Meeting Report

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 functioning of ion channels and transporters (ICTs) 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 ion channels and transporters 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 towards further elucidating the mechanisms and developing therapeutic applications.
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 have been a focus of interest in cancer research for only ~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. Due to the rapid progress made it is now unquestionable that ion channels and transporters 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 ion channels and transporters in cancer. Taking into account that many participants were associated with the EU-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. 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 progression. 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 $^{23}$Na
magnetic resonance imaging 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 ion channels and transporters 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 pre-mitotic cell shrinkage reflecting increased Cl$^-$ efflux via CIC3 channels has been demonstrated to be necessary for the ensuing cell division (3). This is particularly pertinent in light of the roles of CIC3 channels also in glioma cell motility (see below). A toxin targeting these channels is currently in clinical trials (4). Excitingly, this regime increased survival of glioma patients from $\sim$4 to $\sim$12 months. Cellular pH is also dynamically regulated during cell cycle progression, with G2/M transition being particularly pH-sensitive and dependent on increased activity of the Na$^+/H^+$ exchanger NHE1. This is of relevance given the increased activity of NHE1 and other acid extruding transporters demonstrated in many types of cancer. At the meeting, novel evidence from Diane Barber's group (University of California San Fransisco (UCSF), CA) was presented demonstrating that pH sensors are closely involved in control of cell cycle progression and glycolytic flux. Dysregulation of 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 Kv10.1 (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 Kv10.1 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 ion channels and transporters to cell death regulation and chemotherapy resistance in cancer were presented at the meeting. Within the last decade, it has become apparent that ion channels and transporters play central roles in control of cell death-survival balance (2). Thus, sustained opening of K⁺ and Cl⁻ channels is necessary for apoptotic cell shrinkage and the ensuing cell death (7). John E. Cidlowski (NIEHS, Durham, NC) showed that profound alterations in volume regulatory mechanisms lead to the generation of apoptosis resistant cells. Similarly, dysregulation of cellular Ca²⁺ 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 Kv1.3 channels, which can selectively elicit apoptosis in cancer cells, as shown by the group of Ildikò 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 ion channels and transporters 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 that the role of Kv11.1 (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 Allesandra Fiorio Pla (University of Turin, Italy) demonstrated 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 prognostic value of a locally elevated lactate concentration inside the tumor. Cancer cells themselves often exhibit an alkaline intracellular pH compared to that in normal cells, due to compensatory upregulation of acid extruding transporters such as NHE1 or Na⁺-HCO₃⁻ co-transporters.

At the meeting, evidence was presented by Alzbeta Hulikova (University of Oxford, UK) indicating that in many cancer cells, hypoxia reduces NHE1-dependent acid extrusion, and that Na⁺-HCO₃⁻ 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 ion channels and transporters
(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 (9), a concept called dynamic reciprocity (10). Employing 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 β1 and β3 integrins; instead, these β integrin subunits allow the development of resistance to high-dose radiotherapy.

Diane Barber (UCSF, CA) highlighted protonation as a form of post-translational modification driving changes in protein structure, activity and function. Cellular pH sensor proteins feature specific H⁺-sensitive His residues regulating their function at physiological pH. Examples are the three 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 co-localizes and interacts with the HCO₃⁻ transporters NBCe1 and AE2 in order to keep the cytosol alkaline while 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 Na⁺ channels (Naᵥ), especially Naᵥ1.5 and Naᵥ1.7, 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 Naᵥ channel proteins form functional complexes with β 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 co-workers (Imperial College London, UK) demonstrated that tetrodotoxin (TTX)-sensitive Na\textsubscript{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 Ca\textsuperscript{2+}-permeable non-selective 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 (Nijmegen, The Netherlands) proposed that TRPM7 is part of a mechanosensory complex required for efficient formation of metastases. KC\textsubscript{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 KC\textsubscript{Ca}1.1 channel activity, resulting in an activation of CaMKII which then leads to enhanced migration (Mark Steinle, University of Tübingen, Germany). Irradiation-dependent upregulation of K\textsuperscript{+} channels was also presented for non-small cell lung cancer and chronic myeloid leukemia cells (Bastian Roth, Technical University of Darmstadt, Germany).

**Pancreatic Ductal Adenocarcinoma**

Pancreatic ductal adenocarcinoma (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 towards inflammatory signals. Holger Kalthoff (UKSH, Kiel, Germany) reported that the nuclear (transcription) factor-κB
contributes to non-apoptotic signalling 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 (MCTs). Inhibition of either MCTs or of glycolysis reduces the activity of the plasma membrane Ca$^{2+}$-ATPase, allowing the conclusion that this may represent therapeutic approaches to induce [Ca$^{2+}$] overload-mediated cell death in PDAC (James Andrews, University of Manchester, UK). 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 Mg$^{2+}$ homeostasis and/or kinase function. Stephan Reshkin (University of Bari, Italy) demonstrated 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 desmoplasmic 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 CREB, 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 employed 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 accelerates 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 behaviour, *in vivo* and clinical evidence implicating dysregulation of transporters and channels in cancer is now emerging (e.g. references 4, 5, 11). At the meeting, Mustafa Djamgoz (Imperial College London, UK) summarized the *in vivo* evidence for ‘neonatal’ Na\textsubscript{v} expression in breast cancer (Na\textsubscript{v}1.5) and prostate cancer (Na\textsubscript{v}1.7). Data obtained from human prostate cancer biopsies revealed Na\textsubscript{v}1.7 expression could serve as a clinical biomarker. Similarly, significant positive correlation was found between expression of neonatal Na\textsubscript{v}1.5 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 HCO\textsubscript{3}\textsuperscript{−} increased tumor pH and inhibits spontaneous metastases in mice (Robert Gillies, Moffitt Cancer Center, Tampa, FL) 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 (HAPs) 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, AL). 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 Cl⁻ ions through the CaMK-II regulated ClC3 and K⁺-ions through Ca²⁺ activated K⁺ channels. Therefore, disturbing either the channel-regulating Ca²⁺ signals or the channels themselves inhibits cell volume-tuning and thus reduces the ability to metastasize. William Brackenbury (University of York, UK) proposed that repurposing existing antiepileptic drugs that block Naᵥ channels may have the potential to improve patient outcomes in metastatic breast cancer. Especially the oral anticonvulsant phenytoin inhibits Naᵥ-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, ion channels and transporters 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 whilst 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 pathophysiological 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 epidemiological studies on the wide-spread use of drugs known to inhibit cancer-relevant ion channels, such as the antihistamine astemizole that blocks Kv10.1 channels and anticonvulsants and antiarrhythmics for Na,’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 ITC modulators in cancer therapy. Indeed, the future looks very promising for exploiting ICTs as diagnostic and therapeutic targets in cancer.

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References


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