Chapter

Ion Channels and Transporters as Cancer Biomarkers and Targets for Diagnostics with Antibodies

Jessica Iorio, Claudia Duranti and Elena Lastraioli

Abstract

Cancer is a highly heterogeneous disease in terms of both response to therapy and prognosis. The introduction of molecular tools and antibodies had a great impact on cancer management in recent years for both cancer diagnosis and therapy. Ion channels and transporters (ICT) are membrane proteins aberrantly expressed in several human cancers. ICT can now represent potential cancer biomarkers as well as targets for therapeutic and diagnostic purposes. In particular, we will discuss about the potential role of ICTs as biomarkers for solid cancers (evaluated either by immunohistochemistry or molecular biology techniques) and the potential use of antibodies for diagnosis.

Keywords: ion channels, antibodies, biomarkers, cancer, diagnosis

1. Introduction

Ion channels and transporters (ICTs) are emerging as potential cancer biomarkers. Indeed, ICTs are aberrantly expressed in several types of human cancers, and exert a relevant role in mediating interactions between tumor cells and tumor microenvironment. Such interactions drive different functions which in turn regulate neoplastic progression, such as cell proliferation and survival, cell invasiveness and pro-angiogenetic programs [1–3]. Moreover, due to their prevalent expression at the cell surface, ICTs represent good targets for antibodies, to be exploited for diagnostic purposes. Finally, being highly druggable molecules, ICTs may represent novel molecular targets for antineoplastic therapy [4, 5].

The expression and role of different ion channels in tumor cells and their different contribution to tumor progression has been thoroughly described elsewhere [6]. In this chapter, we will focus on the possibility of exploiting ICTs as cancer biomarkers, for diagnostic, prognostic or predictive purposes. Some examples, relative to either solid cancers or hematologic malignancies are provided. We will analyze the possibility of using ICT-targeting antibodies for either *in vitro* or *in vivo* cancer diagnosis.

2. Cancer diagnosis: a focus on antibody-based techniques

The technologies available to help physicians to detect and diagnose cancer has changed dramatically in recent years. In particular, the use of biomarkers has

greatly improved diagnosis through their application for either *in vitro* diagnosis (on tumor specimens or in blood samples) or *in vivo* molecular imaging. According to the National Cancer Institute (NCI) definition (NCI Dictionary of Cancer Terms, http://www.cancer.gov/dictionary?cdrid=46636), a biomarker may be used either to help diagnosis, for example, to identify early stage cancers (Diagnostic) or to forecast how aggressive a condition is (Prognostic), or to predict how well a patient will respond to a define treatment (Predictive).

For the purposes of this chapter, we will briefly summarize the main techniques, either *in vitro* or *in vivo*, which take advantage of the use of biomarkers to obtain diagnostic, prognostic and predictive data on the cancer under study. Notably, most, although not all, of these techniques are based on the use of antibodies, targeting specific cancer-related biomarkers.

2.1 *In vitro* cancer diagnosis

2.1.1 Immunohistochemistry (IHC)

IHC represents an indispensable diagnostic tool to assess the presence or absence, as well as the amount, of a specific molecular tumor marker in a tissue. After appropriate assessment of categorical scoring system and proper validation of the immunohistochemical assay, a given marker can be proposed as a potential diagnostic or prognostic factor. Indeed, many of the cancer biomarkers routinely used in cancer diagnostics are based on this technique.

2.1.2 Flow cytometry (FC)

Using a multiparametric approach, FC immunophenotyping plays an indispensable role in the diagnosis and subclassification of leukemias, as well as for minimal residual disease detection. FC, in fact, provides a rapid and detailed determination of antigen expression profiles; these information along with morphologic assessment, allow to diagnose a particular type of leukemia and/or help in distinguishing from other subtypes. Also, the identification of specific antigens has prognostic and therapeutic relevance in acute leukemias. Moreover, FC immunophenotyping is useful to monitor response to therapy, recurrence and minimal residual disease.

While IHC and FC represent the standard of care in solid cancers and hematologic malignancies, respectively, some remarkable technological breakthroughs of the last 10 years have greatly contributed to improve cancer diagnostics through either the definition of "Omics profile" or the assessment of plasma-based cancer biomarkers:

2.1.3 Omics profiles

The study of tumor genomes using high throughput profiling strategies including (but not limited to) DNA copy number, DNA methylation, and transcriptome and whole-genome sequencing—technologies that may collectively be defined as "omics"—has led to identifying genes and pathways deregulated in cancer, hence revealing those that may be useful for the detection and management of disease. In the near future, such discoveries will lead to the discovery of novel diagnostic, prognostic and predictive markers that will ultimately improve patient outcomes.

2.2 *In vivo* cancer diagnosis: molecular imaging

Besides *ex vivo* procedures (either on surgical/bioptic samples or blood), cancer *diagnosis* is mainly based on imaging procedures, such as *computed tomography,*

magnetic resonance imaging and positron emission tomography. The advent of molecular imaging techniques has progressively allowed more accurate *in vivo* visualization of cancer, based on specific biological and pathological processes. Antibody-based imaging is of great utility since the combination of tumor specificity and different imaging methodologies might improve cancer diagnosis, monitoring and follow up [7–11]. The diagnostic imaging approaches currently used in cancer has been improved by the application of antibodies, thanks to the accuracy that allows antibodies to precisely identifying their targets. Some practical examples of mAbs recognizing cancer-specific biomarkers that are approved by the FDA and/or EMA and are currently used in the clinical setting have been described elsewhere [12]. Monoclonal antibodies (mAbs) have several features (big size, slow pharmacokinetics and blood clearance, not complete penetration and accumulation in tumor tissue) that can delay the time point for imaging. A different class of antibodies (single chain Fragment variable, scFv) might be useful to overcome such limitations and due to the possibility of conjugating the recombinant proteins with fluorescent dyes, scFv antibodies have been proposed for use in imaging applications, especially for cancer diagnostics [8, 11, 13].

3. Ion channels and transporters with clinical relevance in solid cancer

An overview of the main ion channels and transporters expressed in different solid tumors is reported in **Figure 1**.

3.1 Potassium channels

K^+ channels are the class of ion channels mostly de-regulated in cancers. Among them, ***KCa 1.1*** channels (also known as BK channels, encoded by the *KCNMA1* gene) have shown a clinical relevance in breast (BC) and prostate cancer (PCa). In both tumor types, BK overexpression can be traced back to the amplification of the *KCNMA1* gene located in 10q22: in BC, the amplification is restricted to invasive ductal tumors, and is associated with high stage, high grade and unfavorable prognosis [14]. In BC, KCa 1.1 positively correlates with the expression of estrogen

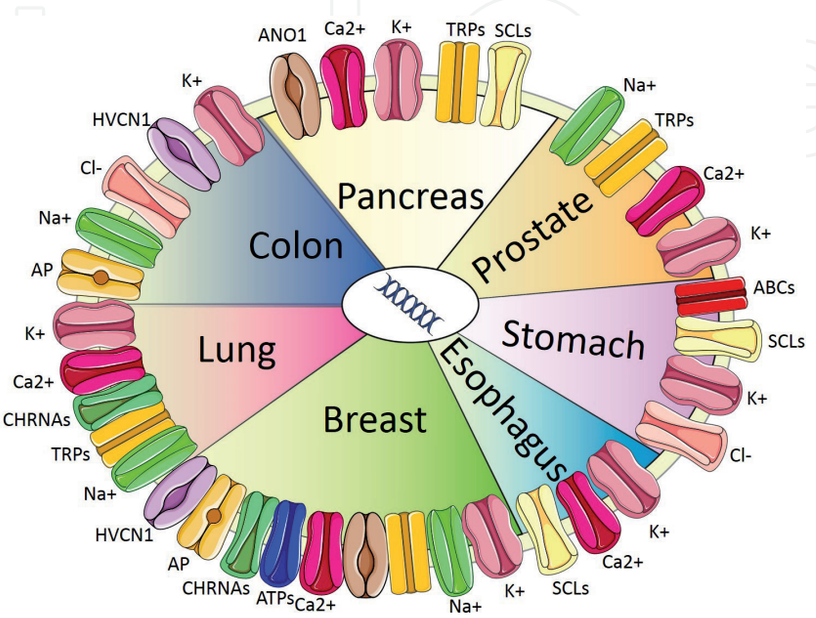

Figure 1.
Schematic representation of the main ICTs expressed in solid tumors.

receptors [15] and their levels are higher in BC metastatizing to brain [16]. In PCa, the *KCNMA1* gene is frequently amplified in late-stage tumors [17] and can be considered a potential biomarker [18]. Another Ca^{2+}-dependent K^+ channel often overexpressed in human cancers is ***KCa3.1*** (encoded by the *KCNN4* gene). $K_{Ca}3.1$ channels are upregulated in BC, especially in high grade tumors [19], in pancreatic cancer (pancreatic ductal adenocarcinoma, PDAC) [20], in colorectal cancer (CRC) [21] as well as in small cell lung cancer (SCLC) [22]. While the clinical relevance of $K_{Ca}3.1$ was hypothesized in CRC [23], although not validated [24], *KCNN4* hypomethylation turned out to be a negative prognostic factor in SCLC [22]. Kv channels are voltage-dependent K+ channels whose expression is often increased in cancer tissues [25]. For example, the expression of **Kv 1.3** (*KCNA3*), markedly increased in PCa in samples with Gleason score of 5–6 (GS5–6), but significantly decreased in the GS8–9 group. This malignancy grade-dependent K^+-channel expression pattern may provide a convenient marker to understand PCa progression level [26]. In PCa, Kv1.3 is mainly expressed in early stages of progression and down-regulated in high grade cancers [27]. Kv1.3 expression is lower in cancer compared with healthy pancreas. Kv1.3 downregulation could be traced back to promoter's methylation and was associated with the presence of metastases [28]. ***K2P9.1*** (KCNK9) belongs to the K2P family and genomic amplification of the gene was shown in a small fraction of BC [29]. **K2P5.1** (KCNK5) is a member of the same family and it was shown to be induced by estrogens in ER-positive BC cells; for this reason, it might represent a therapeutic target for ER-positive BCs [30]. The amplification of the *KCNK9* gene at the 8q23.4 locus justifies the over expression of $K_{2P}9.1$ channels in BC. The overexpression of another K2p channel ***K2p 2.1*** has been demonstrated in PCa and it was shown that it regulates cell proliferation [31]. The expression of inward rectifiers K^+ channels, in particular **Kir3.1** (KCNJ3) channels positively correlated with lymph node metastases in BC [32]. The voltage-gated K^+ channels (VGKC) appear to exert a pleiotropic role in colorectal cancer. In primary human samples, the transcripts of *KCNA3*, *KCNA5*, *KCNC1*, *KCNH1* [33–35], *KCNH2* [36] and *KCNK9* [37] have been detected. A relevant family of VGKC, whose most important members are Kv 10.1 and Kv 11.1 was shown to be highly represented in human cancers. **Kv10.1** (KCNH1) was expressed in esophageal squamous cell carcinoma (ESCC) compared with the corresponding normal tissue, it was associated with depth of invasion and represented an independent negative prognostic factor [38].

Kv11.1 (KCNH2) channels are expressed in gastric cancer (GC) cell lines and primary GCs. In GC cell lines, they regulate tumor proliferation [39]. Consistently, treatment with $K_v11.1$ blockers, like cisapride, and siRNA impairs tumor growth [40, 41]. It was also shown that the mean survival time was shorter in $K_v11.1$ positive patients thus $K_v11.1$ expression was proposed as an independent prognostic factor. We also showed that $K_v11.1$ regulates VEGF-A secretion, with a pathway similar to the one described in CRC [42]. *In vivo* analyses of xenografts obtained with GC cells demonstrated that the treatment with Bevacizumab and $K_v11.1$ blockers dramatically reduces greatly tumor growth. $K_v11.1$ is highly expressed in primary CRC and is associated with invasive phenotype [36]; moreover, along with Glut-1 absence, it represents a negative prognostic factor in TNM I and II CRC [43]. $K_v11.1$ expression is associated with chemosensitivity for several anti-tumor agents (such as vincristine, paclitaxel and hydroxy-camptothecin, doxorubicin). Such chemosensitivity is modulated by erythromycin that is also capable which, to inhibit $K_v11.1$ current [44]. $K_v11.1$ also regulates lung cancer (LC) cell proliferation [45]. $K_v11.1$ is expressed in precancerous and neoplastic lesions of the esophagus and it is associated with malignant progression [46]. $K_v11.1$ channel expression represents a negative prognostic factor in terms of ESCC patients' survival [47].

K_v11.1 are also expressed in PDAC cell lines and primary samples and it negatively affects patients' prognosis [48].

3.2 Sodium channels

Voltage-gated sodium channels (VGSC) were among the first channels to be demonstrated mis-expressed in BC and PCa. In particular, the predominant VGSC in BC is the "neonatal" splice variant of *SCN5A* (**nNaV1.5**), whose activity promotes metastatization [49–51]; consistently, the nNAv1.5 was up-regulated in metastatic BC samples [49, 50, 52]. On the whole, VGSC and in particular nNav1.5 could represent a good specific target for BC treatment. In CRC [53–55], the clinical relevance of Na_v 1.5 expression was established by IHC in CRC samples with respect to healthy colon. VGSC regulates invasiveness and it was shown that *SCNA5* gene modulates genes mediating, among others, cell migration and cell cycle control. Both nNa_v 1.5 and its "adult" counterpart are expressed in CRC and the local anesthetic Ropivacaine, blocks Na_v 1.5 variants [56]. PCa show an aberrant expression of **Nav1.7** (SCN9A), associated with a strong metastatic potential and its activity potentiates cell migration, crucial for the metastatic cascade [57]. Hence, Nav1.7 could represent a useful diagnostic marker [58]. A recent paper [59] showed that EGFR and Nav1.7 are expressed in NSCLC cells and that EGFR-mediated upregulation of *SCN9A* is necessary for the invasiveness of such cells. Nav1.7 has clinical relevance and might represent a novel target for therapy and/or a prognostic biomarker in NSCLC [59]. A recent multicenter study identified two single nucleotide polymorphisms of VGSC genes (*SCN4A*-rs2302237 and *SCN10A*-rs12632942) that were associated with oxaliplatin-induced peripheral neuropathy development [60].

3.3 Calcium channels

Calcium signal remodeling is one of the common features of proliferating cells, including cancer. Indeed many functional studies have provided different calcium signaling that can modulate cell proliferation and resistance to apoptosis [61–63]. Voltage-gated calcium channels (VGCC) that are involved in the regulation of BC cell proliferation. ***CACNA2D3*** gene (encoding the α2δ3 subunit of the voltage gated Ca^{2+} channel) is frequently up-regulated in BC, but in some metastatic cases, its expression is reduced [64]. The mechanisms of *CACNA2D3* contribution to the metastatic process has not being clarified yet. One possible mechanism for the overexpression of some calcium permeable ion channels is through the involvement of hormone receptors, such as ERα. Examples are **ORAI3** [65]. *CACNA2D3*, is frequently downregulated in primary BCs, as a result of methylation in CpG islands [64]. The influence of calcium channels in PCa has been known for over 30 years. Later research identified additional classes of channel proteins having an important regulatory role and affecting malignant transformation (reviewed in [66]). The expression of VGCC (mainly L-type) has been detected in the androgen-responsive LNCaP cells. In these cells Ca^{2+} currents are activated by androgens and mediate the androgen-induced effects [67]. Part of the Ca^{2+} effects depend on K^+ channels stimulation, for example, KCa3.1 blocking inhibits the proliferation of PCa cells [67]. An aberrant methylation of *CACNA2D1/3* gene (encoding the voltage-dependent calcium channel 2 subunit) was demonstrated in GC samples. *CACNA2D3* methylation is associated with diffuse type GC and shorter survival [68]. **ORAI1** and **STIM1**, belonging to the store operated calcium channels (SOC) family, are up-regulated in BC of the basal-like molecular subtype [69]. Moreover, another member of the same family, **STIM2**, is expressed at low levels in BC. Patients with

high STIM1 and low STIM2 have unfavorable prognosis, suggesting that the SOC family has a role in aggressiveness and in the metastatic process [69]. **ORAI3** has recently been associated with ER-positive BC [65] and could represent a novel target for ER-positive BCs [70].

3.4 Transient receptor potential (TRP) channels

TRP channels are non-selective cation channels that can be activated by different stimuli such as pH variations, temperature and pressure among others [71, 72]. Since TRP channels are involved in migration and invasiveness, they contribute to the metastatic process in different tumors [73]. Ca^{2+} influx through TRPCs also occurs and promotes either cell proliferation or apoptosis, depending on TRPC subtype. **TRPC1** whose levels are high in BCs with low proliferation capacity, may not be the optimal target for therapies against aggressive BCs [74]. Significantly elevated (up to 200-fold) mRNA levels of ***TRPC6*** were shown in BC samples compared with paired control samples [74, 75], but no correlations with clinico-pathological features emerged [74]. A similar behavior characterizes TRPC1, whose expression levels decrease during the progression of PCa from androgen-dependent to androgen-independent phase [75]. TRPC6 is overexpressed in ESCC with respect to normal esophageal tissue at both protein and mRNA levels [76]. A recent report evidenced correlations of TRPC6 with T and staging and an association between *TRPC6* mRNA and poor prognosis [77]. **TRPV6** is up-regulated in PgR and ER-negative BCs [78]. Basal-like BCs with high TRPV6 mRNA levels are associated with poor survival [79]. *In vitro* data suggest that TRPV6 may be a potential therapeutic target [79]. TRPV6 is highly expressed in PCa and are associated with the Gleason score and metastatisation [80]. The expression of **TRPV4** is decreased by progesterone [81]. **TRPM7** is highly expressed in BC, and such over expression is associated with poor prognosis in terms of distant metastasis- and recurrence-free survival [82]. In accordance with these observation, *TRPM7* mRNA levels are higher in BC metastases with respect to primary tumors. Also, TRPM7 are overexpressed in pancreatic ductal adenocarcinomas and are associated with lymph node metastases [83]. TRPM7 mRNA and protein are also overexpressed in bladder cancer with respect to normal tissue and are associated with poor prognosis [84]. **TRPA1** is overexpressed also in SCLC patients compared with NSCLC and since it is associated with SCLC patients' survival representing a potential therapeutic target [85].

3.5 Chloride channels

Anoctamin 1 (**ANO1**), the calcium-activated chloride channel, is highly expressed in BC cell lines and primary BCs [86] and the 11q13 region is frequently amplified in BC and it is associated with grading and unfavorable outcome [86].

ANO1 was also shown to play an important role in controlling PDAC cell proliferation [87]. It has been shown that chloride channel accessory 1 and 2 genes (***CLCA1*** and ***CLCA2***) transcripts show widespread downregulation in CRC patients [88]. Therefore CLCA proteins could be tumor suppressors in CRC in analogy with what occurs in BC. **CLC1** is expressed in GC cells where it impairs cell proliferation and stimulates apoptosis, invasion and migration *in vitro* [89]. CLC1 overexpression in primary GC correlates with clinico-pathological parameters (lymph node involvement, stage, lymphatic and perineural invasion) as well as with poor prognosis [90]. **CLIC3** is not expressed in healthy pancreas while it is expressed in PanIN lesions [91] and in PDAC where it has a negative impact on patient survival.

3.6 Ligand-gated channels

The ligand-gated nicotinic acethylcholine receptors (**nAChRs**) are the channel type mostly studied in LC [92]. NSCLC shows altered expression of nicotinic subunits (mainly α1, α5 ανδ α7) compared with normal tissue. Moreover in NSCLC cells, nicotine has mitogenic effects of nicotine, mediated by α7-containing nAChRs [93]. Multiple genome-wide association studies (GWAS) have implicated the 15q25 nAChR gene cluster *CHRNA5-A3-B4* in nicotine dependence and LC [94]. The expression of the ***CHRNA5*** gene which encodes the α5-nAchR was increased in LC tissue and that the p.Asp398Asn polymorphism in the *CHRNA5* gene is associated with LC risk [92] and altered receptor function [95]. Additionally, the p.Asp398Asn polymorphism may influence α5 (CHRNA5) expression as well [92]. A α5-nAChR/HIF-1α/VEGF axis exists in LC and is involved in nicotine-induced tumor cell proliferation. This fact suggests that α5-nAChR may serve as a potential anticancer target in nicotine-associated LC [96].

3.7 Aquaporins (AQP)

AQP1 is expressed in BC and positively correlates with grading, histology, CK14 expression, smooth muscle actin expression, basal-like group and poor outcome, whereas it has significant negative correlation with ER status [97]. AQP1, **AQP3** and **AQP5** are expressed in CRC cell lines. AQP1 and AQP5 are expressed the early steps of CRC progression but also in liver metastases [98]. Moreover, AQP5 expression is associated with grading, nodal involvement and TNM stage [99]. AQP5 is expressed at significant levels in Lauren's intestinal type-GC, where it shows an apical localization [100], whereas AQP3 and **AQP4** are not overexpressed in GC. Shen et al. [101] showed that both AQP3 and AQP5 were overexpressed in GC and were associated with lymph node involvement. Moreover, AQP3 expression was higher in well differentiated tumors. AQP3 is also over-expressed in primary CRC with respect to healthy tissue, and its expression is positively regulated by EGF and is associated with lymph node involvement, metastasis and differentiation [102]. AQP3 and AQP5 are expressed in ESCC, while absent in healthy esophagus [103, 104]: the presence of the two aquaporins is associated with clinico-pathological features and their co-expression represents an independent negative prognostic factor. A recent microarray-based study demonstrated that reduced ***AQP9*** gene expression is related to absence of adjuvant chemotherapy response in CRC patients [38].

3.8 Transporters

The monocarboxylate transporter **SLC16A1** (encoded by the *SLC16A1* gene) is associated to basal-like BC, high histological grade, CK5, CK14, vimentin and Ki67. AQP1 along with SLC16A1 were shown to be associated with tumor aggressiveness of BC [105]. The voltage-gated proton channel Hv1 (**HVCN1**) overexpression in metastatic BC is associated with progression and unfavorable outcome [106]. The same occurs in CRC in which it is associated also with tumor size, lymph node involvement and stage [107]. In stage CRC, a low expression of **SLC7A1** (cationic amino-acid transporters-1, encoded by *SLC7A1* gene) is associated with shorter metastases-free survival [108].

The sodium proton exchanger 1 (**NHE1**, SLC9A1) interacts with EGFR and is involved in PDAC cell invasiveness [109]. It was shown that the Glucose Transporter 1 (**SLC2A1**, GLUT1) is expressed in BE-derived tumors in the late events of tumor progression [110]. SLC2A1 expression described also occurs in ESCC, where it represents a marker of poor prognosis [111]. Moreover, SLC2A1 expression increased

after radiotherapy in ESCC patients [112]. The apical sodium-dependent bile acid transporters (**SLC10A2**), which mediate bile acid transport [113], are not expressed in the normal squamous epithelium of the esophagus [114], whereas their expression increases in Barrett's Esophagus, to decline in EA [115]. Divalent metal transporter1 (DMT1, **SLC11A2**) overexpression was associated with metastatization in EC [116]. One of the main causes of chemotherapy failure is drug efflux mediated by ATP-binding cassette transporters (ABC) [117]. It was recently shown that **ABCG2** together with V-ATPase are overexpressed in ESCC and are associated with grading, TNM stage and metastatization. **ABCB1** and ABCG2 are expressed in primary GC and GC cell lines [118] in which their expression is associated with tumor differentiation. ABCB1 expression is higher in diffuse type GC [119]. ABCG2 represents a target for a several chemotherapy drugs [120]: for example, cisplatin increases *ABCG2* mRNA *in vitro* and this is associated with patients' outcome [121]. In PDAC, **ABCB4, ABCB11, ABCC1, ABCC3, ABCC5, ABCC10** and ABCG2 are up-regulated, while **ABCA3, ABCC6**, CFTR (**ABCC7**) and **ABCC8** are down-regulated: such deregulation contributes to PDAC poor response to therapy [122]. The Solute Carrier transporters (SLC) is a family of transporters frequently deregulated in PDAC. **SLC7A5** (the L-type aminoacid transporter 1) are overexpressed in PDAC and are associated with molecular and clinico-pathological features (such as Ki-67, p53, CD34, CD98, VEGF size, stage) and prognosis [122]. **SLC22A3** and **SLC22A18** are up-regulated in PDAC with respect to healthy pancreas while **SLC22A1**, **SLC22A2**, **SLC22A11**, **SLC28A1**, **SLC28A3** and **SLC29A1** are down-regulated [122]. In particular, SLC28A1 overexpression was associated with poor overall survival whereas SLC22A3 and SLC29A3 overexpression was observed in patients treated with Gemcitabine with longer overall survival. PC patients with low expression of SMCT1 (**SLC5A8**) have poorer survival with respect to patients with high SLC5A8 levels [123]. The human equilibrative nucleoside transporter 1 (**SLC29A1**) is associated to longer time to progression and it was shown that it could predict gemcitabine effects in non-resectable PDAC patients, if evaluated in samples obtained by fine-needle aspiration [124]. Different conclusions were drawn when analyzing SLC29A1 expression in patients treated with chemo-radiotherapy [125]. In GC, **SLC7A5** overexpression was detected and it was found to be associated with clinico-pathological features such as size, lymph node involvement, TNM stage and local invasion [126]. **SLC16A1** was found to be expressed both in healthy stomach and GC, and it could be hypothesized a role in gastric physiology for this transporter [119]. In metastatic GC, **SLC16A3** is down-regulated [119] and is associated with intestinal type. 4F2hc (**SLC3A2**) was found to be over-expressed in GC cell lines and in primary GC, with no significant correlation with clinico-pathological features. Since the study was conducted on a small number of samples, it could not allow definitive conclusions [127].

4. Ion channels and transporters with clinical relevance in hematologic malignancies

As reported for solid tumors, a schematic overview of ion channels and transporters expressed in hematologic tumors is reported in **Figure 2**. Early evidence for the implication of K^+ channels in leukemia cell proliferation was obtained in the myeloblastic leukemia cell line ML-1 [128]. In leukemias, it was shown that **KCa3.1** might represent a useful target since its blockade impairs leukemic cells proliferation [129] while *KCNN4* overexpression was detected in follicular lymphomas [130]. A significant **Kv10.1** expression was detected in myelodysplastic syndromes, CML and almost half of a cohort of AML samples and blocking the channel results in the inhibition of both cell proliferation and migration. Smith

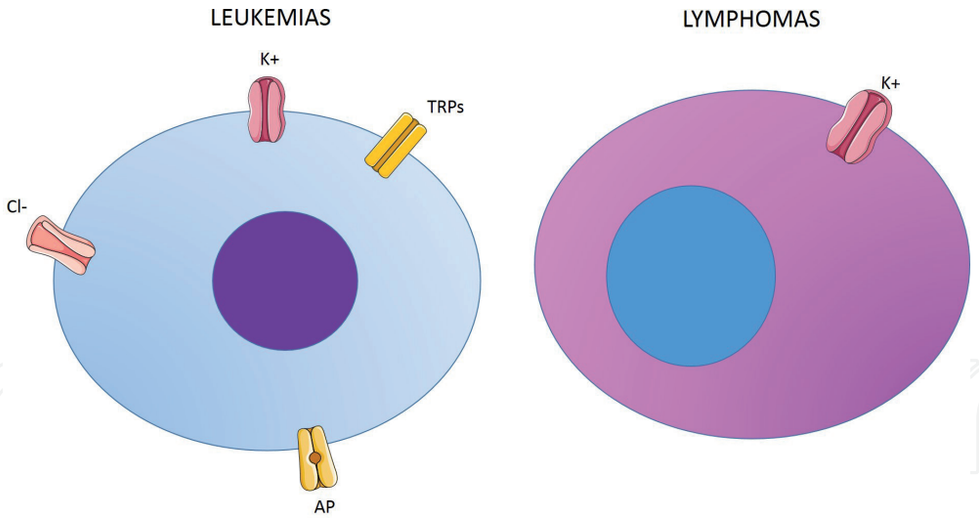

Figure 2.
Cartoon showing the main ICTs expressed in leukemias and lymphomas.

and colleagues [131] carried out an extensive study of the K+ channel transcripts in primary lymphocytes, leukemias (B-cell CLL) and several leukemic cell lines and they found only ***Kv11.1*** was significantly up-regulated. In AML cell lines (FLG 29.1, HL-60 and K562), it was shown that specific block of IKv11.1 led to G1 arrest and impaired their migration on fibronectin-containing ECM [132]. Kv11.1 was also overexpressed in circulating blasts from human AML, in which the block of the channel significantly decreased cell growth [132]. The *hsloBK* splice variant of ***gBK*** has been detected in gliomas [133] and the *herg1b* alternative transcript of *Kv11.1* is overexpressed in human leukemias and neuroblastomas [134, 135]. TWIK-related spinal cord K+ (**TRESK**) channels, members of the double-pore domain K+ channel family, are expressed in Jurkat cells [136] that also express TRPV5 and TRPV6, which were also detected in K562 cells. TRP channels control Ca^{2+} homeostasis in the context of malignant transformation [137] and it was shown that of TRPV5/TRPV6-like channels' activation mediate Ca^{2+} entry and the activation of Ca^{2+}/Calmodulin-dependent kinase II in irradiated K562 cells [138].

During the oxidative burst following activation of K562 cells non-selective cation channel TRPM2 are activated, thus activating **SK4** KCa channels. In parallel, the voltage-gated Cl-channel **ClC-3** is also activated. The overall effect is cell shrinkage because of the osmotic water loss determined KCl outflow [139, 140]. A similar volume-dependent regulation of leukemia cell apoptosis can be operated by volume-regulated chloride currents (**VRCC**). The volume-dependent regulatory mechanisms are accompanied by control of water levels suggesting it could represent an additional modulatory mechanism in the apoptotic cascade [141]. AQPs control osmotic fluxes in a variety of physiological conditions. For instance, AQP5 is overexpressed in CML cells, where it promotes cell proliferation and inhibits apoptosis, perhaps through an effect on cell volume control [142]. Expression of AQP5 increases in parallel with the development of resistance to imatinib mesylate [142].

5. Targeting ion channels and transporters for cancer diagnosis with antibodies

Recently, an antibody directed to a cancer-related ion channel (the purinergic receptor P2X7) was introduced into the clinical settings: it is a polyclonal antibody targeting a conformational epitope of the non-functional channel and it is likely

to be approved as a first-generation therapy. Antibodies targeting ORAI1 were obtained using U2OS cells overexpressing human ORAI1 as immunogens. One of such antibodies impaired cell proliferation of T lymphocytes in peripheral blood [143, 144]. In 2014, a method for the isolation of functional antibodies against Nav1.7 was published [145].

6. Future perspectives

In a recent paper [146], an ICT molecular profile was defined for BC thus opening interesting perspectives in this field. In particular, the expression of 30 ion channel genes was shown to be associated with tumor grade. The authors were able of identifying a "IC30 gene signature" composed of 30 ion channel genes and demonstrated that IC30 might represent a prognostic biomarker predicting clinical outcome in BC, independently from clinical and pathological prognostic factors. The same approach was applied to LC and 37 ion channels genes were identified as differentially expressed in LC in comparison to healthy lung [147]. Moreover, 31 ion channel genes were identified as differentially expressed between lung adenocarcinoma and squamous-cell carcinoma samples, therefore the expression of such genes could be used for NSCLC molecular classification [147]. In NSCLC, it was shown that VDAC1 is an independent prognostic factor and it is associated with shorter overall survival [147]. VDAC1 was also found to be up-regulated in different types of carcinomas [148]. More recently, a paper describing gene expression profile in lymphomas demonstrated that *KCNN4* and *SLC2A1* genes are overexpressed in follicular lymphomas (FL) [130]. In particular, ***SLC2A1*** was proposed to be the hub of a functional network, connecting channels and transporters in FL. Moreover, relapsed FL had 38 differentially expressed ICT genes, among which **ATP9A**, **SLC2A1** and **KCNN4** were under-expressed. In the same paper, it was shown that diffuse large B Cell lymphoma (DLBCL) have a completely different pattern of K^+ channel encoding genes expression along with the overexpression of the fatty acid transporter-encoding gene **SLC27A1**.

Conflict of interest

The authors declare no conflict of interest.

Author details

Jessica Iorio, Claudia Duranti and Elena Lastraioli*
Department of Experimental and Clinical Medicine, Florence, Italy

*Address all correspondence to: elena.lastraioli@unifi.it

References

[1] Arcangeli A, Crociani O, Bencini L. Interaction of tumour cells with their microenvironment: Ion channels and cell adhesion molecules. A focus on pancreatic cancer. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences. 2014;**369**(1638):20130101. DOI: 10.1098/rstb.2013.0101

[2] Lastraioli E, Iorio J, Arcangeli A. Ion channel expression as promising cancer biomarker. Biochimica et Biophysica Acta. 2015;**1848**(10 Pt B):2685-2702. DOI: 10.1016/j.bbamem.2014.12.016

[3] Pedersen SF, Novak I, Alves F. Alternating pH landscapes shape epithelial cancer initiation and progression: Focus on pancreatic cancer. BioEssays. 2017;**39**(6):1-10. DOI: 10.1002/bies.201600253

[4] Arcangeli A, Crociani O, Lastraioli E. Targeting ion channels in cancer: A novel frontier in antineoplastic therapy. Current Medicinal Chemistry. 2009;**16**(1):66-93. DOI: 10.2174/092986709787002835

[5] Wulff H, Castle NA, Pardo LA. Voltage-gated potassium channels as therapeutic targets. Nature Reviews. Drug Discovery. 2009;**8**(12):982-1001. DOI: 10.1038/nrd2983

[6] Prevarskaya N, Skryma R, Shuba Y. Ion channels in cancer: Are cancer hallmarks oncochannelopathies? Physiological Reviews. 2018;**98**(2):559-621. DOI: 10.1152/physrev.00044.2016

[7] Kaur S, Venktaraman G, Jain M. Recent trends in antibody-based oncologic imaging. Cancer Letters. 2012;**315**:97-111. DOI: 10.1016/j.canlet.2011.10.017

[8] Xu W, Liu L, Brown NJ. Quantum dot-conjugated anti-GRP78 scFv inhibits cancer growth in mice. Molecules. 2012;**17**:796-808. DOI: 10.3390/molecules17010796

[9] Sharma SK, Wuest M, Way JD. Synthesis and pre-clinical evaluation of an (18)F-labeled single-chain antibody fragment for PET imaging of epithelial ovarian cancer. American Journal of Nuclear Medicine and Molecular Imaging. 2016;**6**:185-198

[10] Han D, Wu J, Han Y. A novel anti-PSMA human scFv has the potential to be used as a diagnostic tool in prostate cancer. Oncotarget. 2016;**7**:59471-59481. DOI: 10.18632/oncotarget.10697

[11] Mazzocco C, Fracasso G, Germain-Genevois C. In vivo imaging of prostate cancer using an anti-PSMA scFv fragment as a probe. Scientific Reports. 2016;**6**:23314. DOI: 10.1038/srep23314

[12] Duranti C, Arcangeli A. Ion channel targeting with antibodies and antibody fragments for cancer diagnosis. Antibodies. 2019;**8**(2):33. DOI: 10.3390/antib8020033

[13] Holliger P, Hudson PJ. Engineered antibody fragments and the rise of single domains. Nature Biotechnology. 2005;**23**:1126-1136. DOI: 10.1038/nbt1142

[14] Oeggerli M, Tian Y, Ruiz C. Role of KCNMA1 in breast cancer. PLoS One. 2012;**7**(8):e41664. DOI: 10.1371/journal.pone.0041664

[15] Brevet M, Ahidouch A, Sevestre H. Expression of K+ channels in normal and cancerous human breast. Histology and Histopathology. 2008;**23**(8):965-972. DOI: 10.14670/HH-23.965.

[16] Khaitan D, Sankpal UT, Weksler B. Role of KCNMA1 gene in breast cancer invasion and metastasis

to brain. BMC Cancer. 2009;**9**:258. DOI: 10.1186/1471-2407-9-258

[17] Bloch M, Ousingsawat J, Simon R. KCNMA1 gene amplification promotes tumor cell proliferation in human prostate cancer. Oncogene. 2007;**26**(17):2525-2534. DOI: 10.1038/sj.onc.1210036

[18] Altintas DM, Allioli N, Decaussin M. Differentially expressed androgen-regulated genes in androgen-sensitive tissues reveal potential biomarkers of early prostate cancer. PLoS One. 2013;**8**(6):e66278. DOI: 10.1371/journal.pone.0066278

[19] Haren N, Khorsi H, Faouzi M. Intermediate conductance Ca^{2+} activated K^+ channels are expressed and functional in breast adenocarcinomas: Correlation with tumour grade and metastasis status. Histology and Histopathology. 2010;**25**(10):1247-1255. DOI: 10.14670/HH-25.1247.

[20] Bonito B, Sauter DR, Schwab A. $K_{Ca}3.1$ (IK) modulates pancreatic cancer cell migration, invasion and proliferation: Anomalous effects on TRAM-34. Pflügers Archiv. 2016;**468**(11-12):1865-1875. DOI: 10.1007/s00424-016-1891-9

[21] Lai W, Liu L, Zeng Y. KCNN4 channels participate in the EMT induced by PRL-3 in colorectal cancer. Medical Oncology. 2013;**30**:566. DOI: 10.1007/s12032-013-0566-z

[22] Bulk E, Ay AS, Hammadi M. Epigenetic dysregulation of KCa 3.1 channels induces poor prognosis in lung cancer. International Journal of Cancer. 2015;**137**(6):1306-1317. DOI: 10.1002/ijc.29490

[23] Pillozzi S, D'Amico M, Bartoli G. The combined activation of $K_{Ca}3.1$ and inhibition of $K_v11.1$/hERG1 currents contribute to overcome Cisplatin resistance in colorectal cancer cells. British Journal of Cancer. 2018;**118**(2):200-212. DOI: 10.1038/bjc.2017.392

[24] Muratori L, Petroni G, Antonuzzo L. hERG1 positivity and Glut-1 negativity identifies high-risk TNM stage I and II colorectal cancer patients, regardless of adjuvant chemotherapy. OncoTargets and Therapy. 2016;**9**:6325-6332. DOI: 10.2147/OTT.S114090

[25] Bielanska J, Hernández-Losa J, Pérez-Verdaguer M. Voltage-dependent potassium channels Kv1.3 and Kv1.5 in human cancer. Current Cancer Drug Targets. 2009;**9**(8):904-914. DOI: 10.3389/fphys.2013.00283

[26] Ohya S, Kimura K, Niwa S. Malignancy grade-dependent expression of K^+-channel subtypes in human prostate cancer. Journal of Pharmacological Sciences. 2009;**109**(1):148-151. DOI: 10.254/jphs.08208S

[27] Abdul M, Hoosein N. Reduced Kv1.3 potassium channel expression in human prostate cancer. The Journal of Membrane Biology. 2006;**214**(2):99-102. DOI: 10.1007/s00232-006-0065-7

[28] Brevet M, Fucks D, Chatelain D. Deregulation of 2 potassium channels in pancreas adenocarcinomas: Implication of KV1.3 gene promoter methylation. Pancreas. 2009;**38**(6):649-654. DOI: 10.1097/MPA.0b013e3181a56ebf

[29] Mu D, Chen L, Zhang X. Genomic amplification and oncogenic properties of the KCNK9 potassium channel gene. Cancer Cell. 2003;**3**(3):297-302. DOI: 10.1016/S1535-6108(03)00054-0

[30] Alvarez-Baron CP, Jonsson P, Thomas C. The two-pore domain potassium channel KCNK5: Induction by estrogen receptor alpha and role in proliferation of breast cancer cells. Molecular Endocrinology.

2011;**25**(8):1326-1336. DOI: 10.1210/me.2011-0045

[31] Voloshyna I, Besana A, Castillo M. TREK-1 is a novel molecular target in prostate cancer. Cancer Research. 2008;**68**(4):1197-1203. DOI: 10.1158/0008-5472.CAN-07-5163

[32] Stringer BK, Cooper AG, Shepard SB. Overexpression of the G-protein inwardly rectifying potassium channel 1 (GIRK1) in primary breast carcinomas correlates with axillary lymph node metastasis. Cancer Research. 2001;**61**(2):582-588

[33] Hemmerlein B, Weseloh RM, Mello de Queiroz F. Overexpression of Eag1 potassium channels in clinical tumours. Molecular Cancer. 2006;**5**(41):1-13. DOI: 10.1186/1476-4598-5-41

[34] Abdul M, Hoosein N. Voltage-gated potassium ion channels in colon cancer. Oncology Reports. 2002;**9**(5):961-964. DOI: 10.3892/or.9.5.961

[35] Ousingsawat J, Spitzner M, Puntheeranurak S. Expression of voltage-gated potassium channels in human and mouse colonic carcinoma. Clinical Cancer Research. 2007;**13**(3):824-831. DOI: 10.1158/1078-0432.CCR-06-1940

[36] Lastraioli E, Guasti L, Crociani O. herg1 gene and HERG1 protein are overexpressed in colorectal cancers and regulate cell invasion of tumor cells. Cancer Research. 2004;**64**(2):606-611. DOI: 10.1158/0008-5472.CAN-03-2360

[37] Kim CJ, Cho YG, Jeong SW. Altered expression of KCNK9 in colorectal cancers. APMIS. 2004;**112**(9):588-594. DOI: 10.1111/j.1600-0463.2004.apm1120905.x

[38] Ding XW, Wang XG, Luo HS. Expression and prognostic roles of Eag1 in resected esophageal squamous cell carcinomas. Digestive Diseases and Sciences. 2008;**53**(8):2039-2044. DOI: 10.1007/s10620-007-0116-7

[39] Lastraioli E, Gasperi Campani F, Taddei A. hERG1 channels are overexpressed in human gastric cancer and their activity regulates cell proliferation: A novel prognostic and therapeutic target? In: Proceedings of 6th IGCC; Yokohama. 2005. pp. 151-154

[40] Shao XD, Wu KC, Hao ZM. The potent inhibitory effects of cisapride, a specific blocker for human ether-a-go-go-related gene (HERG) channel, on gastric cancer cells. Cancer Biology & Therapy. 2005;**4**(3):295-301. DOI: 10.4161/cbt.4.3.1500

[41] Shao XD, Wu KC, Guo XZ. Expression and significance of HERG protein in gastric cancer. Cancer Biology & Therapy. 2008;**7**(1):45-50. DOI: 10.4161/cbt.7.1.5126

[42] Crociani O, Zanieri F, Pillozzi S. hERG1 channels modulate integrin signaling to trigger angiogenesis and tumor progression in colorectal cancer. Scientific Reports. 2013;**3**:3308. DOI: 10.1038/srep03308

[43] Lastraioli E, Bencini L, Bianchini E. hERG1 channels and Glut-1 as independent prognostic indicators of worse outcome in stage I and II colorectal cancer: A pilot study. Translational Oncology. 2012;**5**(2):105-112. DOI: 10.1593/tlo.11250

[44] Chen SZ, Jiang M, Zhen YS. HERG K+ channel expression-related chemosensitivity in cancer cells and its modulation by erythromycin. Cancer Chemotherapy and Pharmacology. 2005;**56**(2):212-220. DOI: 10.1007/s00280-004-0960-5

[45] Glassmeier G, Hempel K, Wulfsen I. Inhibition of HERG1 K+ channel protein expression decreases cell proliferation of human small cell lung cancer cells. Pflügers Archiv.

2012;**463**(2):365-376. DOI: 10.1007/s00424-011-1045-z

[46] Lastraioli E, Taddei A, Messerini L. hERG1 channels in human esophagus: Evidence for their aberrant expression in the malignant progression of Barrett's esophagus. Journal of Cellular Physiology. 2006;**209**(2):398-404. DOI: 10.1002/jcp.20748

[47] Ding XW, Luo HS, Luo B. Overexpression of hERG1 in resected esophageal squamous cell carcinomas: A marker for poor prognosis. Journal of Surgical Oncology. 2008;**97**(1):57-62. DOI: 10.1002/jso.20891

[48] Lastraioli E, Perrone G, Sette A. hERG1 channels drive tumour malignancy and may serve as prognostic factor in pancreatic ductal adenocarcinoma. British Journal of Cancer. 2015;**112**(6):1076-1087. DOI: 10.1038/bjc.2015.28

[49] Fraser SP, Diss JK, Chioni AM. Voltage-gated sodium channel expression and potentiation of human breast cancer metastasis. Clinical Cancer Research. 2005;**11**(15):5381-5389. DOI: 10.1158/1078-0432.CCR-05-0327

[50] Brackenbury WJ, Chioni AM, Diss JK. The neonatal splice variant of Nav1.5 potentiates in vitro invasive behaviour of MDA-MB-231 human breast cancer cells. Breast Cancer Research and Treatment. 2007;**101**(2):149-160. DOI: 10.1007/s10549-006-9281-1

[51] Gillet L, Roger S, Besson P. Voltage-gated sodium channel activity promotes cysteine cathepsin-dependent invasiveness and colony growth of human cancer cells. The Journal of Biological Chemistry. 2009;**284**(13):8680-8691. DOI: 10.1074/jbc.M806891200

[52] Chioni AM, Fraser SP, Pani F. A novel polyclonal antibody specific for the Na(v)1.5 voltage-gated Na(+) channel 'neonatal' splice form. Journal of Neuroscience Methods. 2005;**147**(2):88-98. DOI: 10.1016/j.jneumeth.2005.03.010

[53] Guzel RM, Ogmen K, Ilieva KM. Colorectal cancer invasiveness in vitro: Predominant contribution of neonatal Nav1.5 under normoxia and hypoxia. Journal of Cellular Physiology. 2019;**234**(5):6582-6593. DOI: 10.1002/jcp.27399

[54] Fairhurst C, Martin F, Watt I. Sodium channel-inhibiting drugs and cancer survival: Protocol for a cohort study using the CPRD primary care database. BMJ Open. 2016;**6**(9):e011661. DOI: 10.1136/bmjopen-2016-011661

[55] Roger S, Gillet L, Le Guennec JY. Voltage-gated sodium channels and cancer: Is excitability their primary role? Frontiers in Pharmacology. 2015;**6**:152. DOI: 10.3389/fphar.2015.00152

[56] Baptista-Hon DT, Robertson FM, Robertson GB. Potent inhibition by ropivacaine of metastatic colon cancer SW620 cell invasion and NaV1.5 channel function. British Journal of Anaesthesia. 2014;**113**(Suppl. 1):i39-i48. DOI: 10.1093/bja/aeu104

[57] Diss JK, Fraser SP, Djamgoz MB. Voltage-gated Na+ channels: Multiplicity of expression, plasticity, functional implications and pathophysiological aspects. European Biophysics Journal. 2004;**33**(3):180-193. DOI: 10.1007/s00249-004-0389-0

[58] Diss JK, Stewart D, Pani F. A potential novel marker for human prostate cancer: Voltage-gated sodium channel expression in vivo. Prostate Cancer and Prostatic Diseases. 2005;**8**(3):266-273. DOI: 10.1038/sj.pcan.4500796

[59] Campbell TM, Main MJ, Fitzgerald EM. Functional expression of the voltage-gated Na^+-channel Nav1.7 is necessary for EGF-mediated invasion in human non-small cell lung cancer cells. Journal of Cell Science. 2013;**126**(Pt 21):4939-4949. DOI: 10.1242/jcs.130013

[60] Argyriou AA, Cavaletti G, Antonacopoulou A. Voltage-gated sodium channel polymorphisms play a pivotal role in the development of oxaliplatin-induced peripheral neurotoxicity: Results from a prospective multicenter study. Cancer. 2013;**119**(19):3570-3577. DOI: 10.1002/cncr.28234

[61] Haustrate A, Hantute-Ghesquier A, Prevarskaya N. TRPV6 calcium channel regulation, downstream pathways, and therapeutic targeting in cancer. Cell Calcium. 2019;**80**:117-124. DOI: 10.1016/j.ceca.2019.04.006

[62] Fliniaux I, Germain E, Farfariello V, Prevarskaya N. TRPs and Ca^{2+} in cell death and survival. Cell Calcium. 2018;**69**:4-18. DOI: 10.1016/j.ceca.2017.07.002

[63] Iamshanova O, Fiorio Pla A, Prevarskaya N. Molecular mechanisms of tumour invasion: Regulation by calcium signals. The Journal of Physiology. 2017;**595**(10):3063-3075. DOI: 10.1113/JP272844

[64] Palmieri C, Rudraraju B, Monteverde M. Methylation of the calcium channel regulatory subunit α2δ-3 (CACNA2D3) predicts site-specific relapse in oestrogen receptor-positive primary breast carcinomas. British Journal of Cancer. 2012;**107**(2):375-381. DOI: 10.1038/bjc.2012.231

[65] Motiani RK, Zhang X, Harmon KE. Orai3 is an estrogen receptor α-regulated Ca^{2+} channel that promotes tumorigenesis. The FASEB Journal. 2013;**27**(1):63-75. DOI: 10.1096/fj.12-213801

[66] Shapovalov G, Skryma R, Prevarskaya N. Calcium channels and prostate cancer. Recent Patents on Anti-Cancer Drug Discovery. 2013;**8**(1):18-26. DOI: 10.2174/1574892811308010018

[67] Vanden Abeele F, Zholos A, Bidaux G. Ca^{2+}-independent phospholipase A2-dependent gating of TRPM8 by lysophospholipids. The Journal of Biological Chemistry. 2006;**281**(52):40174-40182. DOI: 10.1074/jbc.M605779200

[68] Wanajo A, Sasaki A, Nagasaki H. Methylation of the calcium channel-related gene, CACNA2D3, is frequent and a poor prognostic factor in gastric cancer. Gastroenterology. 2008;**135**(2):580-590. DOI: 10.1053/j.gastro.2008.05.041

[69] McAndrew D, Grice DM, Peters AA. ORAI1-mediated calcium influx in lactation and in breast cancer. Molecular Cancer Therapeutics. 2011;**10**(3):448-460. DOI: 10.1158/1535-7163.MCT-10-0923

[70] Faouzi M, Hague F, Potier M. Down-regulation of Orai3 arrests cell-cycle progression and induces apoptosis in breast cancer cells but not in normal breast epithelial cells. Journal of Cellular Physiology. 2011;**226**(2):542-551. DOI: 10.1002/jcp.22363

[71] Clapham DE. TRP channels as cellular sensors. Nature. 2003;**426**(6966):517-524. DOI: 10.1038/nature02196

[72] Nilius B, Owsianik G. The transient receptor potential family of ion channels. Genome Biology. 2011;**12**(3):218. DOI: 10.1186/gb-2011-12-3-218

[73] Canales J, Morales D, Blanco C. TR(i)P to cell migration:

New roles of TRP channels in mechanotransduction and cancer. Frontiers in Physiology. 2019;**10**:757. DOI: 10.3389/fphys.2019.00757

[74] Dhennin-Duthille I, Gautier M, Faouzi M. High expression of transient receptor potential channels in human breast cancer epithelial cells and tissues: Correlation with pathological parameters. Cellular Physiology and Biochemistry. 2011;**28**(5):813-822. DOI: 10.1159/000335795

[75] Nilius B. Transient receptor potential (TRP) cation channels: Rewarding unique proteins. Bulletin et Mémoires de l'Académie Royale de Médecine de Belgique. 2007;**162**(3-4):244-253

[76] Shi Y, Ding X, He ZH. Critical role of TRPC6 channels in G2 phase transition and the development of human oesophageal cancer. Gut. 2009;**58**(11):1443-1450. DOI: 10.1136/gut.2009.181735

[77] Zhang SS, Wen J, Yang F. High expression of transient potential receptor C6 correlated with poor prognosis in patients withesophageal squamous cell carcinoma. Medical Oncology. 2013;**30**(3):607. DOI: 10.1007/s12032-013-0607-7

[78] Bolanz KA, Hediger MA, Landowski CP. The role of TRPV6 in breast carcinogenesis. Molecular Cancer Therapeutics. 2008;**7**(2):271-279. DOI: 10.1158/1535-7163.MCT-07-0478

[79] Peters AA, Simpson PT, Bassett JJ. Calcium channel TRPV6 as a potential therapeutic target in estrogen receptor-negative breast cancer. Molecular Cancer Therapeutics. 2012;**11**(10):2158-2168. DOI: 10.1158/1535-7163.MCT-11-0965

[80] Fixemer T, Wissenbach U, Flockerzi V. Expression of the Ca^{2+}-selective cation channel TRPV6 in human prostate cancer: A novel prognostic marker for tumor progression. Oncogene. 2003;**22**(49):7858-7861. DOI: 10.1038/sj.onc.1206895

[81] Li C, Wu Y, Zhu Q. TRPV4 is involved in levonorgestrel-induced reduction in oviduct ciliary beating. The Journal of Pathology. 2019;**248**(1):77-87. DOI: 10.1002/path.5233

[82] Middelbeek J, Arthur J, Kuipers AJ, Henneman L. TRPM7 is required for breast tumor cell metastasis. Cancer Research. 2012;**72**(16):1-12. DOI: 10.1158/0008-5472.CAN-11-3863

[83] Rybarczyk P, Vanlaeys A, Brassart B. The transient receptor potential melastatin 7 channel regulates pancreatic cancer cell invasion through the Hsp90α/uPA/MMP2 pathway. Neoplasia. 2017;**19**(4):288-300. DOI: 10.1016/j.neo.2017.01.004

[84] Gao SL, Kong CZ, Zhang Z. TRPM7 is overexpressed in bladder cancer and promotes proliferation, migration, invasion and tumor growth. Oncology Reports. 2017;**38**(4):1967-1976. DOI: 10.3892/or.2017.5883

[85] Schaefer EA, Stohr S, Meister M. Stimulation of the chemosensory TRPA1 cation channel by volatile toxic substances promotes cell survival of small cell lung cancer cells. Biochemical Pharmacology. 2013;**85**(3):426-438. DOI: 10.1016/j.bcp.2012.11.019

[86] Britschgi A, Bill A, Brinkhaus H, Choudhury M, Gosling L, Wang S, et al. Bentires-AljCalcium-activated chloride channel ANO1 promotes breast cancer progression by activating EGFR and CAMK signaling. Proceedings of the National Academy of Sciences of the United States of America. 2013;**110**(11):E1026-E1034. DOI: 10.1073/pnas.1217072110

[87] Mazzone A, Eisenman ST, Strege PR. Inhibition of cell proliferation by a selective inhibitor of the Ca(2+)-activated Cl(−) channel, Ano1. Biochemical and Biophysical Research Communications. 2012;**427**(2):248-253. DOI: 10.1016/j.bbrc.2012.09.022

[88] Yang B, Cao L, Liu B. The transition from proliferation to differentiation in colorectal cancer is regulated by the calcium activated chloride channel A1. PLoS One. 2013;**8**(4):e60861. DOI: 10.1371/journal.pone.0060861

[89] Ma PF, Chen JQ, Wang Z. Function of chloride intracellular channel 1 in gastric cancer cells. World Journal of Gastroenterology. 2012;**18**(24):3070-3080. DOI: 10.3748/wjg.v18.i24.3070

[90] Chen CD, Wang CS, Huang YH. Overexpression of CLIC1 in human gastric carcinoma and its clinicopathological significance. Proteomics. 2007;**7**(1):155-167. DOI: 10.1002/pmic.200600663

[91] Dozynkiewicz MA, Jamieson NB, Macpherson I. Rab25 and CLIC3 collaborate to promote integrin recycling from late endosomes/lysosomes and drive cancer progression. Developmental Cell. 2012;**22**(1):131-145. DOI: 10.1016/j.devcel.2011.11.008

[92] Falvella FS, Galvan A, Colombo F. Promoter polymorphisms and transcript levels of nicotinic receptor CHRNA5. Journal of the National Cancer Institute. 2010;**102**(17):1366-1370. DOI: 10.1093/jnci/djq264

[93] Egleton RD, Brown KC, Dasgupta P. Nicotinic acetylcholine receptors in cancer: Multiple roles in proliferation and inhibition of apoptosis. Trends in Pharmacological Sciences. 2008;**29**(3):151-158. DOI: 10.1016/j.tips.2007.12.006

[94] Amos CI, Wu X, Broderick P. Genome-wide association scan of tag SNPs identifies a susceptibility locus for lung cancer at 15q25.1. Nature Genetics. 2008;**40**(5):616-622. DOI: 10.1038/ng.109

[95] George AA, Lucero LM, Damaj MI. Function of human α3β4α5 nicotinic acetylcholine receptors is reduced by the α5(D398N) variant. The Journal of Biological Chemistry. 2012;**287**(30):25151-25162. DOI: 10.1074/jbc.M112.379339

[96] Ma X, Jia Y, Zu S. α5 nicotinic acetylcholine receptor mediates nicotine-induced HIF-1α and VEGF expression in non-small cell lung cancer. Toxicology and Applied Pharmacology. 2014;**278**(2):172-179. DOI: 10.1016/j.taap.2014.04.023

[97] Otterbach F, Callies R, Adamzik M. Aquaporin 1 (AQP1) expression is a novel characteristic feature of a particularly aggressive subgroup of basal-like breast carcinomas. Breast Cancer Research and Treatment. 2010;**120**(1):67-76. DOI: 10.1007/s10549-009-0370-9

[98] Moon C, Soria JC, Jang SJ. Involvement of aquaporins in colorectal carcinogenesis. Oncogene. 2003;**22**(43):6699-6703. DOI: 10.1038/sj.onc.1206762

[99] Wang P, Zhang C, Yu P. Regulation of colon cancer cell migration and invasion by CLIC1-mediated RVD. Molecular and Cellular Biochemistry. 2012;**365**(1-2):313-321. DOI: 10.1007/s11010-012-1271-5

[100] Watanabe T, Fujii T, Oya T. Involvement of aquaporin-5 in differentiation of human gastric cancer cells. The Journal of Physiological Sciences. 2009;**59**(2):113-122. DOI: 10.1007/s12576-008-0017-3

[101] Shen L, Zhu Z, Huang Y. Expression profile of multiple

aquaporins in human gastric carcinoma and its clinical significance. Biomedicine & Pharmacotherapy. 2010;**64**(5):313-318. DOI: 10.1016/j.biopha.2009.12.003

[102] Li A, Lu D, Zhang Y. Critical role of aquaporin-3 in epidermal growth factor-induced migration of colorectal carcinoma cells and its clinical significance. Oncology Reports. 2013;**29**(2):535-540. DOI: 10.3892/or.2012.2144

[103] Kusayama M, Wada K, Nagata M. Critical role of aquaporin 3 on growth of human esophageal and oral squamous cell carcinoma. Cancer Science. 2011;**102**(6):1128-1136. DOI: 10.1111/j.1349-7006.2011.01927.x

[104] Liu S, Zhang S, Jiang H. Co-expression of AQP3 and AQP5 in esophageal squamous cell carcinoma correlates with aggressive tumor progression and poor prognosis. Medical Oncology. 2013;**30**(3):636. DOI: 10.1007/s12032-013-0636-2

[105] Pinheiro C, Albergaria A, Paredes J. Monocarboxylate transporter 1 is up-regulated in basal-like breast carcinoma. Histopathology. 2010;**56**(7):860-867. DOI: 10.1111/j.1365-2559.2010.03560.x

[106] Wang Y, Li SJ, Wu X. Clinicopathological and biological significance of human voltage-gated proton channel Hv1 protein overexpression in breast cancer. The Journal of Biological Chemistry. 2012;**287**(17):13877-13888. DOI: 10.1074/jbc.M112.345280

[107] Wang Y, Wu X, Li Q. Human voltage-gated proton channel hv1: A new potential biomarker for diagnosis and prognosis of colorectal cancer. PLoS One. 2013;**8**(8):e70550. DOI: 10.1371/journal.pone.0070550

[108] Iino I, Kikuchi H, Miyazaki S. Effect of miR-122 and its target gene cationic amino acid transporter 1 on colorectal liver metastasis. Cancer Science. 2013;**104**(5):624-630. DOI: 10.1111/cas.12122

[109] Cardone RA, Greco MR, Zeeberg K. A novel NHE1-centered signaling cassette drives epidermal growth factor receptor-dependent pancreatic tumor metastasis and is a target for combination therapy. Neoplasia. 2015;**17**(2):155-166. DOI: 10.1016/j.neo.2014.12.003

[110] Younes M, Ertan A, Lechago LV. Human erythrocyte glucose transporter (Glut1) is immunohistochemically detected as a late event during malignant progression in Barrett's metaplasia. Cancer Epidemiology, Biomarkers & Prevention. 1997;**6**(5):303-305

[111] Tohma T, Okazumi S, Makino H. Overexpression of glucose transporter 1 in esophageal squamous cell carcinomas: A marker for poor prognosis. Diseases of the Esophagus. 2005;**18**(3):185-189. DOI: 10.1111/j.1442-2050.2005.00489.x

[112] Doki Y, Takachi K, Ishikawa O. Reduced tumor vessel density and high expression of glucose transporter 1 suggest tumor hypoxia of squamous cell carcinoma of the esophagus surviving after radiotherapy. Surgery. 2005;**137**(5):536-544. DOI: 10.1016/j.surg.2005.01.008

[113] Alrefai WA, Gill RK. Bile acid transporters: Structure, function, regulation and pathophysiological implications. Pharmaceutical Research. 2007;**24**(10):1803-1823. DOI: 10.1007/s11095-007-9289-1

[114] Trauner M, Boyer JL. Bile salt transporters: Molecular characterization, function and regulation. Physiological

Reviews. 2003;**83**(2):633-671. DOI: 10.1016/j.surg.2005.01.008

[115] Dvorak K, Watts GS, Ramsey L. Expression of bile acid transporting proteins in Barrett's esophagus and esophageal adenocarcinoma. The American Journal of Gastroenterology. 2009;**104**(2):302-309. DOI: 10.1038/ajg.2008.85

[116] Boult J, Roberts Brookes MJ. Overexpression of cellular iron import proteins is associated with malignant progression of esophageal adenocarcinoma. Clinical Cancer Research. 2008;**14**(2):379-387. DOI: 10.1158/1078-0432.CCR-07-1054

[117] Mohelnikova-Duchonova B, Brynychova V, Oliverius M. Differences in transcript levels of ABC transporters between pancreatic adenocarcinoma and nonneoplastic tissues. Pancreas. 2013;**42**(4):707-716. DOI: 10.1097/MPA.0b013e318279b861

[118] Jiang Y, He Y, Li H. Expressions of putative cancer stem cell markers ABCB1, ABCG2, and CD133 are correlated with the degree of differentiation of gastric cancer. Gastric Cancer. 2012;**15**(4):440-450. DOI: 10.1007/s10120-012-0140-y

[119] Pinheiro C, Longatto-Filho A, Simões K. The prognostic value of CD147/EMMPRIN is associated with monocarboxylate transporter 1 co-expression in gastric cancer. European Journal of Cancer. 2009;**45**(13):2418-2424. DOI: 10.1016/j.ejca.2009.06.018

[120] Schnepf R, Zolk O. Effect of the ATP-binding cassette transporter ABCG2 on pharmacokinetics: Experimental findings and clinical implications. Expert Opinion on Drug Metabolism & Toxicology. 2013;**9**(3):287-306. DOI: 10.1517/17425255.2013.742063

[121] Zhang Q, Li K, Xu JH. Role of ABCG2 expression driven by cisplatin in platinum-containing chemotherapy for gastric cancer. World Journal of Gastroenterology. 2013;**19**(39):6630-6636. DOI: 10.3748/wjg.v19.i39.6630

[122] Kaira K, Sunose Y, Arakawa K. Prognostic significance of L-type amino-acid transporter 1 expression in surgically resected pancreatic cancer. British Journal of Cancer. 2012;**107**(4):632-638. DOI: 10.1038/bjc.2012.310

[123] Helm J, Coppola D, Ganapathy V. SLC5A8 nuclear translocation and loss of expression are associated with poor outcome in pancreatic ductal adenocarcinoma. Pancreas. 2012;**41**(6):904-909. DOI: 10.1097/MPA.0b013e31823f429f

[124] Eto K, Kawakami H, Kuwatani M. Human equilibrative nucleoside transporter 1 and Notch3 can predict gemcitabine effects in patients with unresectable pancreatic cancer. British Journal of Cancer. 2013;**108**(7):1488-1494. DOI: 10.1038/bjc.2013.108

[125] Kawada N, Uehara H, Katayama K. Human equilibrative nucleoside transporter 1 level does not predict prognosis in pancreatic cancer patients treated with neoadjuvant chemoradiation including gemcitabine. Journal of Hepato-Biliary-Pancreatic Sciences. 2012;**19**(6):717-722. DOI: 10.1007/s00534-012-0514-x

[126] Wang J, Chen X, Su L. LAT-1 functions as a promotor in gastric cancer associated with clinicopathologic features. Biomedicine & Pharmacotherapy. 2013;**67**(8):693-699. DOI: 10.1016/j.biopha.2013.05.003

[127] Yang Y, Toy W, Choong LY. Discovery of SLC3A2 cell membrane protein as a potential gastric cancer

biomarker: Implications in molecular imaging. Journal of Proteome Research. 2012;**11**(12):5736-5747. DOI: 10.1021/pr300555y

[128] Lu L, Yang T, Markakis D. Alterations in a voltage-gated K+ current during the differentiation of ML-1 human myeloblastic leukemia cells. The Journal of Membrane Biology. 1993;**132**:267-274. DOI: 10.1007/bf00235743

[129] Grössinger EM, Weiss L, Zierler S. Targeting proliferation of chronic lymphocytic leukemia (CLL) cells through KCa3.1 blockade. Leukemia. 2014;**28**(4):954-958. DOI: 10.1038/leu.2014.37

[130] Magi A, Masselli M, Sala C. The ion channels and transporters gene expression profile indicates a shift in excitability and metabolisms during malignant progression of follicular lymphoma. Scientific Reports. 2019;**9**(1):8586. DOI: 10.1038/s41598-019-44661-x

[131] Smith GAM, Tsui HW, Newell EW. Functional up-regulation of HERG K^+ channels in neoplastic hematopoietic cells. The Journal of Biological Chemistry. 2002;**277**(18):528-534. DOI: 10.1074/jbc.M200592200

[132] Pillozzi S, Brizzi MF, Balzi M. HERG potassium channels are constitutively expressed in primary human acute myeloid leukemias and regulate cell proliferation of normal and leukemic hemopoietic progenitors. Leukemia. 2002;**16**(9):1791-1798. DOI: 10.1038/sj.leu.2402572

[133] Olsen ML, Weaver AK, Ritch PS. Modulation of glioma BK channels via erbB2. Journal of Neuroscience Research. 2005;**81**:179-189. DOI: 10.1002/jnr.20543

[134] Pillozzi S, Brizzi MF, Bernabei PA. VEGFR-1 (FLT-1), beta1 integrin, and hERG K+ channel form a macromolecular signaling complex in acute myeloid leukemia: Role in cell migration and clinical outcome. Blood. 2007;**110**:1238-1250. DOI: 10.1182/blood-2006-02-003772

[135] Crociani O, Guasti L, Balzi MA. Cell cycle-dependent expression of HERG1 and HERG1B isoforms in tumor cells. The Journal of Biological Chemistry. 2003;**278**:2947-2955. DOI: 10.1074/jbc.M210789200

[136] Pottosin II, Bonales-Alatorre E, Valencia-Cruz G. TRESK-like potassium channels in leukemic T cells. Pflügers Archiv. 2008;**456**(6):1037-1048. DOI: 10.1007/s00424-008-0481-x

[137] Vasil'eva IO, Neguliaev IA, Marakhova II. TRPV5 and TRPV6 calcium channels in human T cells. Tsitologiia. 2008;**50**(11):953-957

[138] Heise N, Palme D, Misovic M. Non-selective cation channel-mediated Ca2+entry and activation of Ca2+/calmodulin-dependent kinase II contribute to G2/M cell cycle arrest and survival of irradiated leukemia cells. Cellular Physiology and Biochemistry. 2010;**26**(4-5):597-608. DOI: 10.1159/000322327

[139] Kasinathan RS, Föller M, Lang C. Oxidation induces ClC-3-dependent anion channels in human leukaemia cells. FEBS Letters. 2007;**581**(28):5407-5412. DOI: 10.1016/j.febslet.2007.10.042

[140] Jiang B, Hattori N, Liu B. Expression of swelling- and/or pH-regulated chloride channels (ClC-2, 3, 4 and 5) in human leukemic and normal immune cells. Life Sciences. 2002;**70**(12):1383-1394. DOI: 10.1016/s0024-3205(01)01517-x

[141] Renaudo A, Watry V, Chassot AA. Inhibition of tumor cell proliferation by sigma ligands is associated with K+ channel inhibition

and p27kip1 accumulation. The Journal of Pharmacology and Experimental Therapeutics. 2004;**311**(3):1105-1114. DOI: 10.1124/jpet.104.072413

[142] Chae YK, Kang SK, Kim MS. Human AQP5 plays a role in the progression of chronic myelogenous leukemia (CML). PLoS One. 2008;**3**(7):e2594. DOI: 10.1371/journal.pone.0002594

[143] Lin FF, Elliott R, Colombero A. Generation and characterization of fully human monoclonal antibodies against human Orai1 for autoimmune disease. The Journal of Pharmacology and Experimental Therapeutics. 2013;**345**:225-238. DOI: 10.1124/jpet.112.202788

[144] Cox JH, Hussell S, Søndergaard H. Antibody-mediated targeting of the Orai1 calcium channel inhibits T cell function. PLoS One. 2013;**8**:e82944. DOI: 10.1371/journal.pone.0082944

[145] Lee JH, Park CK, Chen G. A monoclonal antibody that targets a NaV1.7 channel voltage sensor for pain and itch relief. Cell. 2014;**157**:1393-1404. DOI: 10.1016/j.cell.2014.03.064

[146] Ko JH, Ko EA, Gu W. Expression profiling of ion channel genes predicts clinical outcome in breast cancer. Molecular Cancer. 2013;**12**(1):106. DOI: 10.1186/1476-4598-12-106

[147] Grills C, Jitesh PV, Blayney J. Gene expression meta-analysis identifies VDAC1 as a predictor of poor outcome in early stage non-small cell lung cancer. PLoS One. 2011;**6**:e14635. DOI: 10.1371/journal.pone.0014635

[148] Ko JH, Gu W, Lim I. Expression profiling of mitochondrial voltage-dependent anion channel-1 associated genes predicts recurrence-free survival in human carcinomas. PLoS One. 2014;**9**:e110094. DOI: 10.1371/journal.pone.0110094