Ion Channel Blockers and Prostate Cancer Metastasis

Seyhan ALTUN
e-mail: altuns@istanbul.edu.tr
Istanbul University, Faculty of Science, Biology Department, Istanbul,TÜRKİYE

Abstract

Ion channels are transmembrane proteins in the plasma membrane of cells that cause a change in the membrane potential by regulating the ion passage between the inside and outside of the cell. The passage of ions through the membrane is ensured when they open and close. When the ion channels are open, they enable the passive passage of specific ions such as Na^+, K^+, Ca^{2+} and Cl^- through the membrane. Voltage-gated ion channels play a role in the formation and transmission of the action potential. This change in the ion channel activity increases the proliferation of a tumour and/or affects its metastasis. Voltage-gated Na^+ channels (VGSCs) are functionally upregulated in several human carcinomas, including prostate cancer. Prostate cancer is the second most commonly diagnosed male cancer in the world and a leading cause of cancer-related death. It is known that the incidence of prostate cancer is gradually increasing and the deaths that occur result from metastases as in other types of cancer. Studies have focused on the presence of the excess Na^+ ion channels in the membranes of cells with the metastatic capability and on the drugs/agents blocking these channels. The hypothesis that VGSCs play a strengthening role in the metastatic potential of prostate cancer and the metastatic advancement can be repressed by blocking the channels with ion channel blockers was evaluated *in vitro* and *in vivo*. We investigated the effect of VGSC blockers such as tetrodotoxin, lidocaine, gabapentin, ranolazine and riluzole on lung metastases and the migration and invasion of highly metastatic Mat-LyLu and low metastatic AT-2 cells, in the Dunning model.

According to the data obtained; *i*) Experiments conducted with tetrodotoxin proved that the Na_v 1.7 channel activity increases metastasis. *ii*) The fact that this role of the Na+ channel is related to the persistent current was determined with ranolazine experiments. *iii)* It should be noted that drugs such as gabapentin administered for cancer pain are also effective on cancer, and they should be used with care. *iv*) It is necessary to determine the therapeutic effects of lidocaine and riluzole that are effective on Na_v 1.7 by investigating them *in vivo*.

It is necessary to deepen the studies on the effects of VGSC blockers that increase survival by decreasing metastasis on human cells/xenograft models.

Keywords: Prostate cancer, Ion channel blockers, Metastases, Invasion, Migration.

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Functional Effects of Matrix Metalloproteinase-9 on Voltage-Gated Sodium Channel Nav1.5 and β1 Regulation in Human Breast Cancer Cells

Gülgün OKTAY
e-mail: gulgun.oktay@deu.edu.tr
Dokuz Eylul University, School of Medicine, Department of Medical Biochemistry, İzmir, TÜRKİYE

Abstract

Voltage-gated sodium channels (VGSC) are glycosylated transmembrane proteins transmitting fast (transient-I_{NaT}) and prolonged (persistent-I_{NaP}) currents enabling Na^+ flow into cells in order to create action potentials. VGSCs are expressed in conventionally 'excitable' cells, such as neurons and muscle cells, as well as 'non-excitable' cells such as endothelial cells, lymphocytes, osteoblasts, fibroblasts, and renal tubular epithelial cells. In recent years, *in vitro* and *in vivo* studies have shown that VGSCs are overexpressed in a variety of human cancers of *epitelial* origin, including breast, prostate, ovary, cervix, colon, lung and skin cancer, where expression is associated positively with metastatic potential. However, the mechanisms responsible for the functional VGSC expression in cancer cells are not clear. Voltage-gated Na^+ channels exist as pore-forming alpha subunits ('VGSCα's) which are responsible for the ionic permeation and smaller non-pore-forming beta subunits ('VGSCβ's) which are responsible for structural interactions and adhesiveness. It has been suggested that VGSCα subunits are upregulated in parallel with metastatic potential, whereas VGSCβ subunits are expressed more in weakly metastatic cancer cells which are relatively more adhesive.

Breast cancer is the most common type of cancer after lung cancer in women. Metastasis is the major cause of death in breast cancer patients. In tumor metastasis, the degradation and modulation of extracellular matrix by proteolytic enzymes is a critical step. Among these proteolytic enzymes, matrix metalloproteinases (MMPs) play a key role in the digestion of extracellular matrix components (proteolytic activity) and in signal transduction pathways (non-proteolytic effect).

The main aim of our project is to examine mechanistically mutual functional interactions involving matrix metalloproteinase-9 MMP-9), the VGSC subtype $Na_v1.5$ (gene: *SCN5A*), and the VGSCβ subtype, β1(gene: *SCN1B*). Mainly the metastatic human breast cancer MDA-MB-231 cells will be used as a model to shed light on the metastatic process. Thus the following approaches will be adopted:

1. MMP-9 silencing and overexpression; effects on Nav1.5 and β1expression and activity;
2. *SCN5A* silencing and pharmacological inhibition with tetrodotoxin (general VGSC inhibitor) and Ranolazine (specific I_{NaP} inhibitor); effects on MMP-9 expression and activity and SCN1B expression; and
3. *SCN1B* overexpression; effects on MMP-9 and $Na_v1.5$ expression and activity.
4. The effects of all these treatments on the following metastatic cell behaviors will be determined: adhesion, lateral migration and matrigel invasion).

In this project, we shall determine for the first time how MMP-9, which is in the upstream of VGSC, affects alpha ve beta subunit expression and how these effects contribute to the process of invasion and metastasis. In this manner, this *in vitro* study will enlighten further the role of VGSC/I_{NaP} in cancer progression.

Keywords: Voltage-gated sodium channels, Matrix Metalloproteinase-9, Breast cancer cells.

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Voltage Gated and the Oxidative Stress-Induced Ionic Currents in the Neurones of the Ventral Cochlear Nucleus

Ramazan BAL
e-mail: ramazanbal1969@gmail.com
Gaziantep University, Faculty of Medicine, Department of Physiology, Gaziantep, TURKEY

Abstract

The fibers of the auditory nerve project to the cochlear nucleus (CN). The CN divide into two region, the dorsal cochlear nucleus (DCN) and the ventral cochlear nucleus (VCN). The VCN has three cell types, octopus, bushy and stellate cells. Octopus cells are populated caudally in the posterioventral CN (PVCN). The other two cell types are found interminglingly in the anterior part of the ventral CN (AVCN).

Synaptic responses and the firing pattern of these three cells are mainly governed by their unusual biophysical characteristics. In vitro recordings, both bushy and octopus cells respond to DC current pulses at the onset of the stimulus with a single action potential. Whereas, stellate cells responded with a train of action potentials.

Onset responses were found to be resulted from the large resting conductances of, mixed-cation conductance (g_h) and low-voltage activated potassium conductance (g_{KL}). These two conductances are the largest current amplitude recorded in the mammalian CNS neurons. Amplitudes of maximum mixed-cation conductance and low-voltage activated potassium conductance were 150 nS and 512 nS. Even though stellate cells had these ion channels, they are relatively small in amplitude. These cells had calcium channels as well. Furthermore, ventral cochlear nucleus (VCN) neurons had two ion channels induced by oxidative stress, TRPM2 current and ATP-sensitive potassium (KATP) current. The amplitude of the TRPM2 conductance was 5.1 nS, which is induced by intracellular ADPR application. amplitude of the ATP-sensitive potassium maximum conductance in octopus cells was 17.2 nS.

In conclusion, voltage gated ion channels govern the action potentials. Whereas, oxidative stress induced conductances, TRPM2 and KATP, are relatively small, they can change the resting membrane potential and neurons excitability of the neurons with high input resistance such as stellate cells. Small modulatory currents mediated by TRPM2 or K_{ATP} in stellate cells cause large changes in the firing rate, due to their relative high input resistance and low firing thresholds.

Natural Products and Ion Channels

Hatice GUMUSHAN AKTAS
e-mail: haticegumushan@gmail.com, haticeaktas@harran.edu.tr
Harran University, Faculty of Arts & Science, Biology Department, Sanliurfa, TURKEY

Abstract

Ion channels are transmembrane proteins found in the cell and organelle membranes of all living organisms. These proteins allow the transport of ions such as sodium, potassium, calcium, hydrogen, chlorine between inner and outer sides of the cellular membranes. Each channel carries specific ions. Some like potassium leakage channels are constantly open. Others are switched on and off in response to voltage changes or the attaching of a ligand. Also in another group, there are connexins forming gap junctions which allow the transport of various ions and molecules from the cell to the cell, and channels like Piezo which are opened as a response to mechanical stimulation. Ion channels have important roles in all cellular processes such as stimulation of nerve and muscle cells, maintenance of resting potential, transfer of ions and various metabolites between cells, hormone release, sensory conduction, learning, memory, regulation of blood flow, protection of homeostasis, control of cell cycle, apoptosis, and cell movement. Therefore, mutations or the changings of functions that occur in genes encoding these channels play a role in many disorders such migraine, diarrhea, hypertension, depression, and cancer and metastases as.

The medicines which acting on ion channels are used long in the treatment of many diseases such as epilepsy, because of the ion channels play roles in many cellular processes. These drugs have synthetic or natural origin. Natural compounds are small molecules with a biological activity and chemical structure that are produced as a primer or secondary metabolite in any organism. Today, there is an increasing trend towards natural nutrition, which we can called as "return to nature". In this trend, herbal or animal products are consumed for preventive purposes or for treatment.

Natural compounds may be obtained from bacteria or fungi, as well as animal or herbal origin. The most studied Tetrodotoxin (TTX) is a specific blocker for voltage-gated sodium channels (VGSCs). TTX can be isolated from poisonous *Fugu sp.* fish, various amphibians and molluscs. Grayanotoxins, which increase ionic current in VGSCs, are of plant origin. Margatoxin, isolated from a scorpion species, blocks voltage-gated potassium channels. Dehydrosaponin I, which activates the calcium-activated potassium channel, is a secondary metabolite of a plant from the Leguminosae family. Avermectin b1a, which increases the opening of the voltage-gated chlorine channel, is a toxin of *Streptomyces sp*. bacterium. Chlorotoxin, a chlorine channel blocker, is isolated from a scorpion. The muscimol, which exhibits agonist activity on the GABA-A receptor, which is a kind of ligand-gated ion channels, is obtained from *Amanitha muscaria* fungi. Conotoxin is used as local anesthetic, and it is a toxin obtained from sea snail.

In conclusion, the novel natural compounds may have important biological activities on ion channels. Therefore it is necessary to investigate deeply the effects of them.

Keywords: Ion channels, natural compounds, ion channel blockers and openers, cancer, metastases

Environmental Factors and their Mechanisms of Action in Carcinogenesis

Abdurrahim KOCYIGIT
e-mail: abdurrahimkocyigit@yahoo.com
Bezmialem Vakif University, Faculty of Medicine, Department of Medical Biochemistry, Istanbul, TURKEY

Abstract

Cancer is a genetic disorder in which both internal factors (inherited mutations, hormones and immune conditions) and environmental /acquired factors (like tobacco, diet, radiation and infectious organisms) are caused. It has been estimated that only 5–10% of all cancers are due to an inherited gene defects, the others are due to environmental factors. These factors modulate some of the important cellular elements, including genes such as proto-oncogenes, tumor suppressor genes and DNA repair genes, via cellular intermediates. One of the most important cellular intermediates is the reactive oxygen species (ROS) produced in all aerobic organisms and environmental factors such tobacco smoking, irradiation and inflammation. Chronic and cumulative ROS causes harmful changes in various macromolecular components such as DNA, lipids and proteins. ROS can also indirectly mediate indirect attack on DNA and cause DNA damages. The formation DNA damage is located at the center of the carcinogenic process, because if such adducts are confused with cellular repair mechanisms and persistence, they can lead to misspellings and eventual mutations and cancer. It has been shown that there is an 80-90% association between DNA damage and cancer. Major environmental factors that cause cancer by making DNA damage; physical factors (ionizing radiation, ultraviolet rays, asbestos), biological factors (bacteria, viruses and inflammatory diseases), chemical agents (tobacco, alcohol, foods, factors, chemotherapeutics and insecticides) and others (stress, obesity, immune deficiency). These factors usually cause DNA damage by increasing ROS production either directly or indirectly. We can show the DNA damage level by alkaline single cell gel electrophoresis (Comet Assay) which is a sensitive and sensitive test. Thus, we can predict the risk of cancer by environmental factors. People can avoid some cancer-causing exposures, such as tobacco smoke and the sun's rays. But others are harder to avoid, especially if they are in the air we breathe, the water we drink, the food we eat, or the materials we use to do our jobs. Scientists are studying which exposures may cause or contribute to the development of cancer. Understanding which exposures are harmful, and where they are found, may help people to avoid them. As a result, environmental risk factors that cause DNA damage play an important role in the development of cancer, successful management of lifestyle and environmental pollution can reduce cancer risk.

Keywords: Cancer, environmental risk factors, reactive oxygen species, DNA damage

Thiol Disulfide Homeostasis

Özcan EREL
e-mail: erelozcan@gmail.com
Ankara Yıldırım Beyazıt Univ. Med Fac. Medical Biochem. Dept. Atatürk Hospital, Ankara,TURKEY

Abstract

Thiols (mercaptan) are functional sulfhydryl (R-S-H) groups containing a sulfur atom attached to a carbon atom and a hydrogen atom bound to the sulfur atom. The vast majority of the plasma / serum thiol pool consists mainly of albumin and other proteins, while a small portion consists of low molecular weight thiols such as cysteine, cysteinyl glycine, glutathione, homocysteine, and γ-glutamylcysteine.

Thiol groups are oxidized by oxidant molecules under physiological conditions to form a reversible disulfide bond structure. The resulting disulfide bond is also a covalent bond and is also referred to as the -S-S bond or the disulfide bridge. These disulfide bonds can be reduced again to the thiol groups so that the dynamic thiol / disulfide balance is maintained.

The dynamic thiol / disulfide balance has important roles in the regulation of antioxidative protection, detoxification, signal transduction, apoptosis, enzymatic activity, transcription factors and cellular signaling mechanisms. Changes in thiol / disulfide balance are involved in the pathogenetic mechanisms of many diseases such as multiple sclerosis, diabetes mellitus, cardiovascular diseases, cancer, rheumatoid arthritis, chronic kidney disease, FMF, CCHD, tonsillitis, appendicitis, brucella, schizophrenia, Parkinson's disease, Alzheimer's disease and liver diseases.

Thiol disulfide homeostasis can be measured in the laboratory by spectrophotometer and automatic analyzer. This test profile has a high potential to generate new information and has potential to be included in routine test profiles in the near future.

Integration of Transcriptomics and Functional Genomics to Study Drug Resistance and Metastasis in Cancer

Özgür ŞAHİN
e-mail: osahin@fen.bilkent.edu.tr
Bilkent University, Faculty of Science, Department of Molecular Biology and Genetics, Ankara, TURKEY

Breast cancer (BC) is one of the most common cancers with >1,300,000 cases and 450,000 deaths each year worldwide. Two most aggressive subtypes of BC are HER2-overexpressing and triple negative breast cancers (TNBCs). As most cancer-related deaths occur due to resistance to therapies and metastasis, it is crucial to elucidate the molecular mechanisms underlying these processes. In this respect, systems level approaches combining Next Generation Sequencing, network modeling, functional genomics and cancer cell biology provide new avenues for targeting protein or non-coding RNA networks in an efficient way which may lead to blockage of by-pass mechanisms or metastasis spread of the cells.

In my talk, I will first present how we (1) use HER2-overexpressing transgenic mouse models and breast cancer cell lines to develop drug resistant models for HER2-targeting agents, and (2) integrate data from cancer patients' expression profiling, TCGA and RPPA to identify biomarkers of drug response, and (3) perform targeted RNAi screening to identify drug combinations or order of treatments to overcome therapy resistance in this highly aggressive subtype of breast cancer.

In the second part, I will present how we integrate Next Generation Sequencing (RNA-Seq, miR-Seq) data obtained from our mouse models representing chemotherapy resistant or metastatic TNBCs to build 'miRNA-mRNA' or 'miRNA-mRNA-lncRNA' interaction networks. I will then show how we identify key components of these networks using several computational tools to identify novel drug targets, simulate their loss-of-functions and experimentally validate them in order to overcome chemotherapy resistance or block metastasis in TNBCs.

Keywords: breast cancer, drug resistance, metastasis, next generation sequencing, noncoding RNAs

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Specifically Targeting Cancer Cells for Theranostic Applications through Glycobiology, Metabolic Glycoengineering, and Nanotechnology

Hafiz Ahmed, Ph.D.
e-mail: hfzahmed86@gmail.com
GlycoMantra, Biotechnology Center at the University of Maryland Baltimore County, Baltimore, Maryland, USA

Abstract

Cell-cell interactions are essential for the survival of the cell. Cell-cell interactions play crucial role in communicating with each other in response to changes in their microenvironment and the loss of this communication between cells can lead to uncontrollable cell growth and cancer. To specifically target cancer cells for both diagnostic and therapeutic (theranostic) applications, we are employing multiple approaches such as glycobiology, metabolic glycoengineering, and nanotechnology. For example, cell surfaces of more than 90% epithelial cancers contain exposed TF-disaccharide (TFD) in their *O*-glycosylated proteins. In tumors, galectin-3 (gal3) interacts with TFD containing glycoproteins such as MUC2 and plays a significant role in promoting cancer progression and metastasis. In normal cells, TFD is masked by sialic acid and cannot bind with the gal3 and so TFD-gal3 interaction is specific to cancer cells. To prevent cancer metastasis, we have developed a very high affinity gal3 antagonist (GM101) that specifically blocks gal3-TFD interactions. GM101 blocks gal3-mediated angiogenesis, tumor-endothelial cells interactions, and prevents metastasis in mice.

In another approach, we are exploiting cancer cells' abnormal energy metabolism to target cancer cells. We have recently shown that nutrient-deprived cancer cells when supplemented with sialic acid utilize negligible amounts of this sugar for energy compared to their non-malignant counterparts. Instead, malignant cells use sialic acid to maintain cell surface glycosylation through complementary mechanisms. We have determined the nature of sialylation and developed tools to specifically target the cancer cells. Moreover, theranostic nanoparticles are being developed for simultaneous diagnosis of diseases and targeted drug delivery with minimal toxicity.

The Place and Importance of CFSE Method in Determining Cell Cytotoxicity

Faruk SÜZERGÖZ
e-mail: suzergoz@yahoo.com
Harran University, Faculty of Arts and Sciences, Biology Department, Şanlıurfa, TURKEY

Introduction/Aim: Although many advanced molecular biological methods have been tried for cancer treatment; chemotherapy is still one of the preferential method in cancer treatment as a method that maintains its validity. One of the most important parts of the chemotherapeutic agent research is the determination of the ability of herbal or chemical compound to kill or inhibit proliferation of the of cancer cells *in vitro*. Mostly in studies; the cytotoxic or antiproliferative effects of the tested compounds on cancer cells are investigated by similar test methods and the results are jointly evaluated. In recent studies; these two situations are trying to be separated from each other to develop more sophisticated methods for each one. In methods used to determine cell proliferation: based on following of DNA synthesis rate, ATP amount, metabolic activity, change in dyeing properties, specific markers.

Materials and Methods: The technique for analysing cell division using serial dilution of the fluorescein-based dye carboxyfluorescein diacetate succinimidyl ester (CFSE), described by Lyons and Parish in 1994. CFSE consists of a fluorescein molecule containing two acetate moieties and a succinimidyl ester functional group. In this form, it is membrane permeant and non-fluorescent. After diffusion into the intracellular environment; endogenous esterases remove the acetate groups, rendering the molecule highly fluorescent and non-permeant to the cell membrane. In addition, the succinimidyl ester reacts with free amine groups of intracellular proteins, forming dye–protein adducts. CFSE is membrane–permeant fluorescent molecules that are retained within the cells through subsequent rounds of cell division, with each daughter cell receiving approximately half of the fluorescence of the parent. CFSE dye enters the cell and trapped there. Upon division, the fluorescence intensity of each daughter cell is halved which allows for the simple detection of the number of cell divisions by flow cytometry.

Results: CFSE stains cells at high fluorescence intensities (in the FL-1 histogram 103 -104) and CFSE has low toxicity for cells. These properties make CFSE ideal for long-term cell proliferation monitoring. CFSE has very bright fluorescence; therefore location of cells in sections can be determined using confocal or fluorescence microscopy.

Conclusion: CFSE can be used without affecting morphology or physiology to trace generations *in vivo* or *in vitro*.

Keywords: CFSE, Cell proliferation, Flow cytometry

Oncology Nursing: Breast Cancer and Social Support

Fatma ERSİN
e-mail: fatmaersin1@gmail.com
Harran University, Faculty of Health Science, Nursing Department, Sanliurfa, Turkey

Abstract

The studies on breast cancer which is frequently discussed in the field of oncology nursing have gained momentum. A total of 8,2 million cancer-related deaths and 14,1 million new cases of cancer occurred worlwide in the year of 2012 while breast cancer (11.9%) is the second most prevalent cancer in the world. Breast cancer is also the leading cancer in the women in Turkey. Nowadays, cancer is perceived as a life-threatening disease, and it remains a very feared disease in spite of significant improvements in its treatment. It is stated in literature that women with breast cancer experience many problems in the physical, social, psychological, and spiritual fields. Because of this reason, women's daily life activities and quality of life deteriorate. In addition, it was determined that there were some psychosocial problems in these patients when the studies on the women with breast cancer were examined. Anxiety, the thought of self-destruction, social isolation, depression, decrease in self-esteem, hopelessness, sexual dysfunction are frequently mentioned problems in the literature. These problems that arise in the women require the use of supportive approaches in the treatment and care of the patients. At this stage, many women need social support. Social support is important in the recovery process, this condition is independent from the success of the treatment in survival. It is stated in the literature that your social support is very effective in the diagnosis, treatment and rehabilitation of women with breast cancer. At this stage, nursing services come to the forefront, because nurses support the women by identifying the social support needs of the women, and offer the services in this regard. This review was designed to show the effect of social support on the women with breast cancer.

Keywords: Oncology Nursing; Breast Cancer; Social Support, Nurse

Recent Developments in Targeting Carbonic Anhydrase IX for Cancer Therapeutics

İsmail KOYUNCU
e-mail: ismailkoyuncu1@gmail.com
Harran University, Medical Faculty, Department of Medical Biochemistry, Sanliurfa, Turkey

Abstract

The major factor that limits success in cancer therapy is the nonselectivity of the anticancer agents towards tumor cells and tissues. Therefore new studies are conducted for detection of specific novel molecules for cancer cells. Specifically the expression pattern of Carbonic Anhydrase IX (CAIX) in solid tumors has led to the use of CAIX as a biomarker, since it can provide evidence about tumor identification and prognosis. Sulfonamides posses many types of biological activities and have recently been reported to show substantial antitumor activity *in vitro* and/or *in vivo*. There are a variety of mechanisms for the anticancer activity and the most prominent of these is through the inhibition of carbonic anhydrase isozymes.

The aim of this study was to evaluated the effects of CAIX inhibitors, namely amine and imin sulfonamides derivatives newly synthesized aromatic sulfonamides with high affinity for CA IX, on cell proliferation and apoptosis in CAIX-positive and CAIX-negative cell lines and correlate these findings with CAIX expression. We sought to investigate the molecular mechanisms underlying the potential apoptotic effect of CAXI inhibitors. Effect of sulfonamides on cell viability was determined by WST-1 assay and then IC_{50} value of each component was determined. Apoptosis or necrosis induction was determined by flow cytometric annexin V analyse, intracellular free radical induction will be determined by ROS analyse, intracellular pH induction was determined by fluorescens prob analyses; Antiproliferative effects of compounds was determined by BrdU elisa assay, cellular mitochondrial membrane potential was determined by JC-1 assay, CAIX and apoptosis (cleaved caspase 3,8 and 9) was investigated using level at protein level using Western blotting method, respectively.

At the end of the study it was recognized that 4 of the 16 sulfonamide derivative compounds have high cytotoxic and antiprolifetaretive effects on HeLa cells. It was decided that cytotoxic effect was occured by apoptosis and CAIX expression and partial increases in pH would be due to inhibition of those effects with CA-IX. The results of data derived from this study will inspire the projects in which those materials are used and which are on hypoxia, 3-D cell culture medium and consecutively animal cells and especially will serve distinctive research areas in cancer treatment.

Keywords: Sulfonamide, Carbonic Anhydrase-IX, Cancer, Cytotoxicity,

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