrent mutations localize near the potassium selectivity filter of *KCNJ5*. Expression of mutant KCNJ5 in HEK293T and glomerulosa cells causes membrane depolarization attributable to reduced potassium selectivity and increased sodium conductance (Choi et al. 2011). Subsequent depolarization is proposed to activate voltage-gated calcium channels and increase intracellular calcium, thereby promoting aldosterone production and cell proliferation. A high-throughput screen in a HEK293 inducible expression system identified compounds which inhibit mutant KCNJ5. A series of clinically approved macrolides, bacteriostatic antibiotics with established safety profiles and oral bioavailability, suppresses mutant KCNJ5-dependent sodium conductance and normalizes aldosterone production in human adrenocortical cancer cells (Scholl et al. 2017). This study highlights the promise of drug repurposing to target ion channels in cancer.

Mutations in *CACNA1D*, which encodes a L-type voltage-gated calcium channel, occur in 11% of aldosterone-producing adenomas. All seven identified *CACNA1D* mutations affect conserved residues near the transmembrane S6 domain. Expression of mutant CACNA1D in HEK293 cells facilitates channel opening at less depolarized potentials. Through impaired inactivation, this leads to sustained channel activation and increased calcium influx. Thus, *CACNA1D* and *KCNJ5* mutations both lead to increased calcium influx to induce depolarization (Scholl et al. 2013). Given that *KCNJ5* and *CACNA1D* mutations are mutually exclusive, de novo germline mutations occur at the same locations as somatic mutations, and there is a lack of additional somatic mutations in these tumors, single ion channel mutations may be sufficient to initiate aldosterone-producing adenomas. Functional studies to

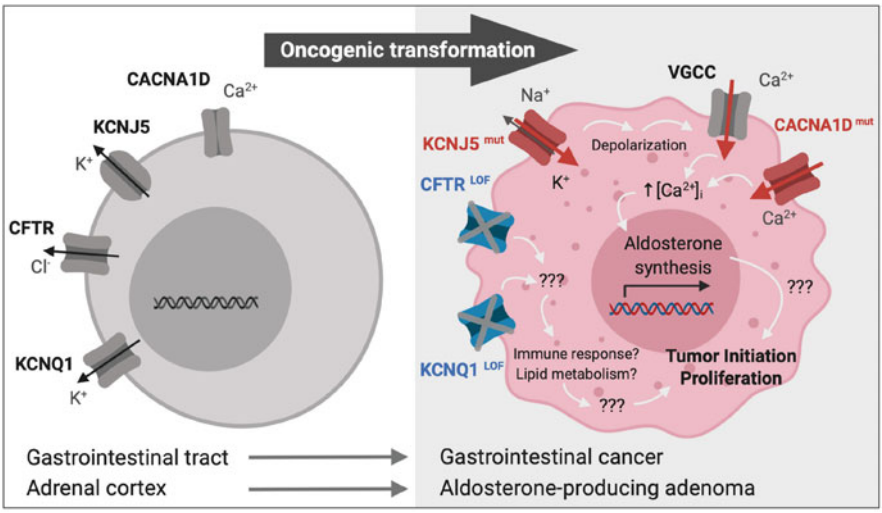

Fig. 1 Ion channels in tumor initiation. Dysregulated expression or function of several ion channels has been implicated in tumor initiation. Sequencing of human endocrine tumors identifies recurrent mutations in potassium channel *KCNJ5* and calcium channel *CACNA1D*. *KCNJ5* mutation promotes sodium over potassium conductance, depolarization, and activation of voltage-gated calcium channels. *CACNA1D* mutations impair channel inactivation and increase calcium influx. Mutations in these channels converge on increasing intracellular calcium and aldosterone production to promote cell proliferation. Through forward genetic screens, KCNQ1 and CFTR loss of function have been identified as tumor initiating events in mouse gastrointestinal cancers. Transcriptomic analysis of resulting tumors implicates perturbed immune response and lipid metabolism. The mechanism by which perturbed potassium and chloride homeostasis induce gastrointestinal cancers remains to be elucidated

induce these mutations in relevant cell types using genetically engineered animal models will fully define the role of these altered ion channels in tumor initiation. Overall, the small number of studies implicating ion channels in tumor initiation indicates that ion channels may be more often co-opted by cancer cells to regulate tumor progression and maintenance, following the oncogenic event that initiated the tumor (Fig. 1).

3.2 Ion Channels in Tumor Progression

As an evolving disease, cancer cells may no longer depend on initiating genes during tumor progression. The demonstration that EAG1 promotes tumor progression in subcutaneous xenograft models is among the first reports to implicate ion channels in tumor progression (Pardo et al. 1999).

3.2.1 Tumor-Promoting Ion Channels

In medulloblastoma, an ion channel network cooperates to regulate cell proliferation (Fig. 2). Voltage-gated potassium channel EAG2 (KCNH5) localizes to the plasma membrane during late G2 phase and mitosis, followed by KCNT2 (a potassium channel activated by sodium and chloride) enrichment to the plasma membrane during metaphase to telophase. Genetic knockdown of either potassium channel reduces human medulloblastoma cell growth in vitro and in mouse xenograft models (Huang et al. 2015). Specifically, EAG2 deficiency causes ectopic cell volume increase, activation of the p38 MAPK pathway, G2 arrest and mitotic catastrophe

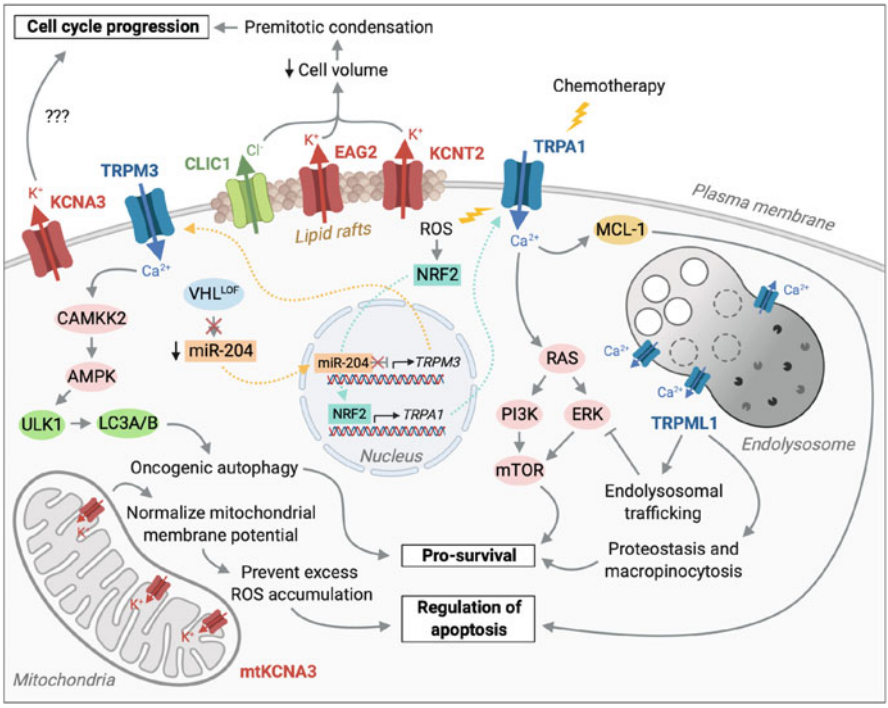

Fig. 2 Cell autonomous functions of ion channels in tumorigenesis. Ion channels in cancer display great diversity with regard to subcellular localization and intracellular signaling. Plasma membrane-localized ion channels CLIC1 and EAG2, both of which are present at lipid rafts, and KCNT2 function cooperatively. During late G2 and mitosis, chloride and potassium efflux reduces premitotic cell volume for cell cycle progression and tumor cell proliferation. Plasma membrane-localized TRP channels TRPM3 and TRPA1 engage autophagy and RAS/PI3K/mTOR signaling respectively to promote tumor cell survival. Potassium channel KCNA3 has localization-dependent functions in cancer. KCNA3 on the plasma membrane promotes cell cycle progression, and KCNA3 at the inner mitochondrial membrane regulates mitochondrial membrane potential and production of reactive oxidative species (ROS) to suppress apoptosis. Endolysosomal channel TRPML1 regulates proteostasis and macropinocytosis through ERK/mTOR signaling to promote tumor cell survival

(Huang et al. 2012). Screening of FDA-approved drugs identifies the antipsychotic thioridazine as an EAG2 channel inhibitor, which reduces tumor progression and prolongs survival in xenograft medulloblastoma mouse models. Furthermore, thioridazine displays efficacy in a human patient with relapsed metastatic medulloblastoma. Thioridazine treatment reduced tumor volume, providing the first proof-of-principle for using an ion channel blocker in treating brain tumor patients (Huang et al. 2015). Ultimately the patient did not tolerate prolonged treatment due to mood lability and depression, which is likely due to the inhibitory effect of thioridazine on dopaminergic and serotonergic receptors. These results demonstrate promise for clinical application of EAG2 inhibition in cancer and highlight the need to develop blockers with improved on-target specificity.

Chloride intracellular channel CLIC1 regulates cell volume homeostasis and cell cycle progression of rapidly dividing medulloblastoma cells (Fig. 2). CLIC1 colocalizes with EAG2 at lipid raft microdomains on the plasma membrane during mitosis. CLIC1 deficiency suppresses in vivo tumor growth in xenograft and genetic mouse models of medulloblastoma. EAG2, KCNT2, and CLIC1 mediate potassium and chloride efflux respectively, to synergistically regulate cell volume, premitotic cytoplasmic condensation, and cell proliferation. Loss of the orthologous clic and eag channels reduces brain tumor growth in *Drosophila* (Huang et al. 2015; Francisco et al. 2020). These results reveal an evolutionarily conserved role for CLIC1 and EAG2 in brain tumor growth and highlight functionally coupled ion channels as vulnerabilities in tumor progression.

Paralytic, which encodes the sole voltage-gated sodium channel in *Drosophila*, regulates the proliferative output of neuroblasts (*Drosophila* neural stem cells). Furthermore, loss of paralytic exerts tumor-suppressive effects in multiple *Drosophila* neuroblast-derived models of brain tumor (Piggott et al. 2019). Sodium channels with similar function in mammalian brain tumors remain unexplored.

Tissue stiffening frequently occurs during solid tumor progression. For example, glioma aggression and patient prognosis correlate with increasing tumor stiffness (Miroshnikova et al. 2016). Mechanosensitive cation channel PIEZO1 is overexpressed in human gliomas. PIEZO1 localizes at focal adhesions of glioblastoma stem cell processes, where its activation induces calcium influx and integrin-FAK signaling to promote extracellular matrix remodeling and tissue stiffening. The stiffer microenvironment elevates PIEZO1 expression to increase glioma cell proliferation. Therefore, PIEZO1 orchestrates a feedforward loop in which it promotes glioma stiffness and malignancy by sensing and responding to heightened tissue stiffness (Fig. 3). Targeting Piezo in *Drosophila* glioma models or PIEZO1 in glioblastoma xenograft mouse models suppresses tumor growth in vivo (Chen et al. 2018).

Potassium channel KCNA3 is expressed in several tumor types (Comes et al. 2013) and represents an actionable target to induce cancer cell death. In subcutaneous models of human lung adenocarcinoma and mouse melanoma, KCNA3 localizes to the plasma membrane and inner mitochondrial membrane, where it regulates cancer cell proliferation and apoptosis, respectively (Jang et al. 2011; Leanza et al. 2012) (Fig. 2). During lymphocyte apoptosis, apoptotic regulator BAX interacts

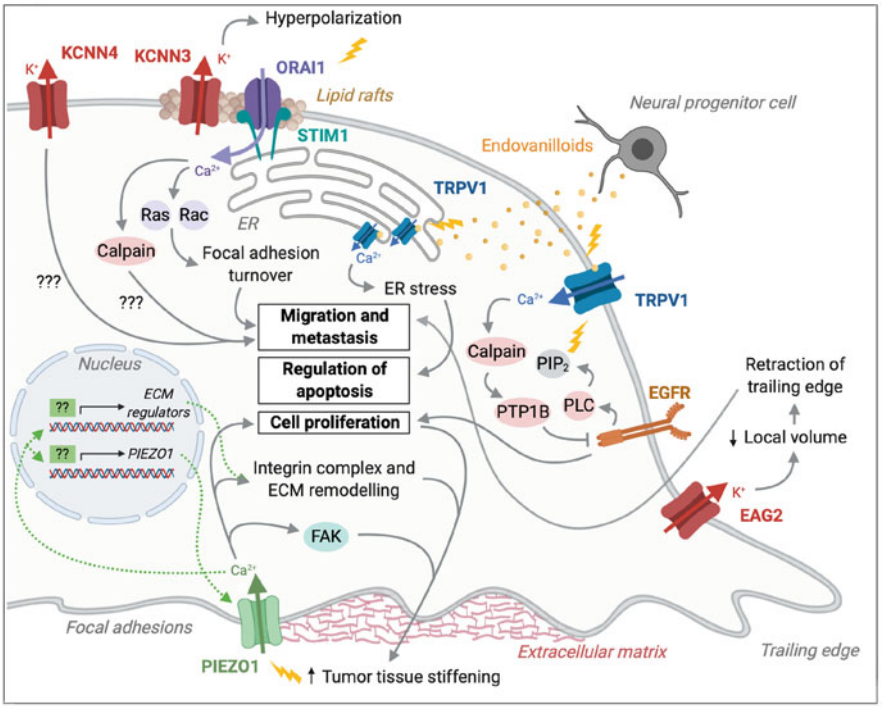

Fig. 3 Ion channel function in tumor cell invasiveness and non-autonomous interactions. Ion channels regulate cancer cell invasiveness and migration through distinct mechanisms. EAG2-mediated potassium efflux leads to local cell volume reduction and retraction of the trailing edge to facilitate cell migration. Potassium channel KCNN4 regulates cell invasiveness, while the mechanism has not been described. Several studies described store-operated calcium channel ORAI1 in cancer metastasis. ORAI1 activates in cooperation with co-localized KCNN3 and STIM1 at the endoplasmic reticulum (ER). ORAI1 activation and subsequent calcium influx enhances focal adhesion turnover. Human brain tumor cells express TRPV1, which can be activated by endovanilloids secreted by neural progenitor cells which home to the tumor. TRPV1 activation induces ER stress and tumor cell apoptosis. In gastric cancer models, TRPV1 activation inhibits EGFR-mediated cell proliferation. By localizing to glioma cell focal adhesions, PIEZO1 senses tumor stiffness to increases its own expression, cell proliferation, and integrin and extracellular matrix (ECM) remodeling to increase tumor stiffness and malignancy

with and inhibits mitochondrial KCNA3, causing mitochondrial membrane hyperpolarization, production of reactive oxidative species, and cytochrome C release (Szabo et al. 2008). Small molecule KCNA3 inhibitors, such as PAP-1, exhibit 23- to 125-fold selectivity over related Kv1 family members (Schmitz et al. 2005). Recently developed synthetic PAP-1 derivatives display increased specificity toward mitochondrial KCNA3 (Leanza et al. 2017). Administration of PAP-1 derivatives elicits tumor-specific apoptosis in leukemic, melanoma, and pancreatic cancer cells in vitro and in orthotopic xenograft models. Importantly, while KCNA3 is expressed

in many organs, its inhibition does not seem to induce apoptosis in non-tumoral organs, including the brain, heart, liver, spleen, and kidney (Leanza et al. 2012, 2013, 2017). Interestingly, siRNA-mediated KCNA3 knockdown fails to induce cancer cell apoptosis (Leanza et al. 2012), highlighting a distinction between pharmacological versus genetic perturbation. In the latter case, other genes regulating ion homeostasis and mitochondrial membrane potential may compensate for KCNA3 deficiency. While the effects of KCNA3 inhibition on normal physiology besides inducing cell death require further examination, these findings highlight KCNA3 as a tumor-specific target.

As reactive oxidative species (ROS) readily accumulate in cancer cells, adaptation to oxidative stress is crucial for tumor progression. ROS signals through oxidant defense factor NRF2 to mediate an anti-oxidant defense program and induce expression of the redox-sensitive cation channel TRPA1. ROS elevation induces TRPA1-mediated calcium influx and upregulates RAS-ERK, PI3K/AKT, and mTOR-dependent pro-survival cues, as well as MCL-1-mediated anti-apoptotic signaling (Fig. 2). TRPA1 knockdown reduces tumor growth in patient-derived xenograft (PDX) breast cancer models. TRPA1 inhibition using AM-0902, an orally bioactive TRPA1 inhibitor, reduces PDX tumor growth in vivo, although the short plasma half-life of AM-0902 may pose challenges for bioavailability (Takahashi et al. 2018). As TRPA1 inhibitors are in clinical trials for pain and respiratory diseases, this study demonstrates the clinical prospect of TRPA1-based therapy in breast cancer.

Endolysosomal cation channel TRPML1 represents a critical dependency in human melanoma cells but not normal melanocytes. TRPML1 loss decreases melanoma cell growth in vitro and in xenograft models through elevating MAPK and mTORC1 activity, ultimately impairing macropinocytosis and inducing proteotoxic stress (Kasitinon et al. 2019). TRPML1 function in tumorigenesis may differ depending on cancer type. For example, TRPML1 inhibition has opposing effects on elevating and attenuating ERK signaling in melanoma cells (Kasitinon et al. 2019) and HRAS-driven human carcinoma cells (Jung et al. 2019), respectively. TRPML1 in HRAS-driven cancers mediates cholesterol distribution, HRAS nanoclustering, and ERK phosphorylation to maintain signaling and proliferation (Jung et al. 2019). These studies suggest a role for TRPML1 in endolysosomal fusion to create a permissive environment for oncogenic signaling. The exact mechanism by which TRPML1 activity in tumor cells regulates cholesterol transport and endolysosomes remains to be defined (Fig. 3). TRPML1 mutations underlie mucolipidosis-type IV, a neurodegenerative lysosomal storage disorder (Bargal et al. 2000; Sun et al. 2000). TRPML1 activation is neuroprotective (Tsunemi et al. 2019), and Trpml1 knockout female mice exhibit luteal cell degeneration, progesterone deficiency, and infertility (Wang et al. 2019). Thus, the consequences of TRPML1 loss in physiology should also be considered in developing TRPML1-based cancer therapies.

Inactivation of the tumor suppressor VHL is common in clear cell renal cell carcinoma (ccRCC) (The Cancer Genome Atlas Research Network 2013; Sato et al. 2013). In VHL-deficient ccRCCs, TRP channel TRPM3 regulates an oncogenic

autophagy network. VHL regulates expression of tumor suppressive microRNA miR-204, derived from intron 6 of the *TRPM3* gene. miR-204 targets and inhibits TRPM3, as well as autophagy regulators LC3B and LC3B2. VHL inactivation and miR-204 downregulation elevate autophagy to sustain tumor growth (Mikhaylova et al. 2012). TRPM3 activation and calcium influx regulate autophagy through CAMKK2 and ULK1 signaling, which converge to positively modulate autophagic regulators LC3A/B (Fig. 2). Genetic knockdown or pharmacological inhibition of TRPM3 reduces tumor growth in orthotopic xenograft models (Hall et al. 2014). Mefenamic acid is an orally bioavailable, FDA-approved, non-steroidal anti-inflammatory drug (NSAID) that specifically inhibits TRPM3 over other TRP channels (Klose et al. 2011). Mefenamic acid reduces TRPM3 expression and autophagy in VHL-inactive human cancer cells in vitro and decreases tumor growth in subcutaneous xenograft models (Hall et al. 2014). These findings demonstrate the potential to target TRPM3 in cancers with VHL deficiency and increased TRPM3 expression. It remains to be determined whether anti-tumor effects of mefenamic acid, which also inhibits cyclooxygenase (COX) enzymes to suppress prostaglandin synthesis, may in part be attributed to a COX-dependent mechanism. Interestingly, upon VHL induction, miR-204 is co-expressed with two short but not full-length *TRPM3* transcripts (Mikhaylova et al. 2012). This may be due to a putative promoter upstream of the first exon of shorter transcripts. The contribution of short and full-length *TRPM3* transcripts to ccRCC tumor progression and its upstream regulatory machinery is intriguing. For example, do short *TRPM3* transcripts encode functional proteins with a role in tumorigenesis, or are they byproducts of miR-204 activation? Concomitant microRNA expression with shorter gene transcripts may be a general mechanism for intronic miRNAs from long genes. Many ion channels are encoded by long genes spanning multiple exons. The existence and function of microRNAs and alternative isoforms from ion channel genes, particularly in cancer, represent an interesting area for future study.

3.2.2 Tumor Suppressive Ion Channels

Neural progenitor cells possess tumor-suppressive function against high-grade astrocytomas (Stock et al. 2012). Neural progenitors display tropism for brain tumors and release endovanilloids, which act non-cell autonomously on TRPV1 channels expressed in human brain tumor cells to induce cell death via the endoplasmic reticulum stress pathway (Fig. 3). This raises the possibility that neural stem cell therapy or TRPV1 activation may be used to treat brain tumors. As proof of concept, the authors demonstrate that administering the blood-brain barrier permeable synthetic TRPV1 agonist arvanil prolongs survival of xenograft tumor-bearing mice. Interestingly, TRPV1 activation inhibits EGFR-mediated epithelial cell proliferation through calcium and PTP1B signaling, and loss of TRPV1 increases tumor formation in the Apc^{min} mouse model of intestinal tumorigenesis, suggesting that TRPV1 activation is also tumor suppressive in intestinal cancer (de Jong et al. 2014).

Given the evidence for ion channel involvement in both promoting and inhibiting tumor growth, most ion channels appear to act as neither oncogenes nor tumor suppressors. Rather, ion channel activity must remain within a proper window to facilitate tumorigenesis. Supra-physiological gain- or loss-of-function is likely detrimental to tumor growth.

3.3 Ion Channels in Metastasis

Tumor metastasis comprises a sequence of events that include dissemination of tumor cells from the primary site, survival in the circulatory system, and invasion and colonization of distant locations for neoplastic growth. Most reported functions of ion channels in tumor metastasis are pertinent to cell migration.

In gliomas, genetic knockdown or pharmacological inhibition of calcium-activated potassium channel KCNN4 reduces tumor cell migration in vitro. KCNN4 knockdown in glioma xenografts reduces invasive growth in vivo (Turner et al. 2014). Therefore, KCNN4 may contribute to the diffuse nature of malignant gliomas (Fig. 3).

Expression of potassium channel EAG2 is upregulated in a subset of metastatic medulloblastoma compared to matched primary tumors. EAG2 localizes to the trailing edge of medulloblastoma cells to promote local potassium efflux and rear cell retraction, which are essential for medulloblastoma cell motility (Fig. 3). Targeting EAG2 reduces medulloblastoma metastasis in mouse xenograft models and decreases metastatic burden in a patient with metastatic medulloblastoma (Huang et al. 2015).

STIM and Orai proteins mediate store-operated calcium entry, a calcium influx mechanism in non-excitable cells. Second messenger signaling induces rapid and transient release of calcium from the endoplasmic reticulum (ER). Decreased ER calcium concentration is sensed by STIM proteins, which cluster near the plasma membrane to complex with the pore-forming Orai for calcium entry. In human breast cancer cells, calcium influx through Orai1 and STIM1 promotes cell migration in vitro through small GTPase-mediated focal adhesion turnover. Store-operated calcium influx may increase FAK tyrosine kinase activity and calcium-dependent protease calpain to regulate focal adhesion dynamics (Fig. 3). Administration of SKF96365, an inhibitor of store-operated calcium entry, reduces breast cancer metastasis from mammary gland to lung in xenograft mouse models (Yang et al. 2009). As SKF96365 also blocks TRPC and low-voltage-activated T-type calcium channels (Singh et al. 2010), additional studies are needed to determine whether the phenotypes can be solely attributed to Orai1. Since genetic or pharmacological manipulations of Orai1/STIM1 also perturbed focal adhesions in mouse embryonic fibroblasts (Yang et al. 2009), future investigation is necessary to determine the full spectrum of phenotypes from pharmacologically targeting this mode of calcium entry.

Orai colocalizes and functionally associates with calcium-activated potassium channel KCNN3 in cancer. In an orthotopic breast cancer xenograft model, KCNN3 knockdown reduces bone metastases without affecting primary tumor growth or lung metastases. KCNN3-Orai1 complexes localize to lipid rafts to regulate calcium entry, calpain activation, and cell migration (Fig. 3). Disruption of KCNN3-Orai1 lipid localization using alkyl-lipid KCNN3 blocker Ohmline impairs calcium influx, migration, and bone metastases (Chantome et al. 2013). Ohmline does not cause systemic toxicity in vivo (Girault et al. 2011). These results suggest that ion channel function depends on precise subcellular localization, which can be leveraged for cancer treatment.

As tumor metastasis involves tumor cell dissemination, intravasation, survival through systemic circulation, extravasation, colonization at a distant site, and eventual metastatic growth, future studies are required to specify which exact steps depend on ion channel function. In addition, it is important to determine whether a particular ion channel is uniquely required for metastasis. If targeting an ion channel reduces the growth of primary tumor and metastasis, it becomes difficult to ascertain whether impaired metastasis is due to a reduction in primary tumor burden or a defect in the metastatic cascade.

3.4 Ion Channels in Therapeutic Resistance and Tumor Recurrence

As described above, TRPA1 overexpression promotes tumor cell survival in environments of high oxidative stress. The anti-cancer agent carboplatin, which generates reactive chemicals to cause cell death, induces TRPA1-dependent increases in intracellular calcium concentration and oscillatory calcium responses. In carboplatin-resistant breast cancer cells, TRPA1 inhibition restores carboplatin sensitivity in vitro and in vivo, highlighting the therapeutic potential of TRPA1-based combination therapy (Takahashi et al. 2018). The contribution of ion channels toward tumor recurrence and resistance has not been sufficiently studied in vivo. Some malignancies, such as glioblastoma, recur in nearly all patients despite first-line treatment (Nabors et al. 2017). Future study to target ion channels in both primary and recurrent tumors will provide the foundation to develop new therapies to improve patient outcome.

3.5 Therapeutic Potential of Targeting Ion Channels

As small molecules and FDA-approved drugs have been used to target ion channels in cancer, we note that stringent criteria must be upheld in defining on-target versus off-target effects. Pharmacodynamic and pharmacokinetic data, such as the

maximum tolerated dose, minimum effective concentration, bioavailability, half-life, and systemic toxicity, must be evaluated. Finally, the IC_{50}/EC_{50} concentrations at which a molecule acts on ion channels should be consistent with the achievable therapeutic concentrations in vivo.

4 Emergent Areas of Ion Channels in Cancer and Outlook

4.1 Ion Channels in Governing Local Ion Milieu

Most studies in the field focus on how ion channels regulate cancer biology through cell autonomous mechanisms. Intratumoral heterogeneity and cell non-autonomous mechanisms are crucial in fueling malignant growth. For example, reciprocal signaling between stem-like and differentiated tumor cells through growth factor secretion creates a tumor ecosystem to drive glioma progression (Wang et al. 2018). Metabolic adaptation of tumor cells, which reside in either hypoxic or oxygenated niches, induces distinct gene expression programs (Allen et al. 2016; Jin et al. 2017). In this regard, one can imagine that tumor heterogeneity in the local ionic milieu and changes in extracellular ion dynamics may exert cell non-autonomous effects (Fig. 4). Indeed, in mouse and human melanomas, dying tumor cells release intracellular potassium into the extracellular environment. Elevated extracellular

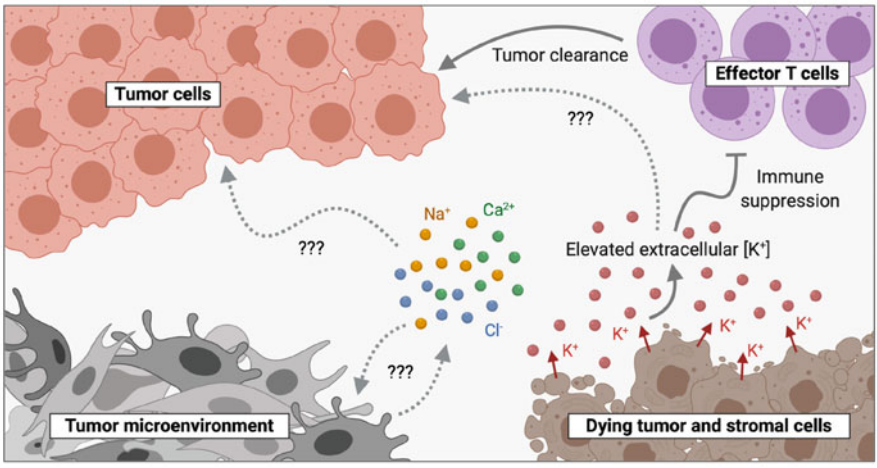

Fig. 4 Ion channels govern local ion milieu and tumor-immune cell interaction. The contribution of ion channels and the extracellular ionic environment to cell non-autonomous interactions in cancer is largely under-explored. In melanoma, tumor cell death releases potassium ions into the extracellular space. Elevation of extracellular potassium suppresses anti-tumor functions of resident T cells through inhibition of Akt/mTOR signaling, dysregulated metabolism, and altered histone acetylation. Whether perturbing the homeostasis of other ion classes has effects in the tumor microenvironment remains to be determined. *Dotted arrows denote hypothetical functions*

potassium suppresses the anti-tumor function of T cells through inhibiting Akt/mTOR and PP2A signaling (Eil et al. 2016). In addition, elevated extracellular potassium limits nutrient uptake and reduces the level of metabolic intermediate acetyl-CoA. Decreased acetyl-CoA suppresses histone acetylation of T-cell effector and exhaustion loci to attenuate their anti-tumor activity. These altered T cells display increased stemness and improved persistence in vivo (Vodnala et al. 2019). These data suggest that manipulation of extracellular potassium levels is a strategy to modulate T-cell-based immunotherapy. In addition to melanoma, it would be of interest to determine the effect of extracellular potassium on infiltrated T cells in other types of tumors and normal organs.

Altered extracellular ion concentrations may shift the membrane potential of cancer cells, activate voltage-gated ion channels, and induce bioelectrical signaling (Tuszynski et al. 2017; Payne et al. 2019). Early studies in chick spinal cord neurons implicate membrane potential in cell cycle progression (Cone and Cone 1976). A correlation exists between membrane potential and differentiation status, where more proliferative cells possess depolarized membrane potentials, while terminally differentiated non-dividing cells reside at more hyperpolarized potentials (Cone 1971). In the developing mouse brain, the membrane potential of neural progenitor cells becomes progressively hyperpolarized during corticogenesis. Inducing hyperpolarization in vivo shifts the neurogenic output and transcriptome of progenitor cells toward a later developmental stage (Vitali et al. 2018). Thus, tumor-specific variation in extracellular ionic balance may explain why certain cancers are more malignant than others (Fig. 4). Future study of the functional impact of local ion milieu in cancer may uncover new ion channel targets to modulate tumor growth through cell-autonomous and cell non-autonomous mechanisms.

4.2 Ion Channels in Regulating Cell-Cell Interactions

Studies from diverse cancer types show that the nervous system is capable of integrating with cancer cells to form an electrical network (Fig. 5). In the central nervous system (CNS), neuronal activity promotes the growth of primary brain tumors and brain metastasis. In orthotopic PDX models, synapses form between neurons and glioma cells (Venkataramani et al. 2019; Venkatesh et al. 2019). AMPA receptor-mediated postsynaptic activity induces glioma cell depolarization, proliferation, and invasion. Interestingly, neuronal activity induces inward potassium currents in glioma (Venkatesh et al. 2019), pointing toward potassium channels as mediators of this process.

In mouse PDX intracardiac injection models of breast-to-brain metastasis, neoplastic cells form functional neuronal synapses, which promote metastatic colonization in an NMDA signaling-dependent manner. In human brain metastases, components of NMDA receptor signaling are elevated when compared to paired primary breast tumors (Zeng et al. 2019). Outside of brain malignancies, innervation of peripheral nerves promotes tumorigenesis in PDX and transgenic mouse models

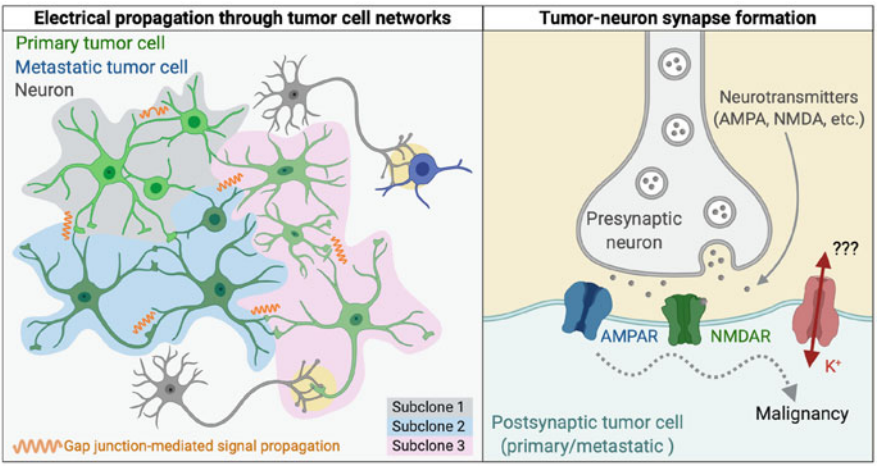

Fig. 5 Ion channels mediate tumor-neuron synapses and propagate electrical signaling. In a network of interconnected tumor cells, electrical activity can occur through gap junction or ion channel-mediated signaling. Propagation of electrical signals mediates interactions between different subclones and buffers tumor cells against therapeutic stress. Surrounding neurons are capable of forming synapses with primary tumor or metastatic tumor cells (*left*). Tumor-neuron synaptic transmission activates neurotransmitter signaling and induces potassium currents and depolarization. Synaptic activity increases tumor proliferation and metastasis (*right*). *Dotted arrows denote hypothetical functions*

of gastric, prostate, and pancreatic cancer in a paracrine manner (Magnon et al. 2013; Hayakawa et al. 2017; Renz et al. 2018). It would be interesting to determine whether such interactions depend on ion channel-mediated signal propagation to proliferate and metastasize.

Bioelectric signaling in cancer is not a single-cell phenomenon. Altered electrical activity in cancer can propagate through an interconnected network. Gap junctions are intercellular channels formed by connexin proteins. Gap junctions between adjacent cells enable rapid cellular communication through passage of ions, second messengers, and other small molecules. PDX models demonstrate that glioma cells form networks through gap junction coupling (Osswald et al. 2015; Venkataramani et al. 2019; Venkatesh et al. 2019). As gap junctions possess distinct ion permeability, differential gap junction expression determines differences in membrane potential and cancer stem cell self-renewal (Hitomi et al. 2015). Such connectivity can buffer interconnected glioma cells against therapeutic stress (Osswald et al. 2015). Furthermore, rapid propagation of electrical state may enable cooperativity among different tumor subclones. We refer readers to excellent reviews on gap junctions in cancer (Aasen et al. 2016; Sinyuk et al. 2018) and the nascent field of cancer neuroscience (Monje et al. 2020).

In summary, ion channel-mediated electrical activities of tumor-tumor interactions and tumor-neuronal synapses should be considered in order to gain a comprehensive view of the dynamic tumor microenvironment. Pharmacologically

manipulating ion channel dependencies may impede malignant growth through impairing tumor electrical activity.

4.3 *Ion Channels in Sensing and Responding to Mechanical Environment*

The mechanical properties of tumors are altered during tumorigenesis (Kumar and Weaver 2009). Pervasive mechanical stress arises from expansion of the tumor mass in confined spaces, infiltration by stromal and immune cells, and increased fluidic components from leaky blood and lymphatic vessels (Northey et al. 2017; Northcott et al. 2018). As solid stress, shear stress, and interstitial fluid pressure co-exist in the tumor, investigating how tumor cells perceive and respond to mechanical microenvironment through mechanosensitive ion channels will uncover new therapeutic opportunities (Fig. 6).

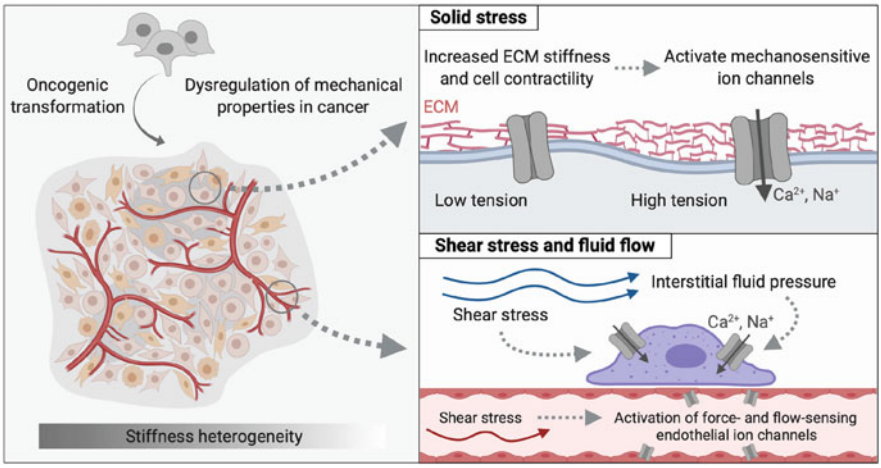

Fig. 6 Mechanosensitive ion channels sense tissue mechanics and regulate tumor malignancy. Aberrant tissue mechanics is a common feature of solid tumors. Tumor stiffness can be sensed by mechanosensitive ion channels. Distinct tumor regions are mechanically heterogeneous (*left*). In a region of high mechanical stress, mechanosensitive ion channels activate intracellular signaling to drive cell proliferation (*top right*). In regions with elevated interstitial fluid pressure or shear stress, flow-sensitive ion channels expressed in tumor cells or tumor vasculature may be activated to promote tumor growth and microenvironmental remodeling (*bottom right*). *Dotted arrows denote hypothetical functions*

4.3.1 Solid Stress

Solid stress arises from expansion of the tumor mass and non-fluid tumor components which compress and distend cells and tissues (Jain et al. 2014). Through mechanoreciprocity, solid stress may propagate to the surrounding stromal tissue to increase extracellular matrix (ECM) tension and induce changes to ECM material properties to drive tumor growth (Butcher et al. 2009). In glioma and pancreatic cancer, increased ECM stiffening and elevated epithelial tension are associated with increased malignancy and shorter patient survival. In both types of tumors, elevated tenascin C and STAT3-mediated mechanosignaling form positive feedback loops by further increasing ECM stiffness and tumor aggression (Laklai et al. 2016; Miroshnikova et al. 2016). Recurrent tumors display further stiffening compared to the primary tumors (Miroshnikova et al. 2016). Mechanical heterogeneity in distinct tumor regions may contribute to the growth of heterogeneous tumor cell populations. Mechanosensitive ion channels Piezo and PIEZO1 are evolutionarily conserved regulators of tumor growth in *Drosophila* and xenograft mouse models of glioma, respectively. Glioma cells perceive microenvironmental stiffness through PIEZO1. PIEZO1 signaling elevates integrin and FAK signaling to increase ECM production, tumor proliferation, and its own expression, forming a feedforward circuit to promote tumor malignancy (Chen et al. 2018).

4.3.2 Shear Stress and Fluid Flow

Dysregulated tumor mechanics impair blood vessel integrity and reduce lymphatic drainage, which lead to increased shear stress and perturbed fluid flow. Decreased circulation can hinder nutrient distribution, drug delivery, and therapeutic response (Tong et al. 2004; Winkler et al. 2004). Fluid mechanics may activate flow-sensitive ion channels, although this notion is yet to be fully elucidated in cancer. In adult mouse neural stem cells of the subependymal zone, flow-sensitive sodium channel ENaC senses ventricular fluid flow to promote proliferation and neurogenic output through Na^+ and Ca^{2+} signaling, activation of calcium-release activated channels, and ERK signaling (Petrik et al. 2018). ENaC knockdown reduces glioma cell migration in vitro (Kapoor et al. 2009), while its in vivo function in cancer remains to be explored. Tumor angiogenesis, a hallmark of cancer, is the ability for cancers to generate or recruit vasculature to provide oxygen and nutrients to the tumor (Hanahan and Weinberg 2000, 2011). Ion channels have been described in endothelial cells, including the shear stress-activated ENaC (Wang et al. 2009; Guo et al. 2016), and mechanosensitive ion channels such as TRPV4 and PIEZOs (Gerhold and Schwartz 2016). Whether ion channel function in tumor vasculature can be therapeutically exploited remains to be investigated.

In summary, ion channels are expressed in a tissue- or cancer-specific manner that can create actionable therapeutic windows (Table 1). The challenges of cancer plasticity and heterogeneity must be considered when investigating context-

dependent ion channel functions. It is important to investigate ion channel function using in vivo model systems that recapitulate physiological features of cancer. Defining the particular stage of tumorigenesis that an ion channel regulates and elucidating its mechanism of action should lead to improved design of targeted therapies. Future studies of non-cell autonomous ion channel functions will uncover how electrical signals propagate through a network of cancer and stromal cells and how ion channels perceive mechanical cues to control tumor malignancy. As this field makes strides forward, we look forward to unlocking the potential to target ion channels in cancer.

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