

autoimmune diseases, effector memory T cells have a unique ion channel pattern, therefore they are promising therapeutic targets. A number of ion channel inhibitors are known as selective inhibitors of T lymphocyte proliferation, but the data available is contradictory. Our aim was to elucidate this phenomenon by investigating how the blockage of ion channels affects the activation and proliferation of T cells treated previously with different concentrations of mitogens.

In our experiments human peripheral blood lymphocytes from volunteers were activated via monoclonal antibodies affecting the TCR-CD3 complex on the cell surface and the co-stimulator molecule CD28. We applied specific ion channel blockers acting on the major cationic channels of the T cell, the Kv1.3, the KCa1.1 and the CRAC channel, either alone or in combination with rapamycin, the inhibitor of the mammalian target of rapamycin (mTOR). Five days after the stimulus flow cytometry measurements were performed to determine the extent of cellular viability and proliferation.

Our measurements indicated that ion channel blockers and rapamycin had a negative dose-dependent effect on the amount of cell division. Simultaneous application of blockers for each channel along with rapamycin proved to be the most effective, which indicates that they affect independent regulation pathways. Upon increasing the rate of stimulation, the anti-proliferative effect of the blockers diminished. This phenomenon was unknown to date and may prove to be important in understanding the fine-tuning of T cell activation.

2963-Pos Board B393

Proton Channels are Present in Cell Membranes of the Breast Cancer Cell Line MDA MB 231 and Affect Recovery from an Acid Load

Leri Morgan¹, Patrick McIntire¹, Vladimir Cherny¹, Susan Smith², Boris Musset³, Thomas DeCoursey¹.

¹Molecular Biophysics, Rush University Medical Center, Chicago, IL, USA,

²Department of Biology & Physics, Kennesaw State University, Kennesaw,

GA, USA, ³Institute of Complex Systems, Forschungszentrum Jülich, Jülich, Germany.

Voltage gated proton channels (HV1) have been implicated in late stage breast cancer (Wang et al, 2012. J. Biol. Chem. 287:13877), where HV1 expression correlated with tumor size and poor prognosis. HV1 knockdown reduced cell proliferation and migration as well as matrix metalloprotease release. However, the presence of functional HV1 on cancer cell membranes has not been demonstrated and the mechanism by which they affect the function of these cells has not been elucidated.

Here we show the definitive presence of functional HV1 on the membranes of MDA MB 231 cells, a highly metastatic triple negative cell line. We performed patch clamp experiments on these cells and were able to detect bona fide voltage- and pH-gated channels that were perfectly selective for protons. The membrane density of the channels in these cells was recorded as 3.5 pA/pF which is roughly 5-fold lower than the 15 pA/pF displayed in neutrophils. In order to show that HV1 expresses at a level sufficient to impact pH regulation within these cells, we acid loaded them using the ammonium prepulse technique and monitored pH recovery utilizing SEER with SNARF-1. Inhibiting HV1 with 1 mM Zn²⁺ slowed recovery from an acid load by 3-fold, demonstrating that the expression of HV1 on these cells affects pH regulation in these cells.

We conclude that one mechanism by which HV1 may influence the pathophysiology of breast cancer is by improving the ability of breast cancer cells to regulate their internal pH.

Supported by the Bears Care and the Brian Piccolo fund.

2964-Pos Board B394

TOK1 Potassium Channels in Phytopathogenic Fungi

William R. Manville¹, Andrew Corran², Anthony Lewis¹.

¹School of Pharmacy and Biomedical Sciences, University of Portsmouth, Portsmouth, United Kingdom, ²Fungicide Biochemistry, Syngenta Limited, Bracknell, United Kingdom.

Fungal plant pathogens are a significant threat to crop yield and global food security and the search for pathogen-specific agricultural fungicide targets is of high priority. TOK1 is a structurally and functionally unique plasma membrane potassium (K⁺) channel with no known homologues in plants or animals and is the only passive K⁺ ion efflux pathway in fungi. Activation of TOK1 leads to ion dyshomeostasis and cell death. However, little is known about TOK1 channels in phytopathogenic fungi. Here we describe the distribution, evolution and molecular conservation of TOK1 homologues across plant fungal phyla, and the cloning and characterization of TOK1 channels from two phytopathogens of significant socio-economic importance. *In-silico* bioinformatics identified genes predicted to encode putative TOK1 protein subunits, conforming to the characteristic eight transmembrane domain two pore domain (8TM/2P) structure, in 204/231 sequenced fungal genomes analysed. Molecular con-

servation of TOK1 primary structure was greatest in both pore domains and flanking pore lining transmembrane domains, TM6 and TM8. MgTOK1 from *Mycosphaerella graminicola* (wheat leaf blotch) and FgTOK1 from *Fusarium graminearum* (wheat head blight) were cloned by RT-PCR into an expression vector. cRNA was transcribed *in-vitro* and injected into *Xenopus laevis* oocytes and ionic currents measured by two-electrode voltage clamp after 24-48 hours. Both cloned TOK1 channels exhibited K⁺ selective, non-inactivating, strongly outwardly-rectifying K⁺ currents whose activation threshold was strictly determined by the transmembrane K⁺ gradient, as revealed by isotonic replacement of extracellular Na⁺ with K⁺. Channels varied in their voltage-dependent activation kinetics and distinct from the canonical TOK1 isolate from *Saccharomyces cerevisiae*, displayed signs of time-dependent deactivation. This appears to be the first reported molecular identification and characterisation of TOK1 K⁺ channels from plant pathogenic fungi.

2965-Pos Board B395

KCa1.1 (BK) Channels on Fibroblast-Like Synoviocytes: A Novel Therapeutic Target for Rheumatoid Arthritis

Mark R. Tanner¹, Zoltan Petho², Rajeev B. Tajhya¹, Redwan Huq¹,

Frank T. Horrigan¹, Percio S. Gulco³, Christine Beeton¹.

¹Department of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX, USA, ²Biophysics and Cell Biology, University of Debrecen, Debrecen, Hungary, ³Division of Rheumatology, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease attacking principally freely-moveable joints and affecting approximately 1.3 million in the US. Recently, joint-resident fibroblast-like synoviocytes in RA (RA-FLS) have been implicated in disease pathogenesis. We have shown that KCa1.1 is the predominant potassium channel expressed by RA-FLS and by FLS from the pristane-induced arthritis (PIA) rat model of RA. Blocking KCa1.1 with paxilline or ibertoxin or reducing its expression with siRNA inhibited the production of pro-inflammatory cytokines, chemokines, and proteases, and the invasiveness of both RA- and PIA-FLS. In contrast, the over-expression of KCa1.1 increased the invasiveness of PIA-FLS and induced the invasiveness of healthy rat FLS. These data demonstrate a crucial role of KCa1.1 in regulating the aggressive behavior of FLS during RA.

We induced two models of RA in rats, moderate PIA and severe complete Freund's adjuvant collagen-induced arthritis. Treatment with the small molecule KCa1.1 blocker paxilline, starting after onset of clinical signs, significantly reduced disease severity in both models. However, paxilline can cross the blood-brain barrier and block all variants of KCa1.1 throughout the body, thereby inducing side effects that preclude its use as a therapeutic for human use without significant modification. A strategy to target KCa1.1 on RA-FLS without side effects in other tissues involves the identification of the β and γ regulatory subunits of KCa1.1 in RA-FLS through qPCR, western blotting, and patch-clamp electrophysiology. This has the potential for the development of blockers that selectively target KCa1.1 on RA-FLS and do not enter the central nervous system.

2966-Pos Board B396

Kidney CLC-K Chloride Channels Inhibitors: Definition of Novel Structural Requirements and Efficacy in CLC-K Polymorphism Associated with Hypertension

Paola Imbrici¹, Antonella Liantonio¹, Giuseppe Fracchiolla¹,

Giuseppe Carbonara¹, Maria Maddalena Dinardo¹, Michael Pusch²,

Diana Conte¹.

¹Department of Pharmacy - Drug Sciences, University of Bari, Bari, Italy,

²Institute of Biophysics, CNR, Genova, Italy.

The human chloride channels CLC-Ka and CLC-Kb play a pivotal role in kidney by controlling chloride and water absorption. Both channels require barttin as an accessory subunit for full activity. Mutations in CLC-Kb and barttin genes lead to severe renal salt loss while CLC-K gain of function polymorphisms could predispose to hypertension. Thus, compounds that selectively bind to CLC-Ka and/or CLC-Kb channels may have a significant therapeutic potential. Recently, we explored the pharmacological profile of CLC-K/barttin expressed in mammalian HEK-293 cells and demonstrated that HEK cells represent a valid biological system to screen CLC-K high affinity blockers (Imbrici et al., Biochim Biophys Acta, 2014). Here, by using molecular modeling and patch-clamp technique, we developed a new series of benzofuran derivatives and evaluated their efficacy on CLC-K channels expressed in HEK 293 cells. Chemical modifications regarding the hydrophobic group at C-5 and C-3 position of the benzofuran nucleus of the lead compounds RT-93 and JBL-44 (IC50 within 10-30 μ M range), allowed us to define the structural requirements to ensure an efficacious CLC-Ka block, finally identifying SRA-36 the most potent compound so far described, with an IC50 of 2.6 ± 1 μ M. Interestingly,