

## Ion Channels and Transporters in Cancer: Pathophysiology, Regulation, and Clinical Potential

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### Abstract

Over the last 15 years it has become increasingly clear that dysregulated expression, splicing, and/or function of ion channels and transporters (ICT) occur in all cancers. Being linked to the widely accepted hallmarks of cancer, ICTs represent novel therapeutic, diagnostic, and prognostic targets. To discuss the current status of the field, a colloquium on "Ion Transport and Cancer" was held, covering the roles of ICTs in cancer cell proliferation, apoptosis, motility, and invasion, and in both the generation of and the interaction of the cancer cells with the tumor environment. Additional sessions dealt with pancreatic ductal adenocarcinoma and transport protein-based therapeutic and diagnostic concepts. There was overall consensus that essential contributions of ICT dysregulation to the cancer process have been demonstrated. Future research should be directed toward further elucidating the mechanisms and developing therapeutic applications. *Cancer Res*; 73(6); 1658–61. ©2012 AACR.

### Introduction

A colloquium on "Ion Transport and Cancer" took place in Würzburg, Germany, from September 9–12, 2012. More than 90 researchers met to present and discuss state-of-the-art data. Ion channels and transporters (ICT) have been a focus of interest in cancer research for only approximately 15 years. A wealth of recent data now suggests (i) that a wide variety of ion transport proteins (including voltage-gated ion channels) is expressed in cancer cells *in vitro* and *in vivo* and (ii) that some of these proteins may be cancer specific. Because of the rapid progress made, it is now unquestionable that ICTs play a significant role in driving the cancer process at all stages. Being involved in nearly all of the "hallmarks of cancer" as defined by Hanahan and Weinberg (1), this opens up new clinical possibilities. During the meeting talks on basic research, preclinical studies, and therapeutic approaches covered the roles of ICTs in cancer. Taking into account that many participants were associated with the European Union-funded Marie Curie Initial Training Network on the role of Ion Transport Proteins in Pancreatic Cancer, IonTraC, one session was dedicated to pancreatic ductal adenocarcinoma (PDAC). Evidence was furthermore presented indicating that it is not always the ionic permeation or transport *per se* that plays a role in cellular processes during cancer development and progres-

sion. Several channels and transporters additionally display kinase activity, exert cell adhesion functions, or serve as structural elements stabilizing subcellular structures such as the cell cortex.

The meeting also emphasized the need for new imaging techniques required to visualize ion distribution/concentrations in patients/live tissue. In this context, Armin Nagel (German Cancer Research Center, Heidelberg, Germany) and Frauke Alves' group (Universitätsmedizin Göttingen, Germany) presented new approaches including <sup>23</sup>Na MRI and novel optical (nano-)probes (near-infrared fluorescence), respectively.

### Cell-Cycle Progression and Proliferation

It has long been appreciated that ion transport dynamics are an integral part of the regulation of cell-cycle progression and proliferation (2). Several types of ICTs are involved. One important factor is the dependence of cell-cycle progression on cell volume, which is in turn tightly controlled by ion transport processes. Marked cell volume changes are an integral part of cell-cycle progression, in a manner at least in part reflecting the differential regulation of specific ion channels (2). In glioma cells, a marked premitotic cell shrinkage reflecting increased Cl<sup>-</sup> efflux via ClC3 channels has been shown to be necessary for the ensuing cell division (3). This is particularly pertinent in light of the roles of ClC3 channels also in glioma cell motility (see below). A toxin targeting these channels is currently in clinical trials (4). Excitingly, this regimen increased survival of glioma patients from approximately 4 to 12 months. Cellular pH is also dynamically regulated during cell-cycle progression, with G<sub>2</sub>-M transition being particularly pH-sensitive and dependent on increased activity of the Na<sup>+</sup>/H<sup>+</sup> exchanger NHE1. This is of relevance given the increased activity of NHE1 and other acid-extruding transporters shown in many types of cancer. At the meeting, novel evidence from Diane Barber's group [University of California,

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San Francisco (UCSF), California] was presented demonstrating that pH sensors are closely involved in control of cell-cycle progression and glycolytic flux. Dysregulation of  $\text{Ca}^{2+}$ -channels, and in particular transient receptor potential (TRP) channels, has also been shown to contribute importantly to dysregulation of cell proliferation in cancer, and Natalia Prevarskaya (INSERM U800, University of Lille, France) showed novel data on TRPV6 to further substantiate this notion. Finally, the potassium channel  $\text{K}_v10.1$  (EAG1), one of the ion channels most widely studied in cancer, is regulated through the cell cycle and in turn regulates cell-cycle progression (5). In fact, it has even been proposed that  $\text{K}_v10.1$  is an oncogene (6).

### Apoptosis

Ion fluxes are also crucial to the regulation of cell death pathways, and several important novel lines of evidence linking ICTs to cell death regulation and chemotherapy resistance in cancer were presented at the meeting. Within the last decade, it has become apparent that ICTs play central roles in control of cell death–survival balance (2). Thus, sustained opening of  $\text{K}^+$  and  $\text{Cl}^-$  channels is necessary for apoptotic cell shrinkage and the ensuing cell death (7). John E. Cidlowski [National Institute of Environmental Health Sciences (NIEHS), Durham, North Carolina] showed that profound alterations in volume regulatory mechanisms lead to the generation of apoptosis-resistant cells. Similarly, dysregulation of cellular  $\text{Ca}^{2+}$  and pH homeostasis are central steps in induction of many (apoptotic and other) death pathways. Thus, inhibition of acid/base transport can be exploited to sensitize cancer cells to chemotherapy, as presented by the group of Stine Pedersen (University of Copenhagen, Denmark). Other examples presented at the meeting included the inhibition of mitochondrial  $\text{K}_v1.3$  channels, which can selectively elicit apoptosis in cancer cells, as shown by the group of Ildikó Szabo (University of Padova, Italy).

### Cancer Cell Metabolism and Tumor Microenvironment

The tumor microenvironment exhibits physical and chemical characteristics distinct from those of most normal tissues and contains numerous cell types in addition to the cancer cells themselves. All of these properties contribute importantly to the cancer phenotype, and ICTs are emerging as relevant players in the interplay between the cancer cells and the tumor microenvironment. An important example was presented by the group of Annarosa Arcangeli (Università degli Studi di Firenze, Florence, Italy), who showed the role of  $\text{K}_v11.1$  (hERG1) potassium channels in cancer cells in stimulating angiogenesis by promoting VEGF-A secretion from colorectal cancer cells. Also, TRP channels are important for tumor angiogenesis, and data shown by Alessandra Fiorio Pla (University of Turin, Italy) showed the specific role of TRPV4 in arachidonic acid–induced migration of tumor-derived, but not of normal, endothelial cells.

Fundamental characteristics of the microenvironment of solid tumors include hypoxia, acidic extracellular pH, and elevated lactate concentrations. Ulrike Sattler (Johannes Gutenberg University, Mainz, Germany) pointed to the prog-

nostic value of a locally elevated lactate concentration inside the tumor. Cancer cells themselves often exhibit an alkaline intracellular pH compared with that in normal cells, because of compensatory upregulation of acid-extruding transporters such as NHE1 or  $\text{Na}^+\text{-HCO}_3^-$  cotransporters. At the meeting, evidence was presented by Alzbeta Hulikova (University of Oxford, United Kingdom) indicating that in many cancer cells, hypoxia reduces NHE1-dependent acid extrusion, and that  $\text{Na}^+\text{-HCO}_3^-$  cotransport dominates the regulation of steady-state pH in most cancer cells studied. This points to a central role of these transporters in cancer, and emphasizes the need to study ion transport specifically in the context of the tumor microenvironment.

### Tumor Cell Motility and Invasion

Cell motility and invasion, major steps in the metastatic cascade, require functional integration of (i) a fine-tuned interaction with the tumor microenvironment mediated by, for example, integrins and growth factor receptors; (ii) well-organized cytoskeletal dynamics; (iii) spatially and temporally precisely regulated activity of ICTs (8); and (iv) nuclear and chromatin structures contributing to gene expression. Cell-intrinsic pathways and those initiated by the microenvironment converge to regulate motility (reviewed in ref. 9), a concept called "dynamic reciprocity" (10). Using intravital infrared multiphoton imaging, Peter Friedl's group (Radboud University Nijmegen Medical Center, the Netherlands) found surprisingly that collective melanoma cell invasion does not depend on  $\beta 1$  and  $\beta 3$  integrins; instead, these  $\beta$  integrin subunits allow the development of resistance to high-dose radiotherapy.

Diane Barber (UCSF) highlighted protonation as a form of posttranslational modification driving changes in protein structure, activity, and function. Cellular pH sensor proteins feature specific  $\text{H}^+$ -sensitive His residues regulating their function at physiologic pH. Examples are the 3 cytoskeleton-associated proteins cofilin, FAK, and talin, as well as the polarity-inducing Cdc42. Many cancer cells regulate pH also at their surface by expressing the membrane-bound carbonic anhydrase IX (CAIX) under hypoxic conditions. CAIX accumulates at protruding lamellipodia and contributes considerably to cell migration (Elisaka Svastova, Slovak Academy of Sciences, Bratislava, Slovakia). CAIX colocalizes and interacts with the  $\text{HCO}_3^-$  transporters NBCe1 and AE2 to keep the cytosol alkaline although acidifying the cell surface. Notably, CAIX expression is also upregulated by EGF and PI3K.

Numerous ion channels have been associated with tumor cell motility. Voltage-gated  $\text{Na}^+$  channels ( $\text{Na}_v$ ), especially  $\text{Na}_v1.5$  and  $\text{Na}_v1.7$ , are ectopically expressed in epithelial cancers such as breast, lung, prostate, and cervix cancer. Sébastien Roger's group (INSERM921, Tours, France) introduced the intriguing idea that  $\text{Na}_v$  channel proteins form functional complexes with  $\beta$  auxiliary subunits and NHE1 in specific membrane areas to promote breast cancer cell invasiveness by increasing invadopodial NHE1 activity, which then generates an optimum pericellular pH for the activity of acidic cysteine cathepsins. Scott Fraser and coworkers

(Imperial College London, United Kingdom) showed that tetrodotoxin-sensitive  $\text{Na}_v$ s are upregulated in metastatic ovarian cancer cells and promote the cells' invasiveness. Several other channels were shown to contribute to invasiveness and metastasis. The ATP-gated cation-permeable P2X7 receptor is strongly expressed in highly aggressive human MDA-MB-435s breast cancer cells, and its inhibition reduces MDA-MB-435s invasion (Bilel Jelassi, INSERM 921, Tours, France). The TRP channel TRPM7 functions both as a  $\text{Ca}^{2+}$ -permeable nonselective cation channel and as a kinase. High TRPM7 expression is required for metastasis in mice and predicts poor outcome in many breast cancer patients. As TRPM7 regulates myosin-II-based cellular tension, thereby impacting on focal adhesion formation, cell polarization, and directional migration, Frank van Leeuwen (Radboud University Medical Center, Nijmegen, the Netherlands) proposed that TRPM7 is part of a mechanosensory complex required for efficient formation of metastases.  $\text{K}_{\text{Ca}}1.1$  (BK) channels are required for glioblastoma cell migration and invasion. Ionizing radiation turns out to be a counterproductive therapeutic approach in this context as it stimulates  $\text{K}_{\text{Ca}}1.1$  channel activity, resulting in an activation of CaMKII that then leads to enhanced migration (Mark Steidle, University of Tübingen, Germany). Irradiation-dependent upregulation of  $\text{K}^+$  channels was also presented for nonsmall cell lung cancer and chronic myeloid leukemia cells (Bastian Roth, Technical University of Darmstadt, Germany).

## PDAC

PDAC is one of the most lethal cancers due to late diagnosis and severely limited treatment options. The malignancy of PDAC originates at least in part from an unusually high susceptibility toward inflammatory signals. Holger Kalthoff (Universitätsklinikum Schleswig-Holstein, Kiel, Germany) reported that the NF- $\kappa$ B contributes to nonapoptotic signaling of death receptors that typically, upon activation by binding death ligands, induce apoptosis in PDAC. Targeting this pathway should be taken advantage of in future therapeutic concepts. Also in PDAC, excess lactic acid is extruded via monocarboxylate transporters (MCT). Inhibition of either MCTs or of glycolysis reduces the activity of the plasma membrane  $\text{Ca}^{2+}$ -ATPase, allowing the conclusion that this may represent therapeutic approaches to induce  $[\text{Ca}^{2+}]$  overload-mediated cell death in PDAC (James Andrews, University of Manchester, United Kingdom). A promising biomarker for PDAC could be TRPM7. Pierre Rybarczyk (Université de Picardie Jules Verne, Amiens, France) showed that TRPM7 affects PDAC cell migration and invasion by regulating  $\text{Mg}^{2+}$  homeostasis and/or kinase function. Stephan Reshkin (University of Bari, Italy) showed that expression and activity of NHE1 correlate with the degree of aggressiveness of human PDAC cell lines. Upon stimulation with EGF, the NHE-regulatory factor 1 (NHERF1) is physically complexed with both EGFR and NHE1 indicating the formation of an NHE1/EGFR/NHERF1 axis that could be targeted by innovative therapeutic strategies in PDAC. A hallmark of PDAC is the strong desmoplastic reaction resulting from the interaction of cancer and pancreatic stellate cells (PSC). Ivana Novak (University of Copenhagen, Denmark) reported that the purinergic P2X7 receptor and its ligand ATP

are important regulators of PSC proliferation and death. An interaction between pancreatic cancer and stellate cells is required for matrix metalloproteinase expression and activity in both cell types (Hans-Jörg Habisch, Uniklinikum Ulm, Germany). Interestingly, in PSC the phosphorylation status of the kinases cAMP-responsive element binding protein, Akt, Rsk, S6K, and FAK and possibly also the secretion of variable amounts of the matrix metalloproteinases (MMP) 1, 2, 3, 7, and 14 are affected differently by pancreatic cell-conditioned medium depending on the used pancreatic cancer cell line. This further supports the view that PDAC cells stimulate PSC that, in return, contribute to PDAC development by secreting MMPs, which accelerate local degradation of the extracellular matrix.

## In Vivo and Clinical Evidence

In addition to the impressive *in vitro* data for the role of ICTs in cancer cell behavior, *in vivo* and clinical evidence implicating dysregulation of transporters and channels in cancer is now emerging (e.g., refs. 4, 5, 11). At the meeting, Mustafa Djamgoz (Imperial College London, United Kingdom) summarized the *in vivo* evidence for "neonatal"  $\text{Na}_v$  expression in breast cancer ( $\text{Na}_v1.5$ ) and prostate cancer ( $\text{Na}_v1.7$ ). Data obtained from human prostate cancer biopsies revealed  $\text{Na}_v1.7$  expression could serve as a clinical biomarker. Similarly, significant positive correlation was found between expression of neonatal  $\text{Na}_v1.5$  protein in primary tumors of breast cancer patients and both metastatic potential and survival.

## New Therapeutic and Diagnostic Potential

The mechanistic insights and the *in vivo* evidence for ICT involvement in cancer have raised novel clinical possibilities as regards both diagnosis and therapy. The typically acidic tumor environment itself could be a therapeutic target. The observation that oral uptake of  $\text{HCO}_3^-$  increased tumor pH and inhibits spontaneous metastases in mice (Robert Gillies, Moffitt Cancer Center, Tampa, Florida) has led to a still ongoing phase I clinical trial. In patients suffering from renal cell carcinoma, CAIX, which is highly expressed in many tumors but essentially absent in most healthy cells, has been successfully targeted by the specific monoclonal antibody G250 (Egbert Oosterwijk, Nijmegen Center for Molecular Life Sciences, the Netherlands). Also hypoxia *per se* can be a therapeutic target. The efficacy of hypoxia-activated prodrugs can be enhanced by promoting tumor hypoxia. Targeting volume- and shape-regulating processes may be an option as well, particularly in gliomas (Harald Sontheimer, University of Alabama Birmingham, Alabama). They metastasize by moving through small and tortuous extracellular spaces along cerebral blood vessels. This requires a constant (re)adjustment of cell shape/local volume by a concerted release of  $\text{Cl}^-$  ions through the CaMK-II-regulated  $\text{ClC}3$  and  $\text{K}^+$  ions through  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels. Therefore, disturbing either the channel-regulating  $\text{Ca}^{2+}$  signals or the channels themselves inhibits cell volume tuning and thus reduces the ability to metastasize. William Brackenbury (University of York, United Kingdom) proposed that repurposing existing antiepileptic drugs that block  $\text{Na}_v$  channels may have the potential to improve patient outcomes in metastatic breast cancer, especially the oral

anticonvulsant phenytoin, which inhibits  $\text{Na}_v$ -dependent migration and invasion in highly metastatic MDA-MB-231 breast cancer cells.

### Conclusions and Recommendations for Future Research

The meeting concluded with a joint discussion of the essential recommendations for future research. First of all, it was unanimously concluded that there is no longer any doubt about the contribution of dysregulated expression, splicing, and/or function of ICTs to the cancer process. Such changes occur in all cancers, and contribute importantly to the "hallmarks of cancer." Thus, ICTs that, for a long time, have been known to be important drug targets in other pathologies such as cardiovascular disorders and epilepsy, show great promise as potential drug targets in oncology. It was concluded that although the field of ICTs and cancer is now well established, further work is required in several key areas. First, the experimental models should be refined further by combining, for instance, genetic tumor mouse models with the respective ion channel/transporter mouse models. Second, further mechanistic insight should be sought into the precise combination of ICTs and series of events leading from dysregulation to the pathophysiologic outputs in a "systems" approach. Third, the current knowledge on ICTs should be translated into a clinical context, for example, by undertaking further *in vivo* tests and human tissue analyses. This can be extended to epidemiologic studies on the widespread use of drugs known to inhibit cancer-relevant ion channels, such as the antihistamine

astemizole that blocks  $\text{K}_v10.1$  channels and anticonvulsants and antiarrhythmics for  $\text{Na}_v$ s and metastatic disease. ICTs are easily detectable and are frequently dysregulated as a very early event in cancer development, pointing to substantial diagnostic potential. Most ICTs implicated in cancer are located at the plasma membrane, a convenient site for drug targeting. The fact that, for decades, ion channel and transporter modulators have been used highly successfully for the treatment of a wide range of diseases including congestive heart failure, cardiac arrhythmia (cardiac/digitalis glycosides), or hypertension (diuretics) further argues for the feasibility of applying ICT modulators in cancer therapy. Indeed, the future looks very promising for exploiting ICTs as diagnostic and therapeutic targets in cancer.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

### Authors' Contributions

**Conception and design:** S.F. Pedersen, C. Stock

**Writing, review, and/or revision of the manuscript:** S.F. Pedersen, C. Stock  
**Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases):** S.F. Pedersen, C. Stock

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