BM1406: Ion Channels and Immune Response toward a global understanding of immune cell physiology and for new therapeutic approaches (IONCHAN-IMMUNRESPON)

Fourth MEETING
23-24th March 2017

University of Belgrade, Rectorate building

Belgrade, SERBIA

Belgrade meeting
COST Action N° BM1406
Rectorate Building, University of Belgrade
March 23th -24th 2017
Scientific program

Thursday 23th March

8:30 **Registration**

09:00 **Welcome Address**

Chair: Francesco Di Virgilio (University of Ferrare, Italy)

09:10 –10:10 **Keynote speaker Marco Rossato (University of Padova, Italy)**

10:10 – 10:30 Break and Posters

10:30 – 11:00 **Gyorgy Panyi (University of Debrecen, Hungary)**
Therapeutic targeting of ion channels in immune cells

11:00 – 11:30 **Vincent Jaquet (University of Geneva, Switzerland)**
NOX inhibitors: characterization and potential applications for therapy in human diseases

11:30 – 12:00 **Ankita Agrawal (University of Copenhagen, Denmark)**
Effects Of P2X7R Activation On Receptor And Cell Functions In Human Myeloma Cell Lines

12:00 – 12:30 **Romain Krzysiek (INSERM Unit 996, CHU Bicêtre, Paris, France)**
Characterization of tumour infiltrating Foxp3+ Tregs-specific channelome by whole-genome transcriptional profiling

12:30 – 14:00 Lunch

Young Researcher's session

14:00 – 14:20 **Martinez J.** *P2X7 receptor associates with mitochondrial dysfunction in monocytes during severe sepsis*

14:20 –14:40 **Ellegard M.** *The role of the P2X7 receptor in bone remodelling*

14:40 – 15:00 **Visic P.** *The regulation of the store operated calcium entry (SOCE) process by Sigma-1R*

15:00 – 15:20 **Pislar A.** *Cathepsin X Involvement in Neuroinflammation-Induced Neurodegeneration*

15:20 – 15:40 **Rodrigues C.** *Aquaporins are modulated by oxidative stress in cancer cells*

15:40 – 16:00 **Damjanovic A.** *The effects of Mahonia aquifolium extracts on doxorubicin cytotoxicity against lung adenocarcinoma cells.*

16:00 – 16:30 Break and Posters

BM1406 MC Meeting

16:30 – 18:30 **Management Meeting for the Country representatives**

20:00 – Social dinner

Friday 24th March

Chair: Ruth Murrel-Lagnado (University of Sussex, England)

9:00 – 10:00 **Keynote speaker Stefan Feske (NY Medical School, USA)**
Role of CRAC channels in T lymphocyte functions

10:00-10:30 Break and Posters

10:30 – 11:00 **Ruth Murrel-Lagnado (University of Sussex, England)**
P2X receptors and lysosome function

11:00 – 11:30 **Andriana Kavallari (University of Athens, Greece)**
Ion channels in mast cell pharmacology

11:30 – 12:00 **Ana Čipak Gašparović (*Rudjer Boskovic Institute, Zagreb, Croatia)***
Yeast aquaporin is implicated in oxidative stress response

12:00 – 12:30 **Prof. Pavle Andjus (University of Belgrade, Serbia)**
IP3R and store-operated calcium entry in astrocytes and the effect of IgGs from amyotrophic lateral sclerosis patients

12:30 – 14:00 Lunch

Chair: Jelena Antic Stankovic (University of Belgrade, Serbia)

14:00 – 15:00 **Romain Lara (Biosceptre International, Cambridge UK)**
NfP2x7 a cancer specific therapeutic target

15:00 –15:30 **Erin Nuray (University of Akdeniz, Antalya Turkey)**
TRPV1 channels of sensory nerves, tumour cells and immune cells: Do all work against breast cancer?

15:30 – 16:00 **Ozlen Konu (University of Bilkent, Ankara Turkey)**
Analysis of synergism and antagonism between miR-495-3p and cholinergic receptor nicotinic alpha 5 (CHRNA5) in MCF7

16:00 – 16:30 **Paula Nunes-Hasler (University of Geneva, Schwitzerland)**
STIM1 promotes phagosomal maturation and antigen cross- presentation in dendritic cells.

16:30 - 17:00 Break and Posters

Round Table

17:00 – 18:00 **Round table "Inhibitors of ion channels as therapeutics"**
Organizers F. Di Virgilio and Ruth Murrel-Lagnado

18:00 Closing remarks

First Name	Last Name	Abstract (subject)
Ankita	Agrawal Ej Efternavn	Effects Of P2X7R Activation On Receptor And Cell Functions In Human Myeloma Cell Lines
Pavle	Andjus	IP3R and store-operated calcium entry in astrocytes and the effect of IgGs from amyotrophic lateral sclerosis patients
Iva	Bozic	Voltage gated potassium channel Kv1.3 is upregulated on astrocytes during experimental autoimmune encephalomyelitis
Sabrina	Brechard	Regulation of neutrophil cytokine secretion by Ca2+ signalling
Stéphanie	Chadet	Role of the P2X4 receptor in breast cancer cell invasiveness
Ana	Čipak Gašparović	Yeast aquaporin is implicated in oxidative stress response
Agota	Csoti	Characterization of a novel high-selectivity Kv1.3 inhibitor peptide
Ana	Damjanovic	The effects of Mahonia aquifolium extracts on doxorubicin cytotoxicity against lung adenocarcinoma cells, in vitro
Maria	Ellegaard	The role of the P2X7 receptor in bone remodelling
Stefan	Feske	Role of CRAC channels in T lymphocyte functions
Ivana	Gadjanski	Expression pattern of CaV1.2 type of voltage-dependent calcium channels is altered after cortical injury in rats
Peter	Hadju	Targeting Kv1.3 channels of T cells in autoimmune diseases
Vincent	Jaquet	NOX inhibitors: characterization and potential applications for therapy in human diseases
Ester	Katja	Measurements of ion fluxes in breast tumor cells using Microelectrode flux estimation
Andriana	Kavallari	Ion channels in mast cell pharmacology
Ozlen	Konu	Analysis of synergism and antagonism between miR-495-3p and cholinergic receptor nicotinic alpha 5 (CHRNA5) in MCF7 breast cancer cells
Roman	Krzysiek	Characterization of tumor infiltrating Foxp3+ Tregs-specific channelome by whole-genome transcriptional profiling
Romain	Lara	NfP2x7 a cancer specific therapeutic target
Cedric	Louvet	Intracellular cation channels in RORγt+ cells
Lukasz	Majewski	Neuronal overexpression of STIM1 in mouse brain shapes hippocampal synaptic plasticity and animal behavior
Juan Jose	Martinez Garcia	P2X7 receptor associates with mitochondrial dysfunction in monocytes during severe sepsis
Ruth	Murrell-Lagnado	P2X receptors and lysosome function
Mirjaná	Nacka-Aleksic	Relevance of sexual bias in pathogenesis of autoimmune neuroinflammation for immunomudulatory/anti-oxidant agent action: lessons from dimethyl fumarate administration
Paula	Nunes-Hasler	STIM1 promotes phagosomal maturation and antigen cross- presentation in dendritic cells.

Erin	Nuray	TRPV1 channels of sensory nerves, tumour cells and immune cells: Do all work against breast cancer?
Fotini	Paliogianni	Ca2+calmodulin-dependent protein kinase II (CaMKII) : an enzyme in the crossroads of T cell activation and anergy.
Gyorgy	Panyi	Therapeutic targeting of ion channels in immune cells
Mina	Perić	Potassium inwardly rectifying channel Kir4.1 in microglial cells in the rat model of ALS
Anja	Pišlar	Cathepsin X Involvement in Neuroinflammation-Induced Neurodegeneration
Claudia	Rodrigues	Aquaporins are modulated by oxidative stress in cancer cells
Marco	Rossato	TRP ion channels in thermosensation, thermoregulation and metabolism
Anna	Rubartelli	High levels of secreted ATP are responsible for pro-inflammatory cytokine secretion by stressed inflammatory cell
Rainer	Schindl	Transcriptional activation induced by cancer database derived Orai1 mutants (WG1/WG2)
Petra	Visic	The regulation of the store operated calcium entry (SOCE) process by Sigma-1R (with Ruth Murrell-Lagnado)
Orsolya	Vörös	T cell cation channels in the immunological synapse
Iga	Wasilewska	Expression of genes involved in SOCE in Danio rerio

Effects Of P2X7R Activation On Receptor And Cell Functions In Human Myeloma Cell Lines

Ankita Agrawal 1*, Lars Schack Kruse 1, Annette Vangsted 2, Alison Gartland 3 and Niklas Rye Jørgensen 1

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3 The Mellanby Centre for Bone Research, Department on Human Metabolism, The University of Sheffield, Sheffield, UK.

Purpose: Myeloma is a malignancy of plasma cells in the bone marrow. Even with the widespread use of novel agents, myeloma remains incurable and new treatment strategies are needed. Expression and function of P2X7R is implicated in leukemia and osteosarcoma and a high number of loss of function alleles in P2X7R gene are associated with a greater risk of myeloma than individuals not carrying these variant alleles. Therefore, the purpose of the study was to characterize P2X7R function in human myeloma cell lines (hMCL).

Methods: hMCL RPMI8226, U266 and NCIH929 were stimulated with 500 µM or 300 µM BzATP to assess YO-PRO-1 uptake or change in FLUO-4 fluorescence respectively with or without pre-treatment with P2X7R antagonists on NOVOstar. Treatment with 300 µM BzATP followed by combined DAPI/ BrdU staining for quantitation of cell cycle phases by flow cytometry after 1 and 3 days, proliferation by colorimetric tetrazolium based assay and apoptosis by Annexin V/ PI assay after 2 days were analyzed.

Results: BzATP caused significant YO-PRO-1 uptake in all 3 hMCL compared to non-stimulated cells. Dye uptake was blocked in the presence of P2X7R antagonists at concentrations greater than 1 µM in RPMI8226 and U266 but not in NCIH929. Significant increase in intracellular calcium concentrations due to BzATP was inhibited in the presence of P2X7R antagonists only in RPMI8226 hMCL. RPMI8226 showed a marked increase in percentage of cells in S phase of the cell cycle, with a concomitant reduction in the cells in G2/M and G1 phase after 1 day of BzATP treatment which was not seen after 3 days nor in the other hMCL. BzATP caused a significant reduction in proliferation with RPMI8226 more sensitive than the U266 and NCIH929. This inhibition of proliferation was accompanied by induction of cell apoptosis as assessed by flow cytometry. The increase in percentage of apoptotic cells was variable between the experiments but consistently more than the untreated controls and comparable to that induced by an antineoplastic agent bortezomib in RPMI8226, U266, less so in NCIH929.

Conclusions: P2X7R activation alters cell cycle progression in hMCL RPMI8226 whilst inhibiting cell proliferation and induction of apoptosis. Effects in U266 and NCIH929 are less pronounced and could be mediated by other P2 receptors. The data demonstrates the potency of P2X7R activation in controlling myeloma cell growth and highlights the potential of P2 receptors as targets in myeloma.

This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

IP3R and store-operated calcium entry in astrocytes and the effect of IgGs from amyotrophic lateral sclerosis patients

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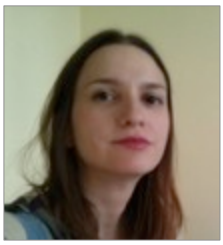
Astrocytes are considered essential in the etiopathogenesis of amyotrophic lateral sclerosis (ALS) with a particular role in underlining neuroinflammation. Previously, we and others demonstrated that IgGs isolated from ALS patients cause bursts of synaptic currents in cultured neurones (1), and on the other hand, enhance the mobility of acidic vesicles in cultured astrocytes (2), all in a Ca^{2+}-dependent manner. Here we will present data on the impact of purified sporadic ALS IgGs on $[Ca^{2+}]_i$ in astrocytes. Confocal time-lapse images were acquired and fluorescence of a non-ratiometric Ca^{2+} indicator (Fluo 4) was recorded before and after the application of IgGs. ALS IgGs (0.1 mg/ml) evoked abrupt Ca^{2+} transients in $[Ca^{2+}]_i$, albeit in ~50% of tested astrocytes. The probability of observing a response and its peak amplitude were independent of $[Ca^{2+}]_e$, however, transients increase was ~3 times faster and the their time integral was ~2-fold larger in 2mM compared to 0 Ca^{2+}_e. Activation of inositol-1,4,5-triphosphate receptors with ensuing second messengers was necessary and sufficient to initiate $[Ca^{2+}]_i$ transients. Store-operated calcium entry prolonged the transient increase in $[Ca^{2+}]_i$. Thus, ALS IgGs can mobilize both, intra- and extracellular Ca^{2+} into the cytosol offering potential targets for therapy of neuroinflammation in ALS.

1) Bataveljic D, Milosevic M, Radenovic L, Andjus P. Novel molecular biomarkers at the blood-brain barrier in ALS. Biomed Res Int. 2014:907545 (2014).
2) Stenovec M, Milošević M, Petrušić V, Potokar M, Stević Z, Prebil M, Kreft M, Trkov S, Andjus PR, Zorec R. Amyotrophic lateral sclerosis immunoglobulins G enhance the mobility of Lysotracker-labelled vesicles in cultured rat astrocytes. Acta Physiol (Oxf). 2011 203:457-471 (2011)

This abstract fits best into: WG2 (diseases)

Voltage gated potassium channel Kv1.3 is upregulated on astrocytes during experimental autoimmune encephalomyelitis



Iva Bozic[1], Katarina Tesovic[1], Marija Jovanovic[1], Marija Adzic[2], Ivana Bjelobaba[1], Danijela Savic[1], Danijela Laketa[2], Sanja Pekovic[1], Irena Lavrnja[1].
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Multiple sclerosis (MS) is a chronic demyelinating disease of the central nervous system, characterized with a strong autoimmune inflammatory component. Voltage gated potassium channel $K_V1.3$ is expressed in immune cells, such as microglia, macrophages, T and B lymphocytes, and has been implicated in their activation in autoimmune diseases, including MS. We investigated the expression of $K_V1.3$ during experimental autoimmune encephalomyelitis (EAE), animal model of MS with correlative imunological features. Our results indicate that $K_V1.3$ is upregulated in the spinal cord during onset and peak of the disease, both on gene and protein level. Its expression subsided in the postsymptomatic phase of EAE. Double immunofluorescence labeling of $K_V1.3$ with astrocytic marker, glial fibrilary acidic protein (GFAP), showed that enhanced expression of $K_V1.3$ during EAE was partly due to upregulation of this channel on astrocytes. $K_V1.3$ was found on reactive astrocytes with hypertrophied cell bodies at the peak of the disease, while at the end of EAE it was mostly present on astrocytes with fibrous morphology (Fig. 1). These results implicate that $K_V1.3$ channels are involved in the complex role that reactive astrocytes have during the course of EAE (Lavrnja et al., 2015; Lavrnja et al., 2012).

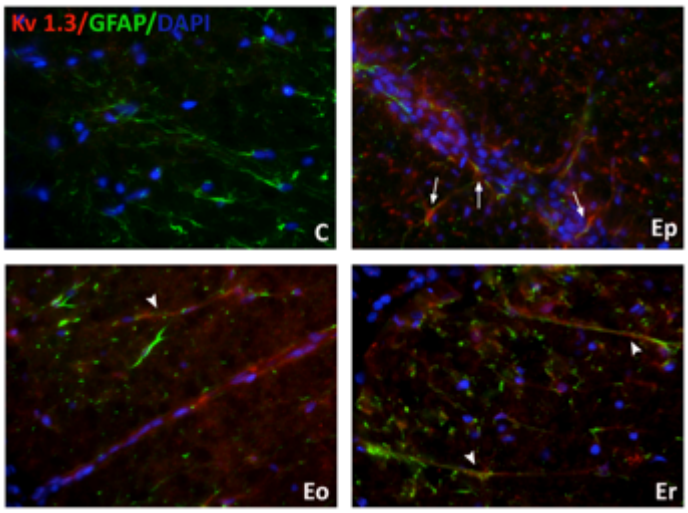

Figure 1. Immunofluorecent staining of $K_V1.3$ (red), GFAP (green) and DAPI (blue) in the spinal cords of control animals (C) and at onset (Eo), peak (Ep) and recovery period (Er) of EAE. Arrows mark hypertrophied astrocytes, arrowheads denote fibrous astrocytes.

References:

1) Lavrnja I, Laketa D, Savic D, Bozic I, Bjelobaba I, Pekovic S, Nedeljkovic N. Expression of a second ecto-5'-nucleotidase variant besides the usual protein in symptomatic phase of experimental autoimmune encephalomyelitis. J Mol Neurosci. 2015 Apr;55(4):898-911.
2) Lavrnja I, Savic D, Bjelobaba I, Dacic S, Bozic I, Parabucki A, Nedeljkovic N, Pekovic S, Rakic L, Stojiljkovic M. The effect of ribavirin on reactive astrogliosis in experimental autoimmune encephalomyelitis. J Pharmacol Sci. 2012;119(3):221-32.

This abstract fits best into WG1

Regulation of neutrophil cytokine secretion by Ca^{2+} signalling

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Abstract text: Neutrophils are the pivot of the cell-mediated innate immunity responsible for the killing of invading pathogens. To fulfill this role, they are able to deploy a sophisticated arsenal including release of antimicrobial enzymes during the degranulation process, production of reactive oxygen species and formation of neutrophil extracellular traps. On the other hand, neutrophils can accumulate in tissues and become inappropriately activated to secrete their cytotoxic products contributing to host tissue damage.

All the defense mechanisms of neutrophils against pathogenic microorganisms have a common feature: they have been described to be governed by Ca^{2+} signals. The store-operated Ca^{2+} entry process has been pointed as the major pathway to maintain long-lasting Ca^{2+} signals that regulate immune cell functions.

According to current understanding, neutrophils are also able to secrete an impressive array of mediators (*e.g.* cytokines), which can recruit other immune cells (dendritic cells, T and B cells, natural killer cells, macrophages) and amplify the pro-inflammatory response. The related molecular mechanisms and the role of Ca^{2+} signalling involved in the cytokine secretion are poorly understood. Our current studies highlight the role of Orai1 channels and open new perspectives on the regulation of cytokine secretion by miRNA.

This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

Role of the P2X4 receptor in breast cancer cell invasiveness

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Purinergic signalling has been associated with cancer cell invasiveness and metastasis formation. A key process in metastasis formation is the proteolytical disruption of the extracellular matrix, by extracellular proteases. Among the proteases, extracellular cathepsins are highly up-regulated in a wide variety of cancers, including breast cancer (1). The classical localization of cathepsins is within lysosomes. In a previous study, we have shown that extracellular ATP activates the purinergic receptor P2X7 in highly metastasizing breast cancer cells. We found that this receptor increase cell migration and cell invasiveness *in vitro* and *in vivo* (2). However, the signalling pathway regulating cathepsin release and promoting cell spreading remained to be identified.
In this study, we are interested in the role of P2X4 and its cross-talk with P2X7. We showed that P2X4 receptor is mainly targeted to lysosomes and its downregulation prevents the ATP-mediated cell invasiveness (Fig 1). We also found that P2X4 inhibition led to a decrease of cathepsin release, by 61%. The release of cathepsins was associated with a lysosomal redistribution from perinuclear to submembrane localization. Our preliminary results highlight a potential new signalling pathway, involving P2X4 and P2X7 receptors, and participating in the metastatic potential of mammary cancer cells.

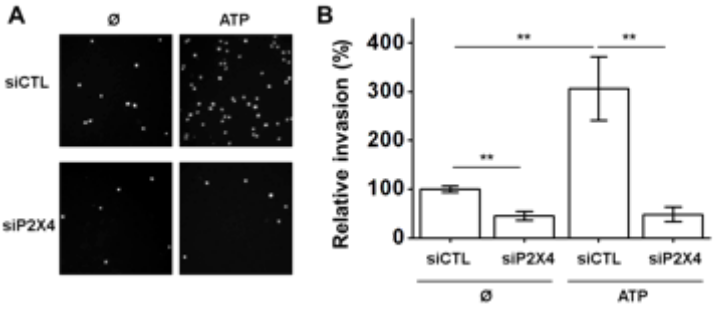

Fig. 1 Role of P2X4R in MDA-MB-435s cell invasiveness. After transfection with siRNA (siCTL or siP2X4), experiments were performed through Matrigel-coated inserts, without (Ø) or with ATP (3 mM). **(A)** Pictures of invading cells (DAPI staining). **(B)** Relative invasion (N=4).

1) Mohamed MM, Sloane BF. Cysteine cathepsins: multifunc-tional enzymes in cancer. Nat Rev Cancer 6: 764–775 (2006).
2) Jelassi *et al*. P2X(7) receptor activation enhances SK3 channels- and cystein cathepsin-dependent cancer cells invasiveness. Oncogene, 30, 2108–2122 (2011).

Yeast aquaporin is implicated in oxidative stress response

Claudia Rodrigues[1,2], Ivana Tartaro Bujak[3], Branka Mihaljević[3], Graça Soveral[1,2], Ana Cipak Gasparovic[3]
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Reactive oxygen species, especially hydrogen peroxide (H_2O_2), contribute to functional molecular impairment and cellular damage, but also are necessary in normal cellular metabolism, and in low doses play stimulatory role in cell proliferation and stress resistance. In parallel, reactive aldehydes such as 4-hydroxynonenal (HNE), are lipid peroxidation breakdown products which also contribute to regulation of numerous cellular processes.
Recently, channeling of H_2O_2 by some mammalian aquaporin isoforms has been reported and suggested to contribute to aquaporin involvement in cancer malignancies, although the mechanism by which these membrane water channels are implicated in oxidative stress is not clear. Therefore, we have used two yeast models to evaluate the interplay between membrane content and aquaporins. Yeast transformed with the delta desaturase gene showed increased sensitivity to oxidative stress which changed to resistance after 3 days. In addition, this model also showed increased AQY1 expression. In order to evaluate AQY1 role in the response to oxidative stress, we used yeast overexpressing AQY1. Our results strongly suggest that aquaporins are important players in oxidative stress response and could be contribute to regulation of cellular processes by regulation of H_2O_2 influx.

This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

Characterization of a novel high-selectivity Kv1.3 inhibitor peptide

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Ion channels expressed in T lymphocytes play key roles in the control of the membrane potential and calcium signaling. The physiological function of effector memory T lymphocytes can be modulated selectively by peptide toxins acting on the Kv1.3 K^+ channels. Since Kv1.3 specific peptide toxins are considered to have a significant therapeutic potential in the treatment of autoimmune diseases, the discovery of new toxins is highly motivated (1). Vm24, isolated from the venom of Vaejovis mexicanus smithi), inhibits Kv1.3 with high affinity (K_d = 2.9pM). However, other biologically important channels, such as hKCa3.1. mKv1.1 and hKv1.2 were partially blocked by the peptide at 10nM concentration, whereas other channels tested were unaffected (2). A novel peptid toxin from the same scorpion named sVmKTx half-blocked the Kv1.3 currents in 770pM concentration. In contrast, we could not observe significant effects of sVmKTx (100nM) on currents of the following ion channels: hKv1.1, hKv1.2, hKv1.4, hKv1.5, rKv2.1. hKCa3.1, hKCa1.1 and hNav1.5.

Our experiments confirm a higher selectivity of sVmKTx for Kv1.3 at a cost of a decrease in the Kv1.3 affinity.

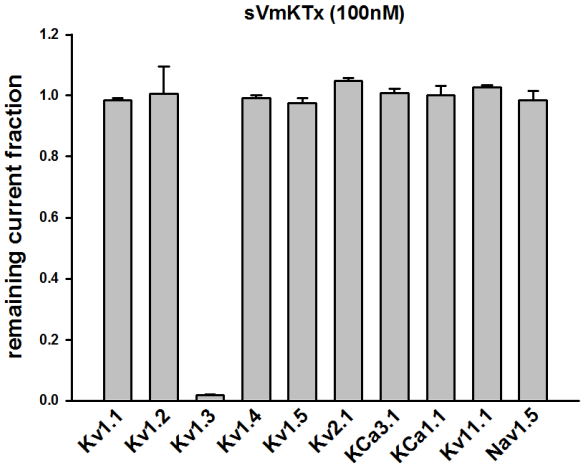

Fig. 1. sVmKTx selectivity for Kv1.3. The novel peptide fully blocks the Kv1.3 channel in 100nM concentration but leaves the other tested channels unaffected. Data are presented as mean ± S.E.M. , remaining current fraction is the I/I_0, where I and I_0 are the peak currents in the presence and absence of sVmKTx, respectively..

1)Panyi G, Possani LD, Rodriguez de la Vega RC, Gaspar R, Varga Z. K+ channel blockers: novel tools to inhibit T cell activation leading to specific immunosuppression. Curr Pharm Des 2006;12:2199-220.
2) Varga Z, Gurrola-Briones G, Papp F, Rodriguez de la Vega RC, Pedraza-Alva G, Tajhya RB, et al. Vm24, a Natural Immunosuppressant Peptide Potently and Selectively Blocks Kv1.3 Potassium Channels of Human T Cells. Mol Pharmacol.

This abstract fits best into: WG1 (cells), WG2 (diseases), WG3 (drugs)

The effects of *Mahonia aquifolium* extracts on doxorubicin cytotoxicity against lung adenocarcinoma cells, *in vitro*

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The species belonging to genus *Mahonia*, including *Mahonia aquifolium* plant, were shown to have antimicrobial, anti-inflammatory, antioxidant effects, and have been used in traditional Chinese and North American medicine. Based on *Mahonia aquifolium* chemical composition, and previously obtained results about plants from this genus,, this plant is belived to possess anticancer properties, which we confirmed and reported.

The objectives of our research were to investigate the effects of *Mahonia aquifolium* extracts on doxorubicin cytotoxic activity, and to determine influence of extracts on doxorubicin cellular uptake and retention.

We performed MTT assay, analyzed cell cycle phase distribution, and doxorubicin uptake/retention with flow cytometry, following the treatment of lung adenocarcinoma cells (A549 cells) with doxorubicin, *M. aquifolium* extracts, or their combination.

MTT assay showed strong synergistic effects of doxorubicin/water or ethanol extract combination treatments. Furthermore the application of this treatment led to an increase in cell numbers in subG1 phase of cell cycle. The results demonstrated that both extracts induced doxorubicin retention in treated A549 cells.

Our results suggest that *Mahonia aquifolium* extracts can induce the cytotoxic activity of commonly used therapeutic doxorubicin, thus potentially allowing the application of lower doses *in vivo,* and lower doxorubicin toxic effects on normal tissues.

1.**Damjanović A**, Zdunić G, Šavikin K, Mandić B, Jadranin M, Matić IZ, Stanojković TP. Evaluation of the anti-cancer potential of *Mahonia aquifolium* extracts via apoptosis and anti-angiogenesis. Bangl J Pharmacol 2016; 11:741-9.

2.Simić MR., **Damjanović AB.**, Kalinić MD., Tasić GD., Erić SM., Antić Stanković JA, Savić VM. Synthesis, cytotoxicity and computational study of novel protoberberinederivatives. J. Serb. Chem. Soc. 2016; 81:103-23.

This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

The role of the P2X7 receptor in bone remodelling

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The ionotropic ATP-gated P2X7 receptor (P2X7R) is involved in the regulation of many physiological functions including bone metabolism. Single Nucleotide polymorphisms in the P2X7 gene has been shown to be associated with post-menopausal bone loss and the risk of vertebral fractures in the Danish Osteoporosis Prevention Study (1). Moreover, in vitro data suggest that P2X7R regulates osteoblast activity as well as the response of the cells to mechanical stimuli (2).
To investigate the potential of the purinergic P2X7 receptor antagonist AFC-5278 (AFC) in treating osteoporosis, we used a rat model of post-menopausal bone loss. Ovariectomized rats were treated with AFC (50, 150 or 300 mg/kg/day) or vehicle five days a week and after six weeks the following examinations were done: DXA (bone mineral density), bone strength, µCT (microstructural analysis) and serum bone turnover markers (CTX-I and PINP). The highest dose of AFC decreased cortical porosity and trabecular spacing; however no effects were seen on bone strength. Finally, the two highest doses of AFC reduced bone resorption significantly as determined by the serum marker CTX-I.
In conclusion, modulation of the P2X7 receptor in vivo reduces bone resorption and could therefore have potential in treating bone diseases such as osteoporosis.

1) Jørgensen NR, Husted LB, Skarratt KK, Stokes L, Tofteng CL, Kvist T, Jensen JE, Eiken P, Brixen K, Fuller S, Clifton-Bligh R, Gartland A, Schwarz P, Langdahl BL, Wiley JS. Single-nucleotide polymorphisms in the P2X7 receptor gene are associated with post-menopausal bone loss and vertebral fractures. Eur J Hum Genet. 2012 Jun;20(6):675-81

2) Agrawal A, Henriksen Z, Syberg S, Petersen S, Aslan D, Solgaard M, Nissen N, Larsen TK, Schwarz P, Steinberg TH, Jørgensen NR. P2X7Rs are involved in cell death, growth and cellular signaling in primary human osteoblasts. Bone. 2017 Feb;95:91-101

Role of CRAC channels in T lymphocyte functions

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Ion channels mediate the transport of charged ions across hydrophobic lipid membranes. In immune cells, Ca^{2+} is a second messenger known to be critical for the function of T and B lymphocytes and cells of the innate immune system. Ca^{2+} signals are essential for many immune functions including the proliferation, differentiation and death of immunocytes, effector functions or the regulation of gene expression. In vivo, Ca^{2+} signals regulate immune responses to infection, immunological tolerance and autoimmunity, inflammation and antitumor immunity. The predominant Ca^{2+} channel mediating Ca^{2+} influx following antigen receptor stimulation in many immune cell types is the CRAC channel, which is formed by ORAI1 and its homologues ORAI2 and ORAI3, and which is activated by STIM1 and STIM2. Other Ca^{2+} channels may also contribute to immune cell function including P2X receptors, TRP channels and voltage-gated Ca^{2+} channels, although their regulation and contribution to immunity are less well defined. Other divalent cations such as Mg^{2+} and Zn^{2+} have important roles in regulating immune cell function and development, too. In addition, monovalent ions such as Na^{+}, K^{+} and Cl^{-} mainly regulate the membrane potential, which indirectly controls the influx of Ca^{2+} and immune cell signaling. Studies investigating human patients with mutations in ion channels, analysis of gene-targeted mice, or pharmacological experiments with ion channel inhibitors have revealed important roles of ionic signals in lymphocyte development and in innate and adaptive immune responses. In this talk, we will discuss the role of CRAC channels encoded by ORAI proteins and other ion channels in lymphocyte function.

This abstract fits best into: WG2 (diseases)

Expression pattern of $Ca_V1.2$ type of voltage-dependent calcium channels is altered after cortical injury in rats

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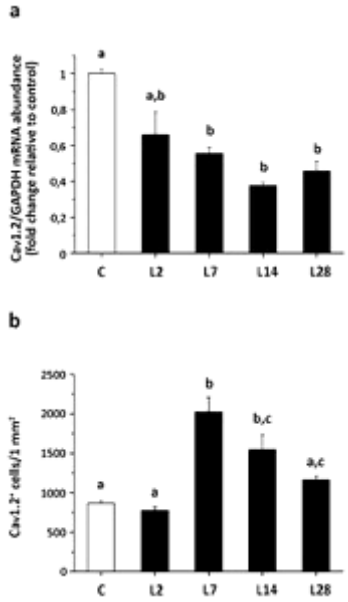

It is known that Ca^{2+} entry into the cells occurs via various types of voltage-dependent calcium channels (VDCCs). The aim of this study was to investigate temporal and cellular pattern of expression of $Ca_V1.2$ type of VDCCs after the sensorimotor cortex ablation (SCA). Expression profiling of $Ca_V1.2$ revealed reduction of $Ca_V1.2$ mRNA levels. However, quantitative analysis of $Ca_V1.2$ immunostaining demonstrated upregulated expression at 7 and 14 days post-injury (dpi) on distinct cell populations in injured cortex. Upregulation of $Ca_V1.2$ expression was detected at 7 and 14 dpi on reactive astrocytes. The most prominent expression of $Ca_V1.2$ coincided with a peak of reactive astrogliosis at 14 dpi and was detected in reactive astrocytes and astrocytic processes that form glial scar around the lesion site. Additionally, a subset of activated microglia/macrophages around the lesion site expressed $Ca_V1.2$, while neuronal expression remained without prominent changes. The results of this study demonstrate that SCA alters expression of $Ca_V1.2$ type of VDCCs on different cell types in proximity to the injury site in a time dependent manner, pointing to complexity of intercellular regulation of Ca^{2+} homeostasis after SCA. Consequently, modulation of $Ca_V1.2$ channels expression may be a potential target for the treatment of brain injury.

Fig. 1 (a) Levels of $Ca_V1.2$ mRNA in control (C) and injured cortices at 2 (L2), 7 (L7), 14 (L14) and 28 (L28) dpi are expressed relative to GAPDH-mRNA. **(b)** Number of $Ca_V1.2^+$ cells around the lesion site per 1 mm^2 ± SEM

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This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

Targeting Kv1.3 channels of T cells in autoimmune diseases

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Effector memory T (T_{EM}) cells are key players in pathology of various autoimmune diseases. Kv1.3 ion channels of T_{EM}'s control the Ca^{2+}-signalling that is necessary for the activation and effector functions. Ca^{2+}-dependent activation includes NFAT stimulation and upregulation of CD40L expression, the latter binds to CD40 on B cells/dendritic cells leading to autoimmune responses and inflammation. Hence, the inhibition of the Kv1.3 function could be used as a novel treatment in autoimmune disorders. We could synthetize siRNA-loaded (against Kv1.3), functionalized lipid nanovesicles (Kv1.3-NPs) that specifically targeted T_{EM} cells through the CD45RO cell surface marker. Treatment with these Kv1.3-NPs resulted in the downregulation of Kv1.3 level and decreased Ca^{2+}-response in T_{EM}'s [1]. Subsequently, the NFAT translocation to the nucleus and CD40L expression was also lessened upon Kv1.3-NP incubation in activated T_{EM}. Moreover, the cytokine production was inhibited, and a memory to naïve subtype re-differentiation could be observed [2]. Our findings show that lipid nanovesicles could be a promising/innovative approach in the therapy of the autoimmunity.

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NOX inhibitors: characterization and potential applications for therapy in human diseases

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The identification of NOX enzymes emerged from human mutations in the phagocyte NADPH oxidase (NOX2). These mutations lead to chronic granulomatous disease (CGD), a severe immune deficiency that originally would make NOX2 appear like the furthest thing from a drug target. It was only when other NOX isoforms were identified (NOX1-5 and DUOX1-2) that they began to be recognized as important ROS generators leading to oxidative damage in numerous pathological states. The list of diseases where NOX are thought to play a detrimental role is ever growing, including diseases with unmet medical needs, such as diabetic end-organ damage, stroke and fibrotic diseases. This talk focuses on the discovery path of novel NOX inhibitors from proof of principle studies, identification of small molecules inhibitors, preclinical

This abstract fits best into: WG1 (cells), WG2 (diseases), WG3 (drugs)

Measurements of ion fluxes in breast tumor cells usin Microelectrode flux estimation

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Based on the previous *in vitro* screening (1), we identified proprietary crown-ether compound 613 as a potential antitumor drug. Since crown ethers can act as K^+ ionophores, we hypothesized that anticancer activity towards breast tumor cells by 613 could be driven by disruption of potassium transport.

Potassium homeostasis plays an important role in maintaining of the resting membrane potential, enzyme activities and has an impact on cell proliferation and differentiation. K^+channels are involved in the promotion of tumorigenesis.

Flow cytometric measurements using the cationic dye $DiOC_6(3)$ showed that compound 613 modulates membrane potential in breast cancer stem cell model (HMLE cells). It is extremely difficult to patch clamp HMLE cells, thus we used Microelectrode Flux estimation (MIFE), a non invasive method based on measuring the concentration changes of ions close to the cells to examine ion fluxes (review in 2). Specifically, we analysed fluxes of potassium and sodium ions through the plasma membrane of monolayers of HMLE cells and breast tumor cell lines MCF7 and SUM159. Furthermore, membrane potential changes induced by 613 were assessed by using fluorescent potentiometric dyes. The results indicate that 613 in fact changes permeability of the plasma membrane of the examined cells for monovalent cations.

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Ion channels in mast cell pharmacology

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IgE-dependent mast cell activation is a dynamic process that primarily governs type I hypersensitivity reactions implicated in allergic diseases such as asthma, rhinitis, conjunctivitis, urticaria and anaphylaxis. Mast cell degranulation results in the release of preformed proinflammatory mediators, such as histamine, as well as of a variety of newly synthesized substances, including leukotrienes and cytokines. In addition, differential mediator release from mast cells may be triggered by a plethora of non-IgE-dependent stimuli, thus increasing the complexity of the aetiopathological mechanisms underlying tissue inflammation and modulation of innate and adaptive immunity. The pivotal role of the versatile mast cell in these processes is strongly associated with Ca^{2+} signalling. Ca^{2+} channels are responsible for the influx of extracellular Ca^{2+} characterizing mast cell activation and mediator release, whereas and interaction of a range of additional components, including K^+, Cl^- and transient receptor potential channels regulate Ca^{2+} signalling. Interestingly, the elucidation of the crucial role of ion channels in mast cell biology offers new insights for the development of novel pharmacological tools with clinical exploitation potential.

This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

Analysis of synergism and antagonism between miR-495-3p and cholinergic receptor nicotinic alpha 5 (CHRNA5) in MCF7 breast cancer cells

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Abstract text: miR-495-3p is an important microRNA shown to have a role in inhibition of cancer cell proliferation and in discrimination of early breast cancer. On the other hand, increased levels of ligand-gated ion channel cholinergic receptor nicotinic alpha 5 (CHRNA5) is associated with cancer. Our previous studies have shown that CHRNA5 depletion via siRNA resulted in G1 cell cycle arrest in MCF7 breast cancer cells (TUBITAK 111T316). In the present study, we have performed an Affymetrix microarray analysis of MCF7 cells treated with the synthetic miR-495-3p mimic, alone or in combination with CHRNA5 siRNA. Treatment with miR-495-3p mimic resulted in both synergistic and antagonistic associations with CHRNA5 siRNA application based on bioinformatics analyses using Ingenuity Pathway Analysis (IPA®) and miRNet (www.mirnet.ca). Functional interactions between the miR-495-3p and CHRNA5 were studied using qPCR analysis of selected targets of miR-495-3p.
This study was funded by a research grant from TUBITAK (114S367).

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This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

Intracellular cation channels in RORγt^{+} cells

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We explore the role of intracellular ion channels encoded by the homologs *Tmem176a* and *b*, two of the rare genes directly controlled by the transcription factor RORγt. These genes are indeed strongly expressed in RORγt^{+} cells including Th17 (Figure 1), Tγδ17 or ILC3s compared to type 1 or 2 polarizations (Drujont *et al.* 2016). Our preliminary results show that double KO mice (that we recently generated : Lemoine *et al.* 2016), but not *Tmem176b* single KO mice, develop exacerbated pathology in a model of acute colitis, indicating a strong functional redundancy between *Tmem176a* and *b*. We hypothesize that this ion flux is implicated in a yet unknown cellular mechanism in RORγt^{+} cells required for efficient issue protection, repair and/or immunoregulation.

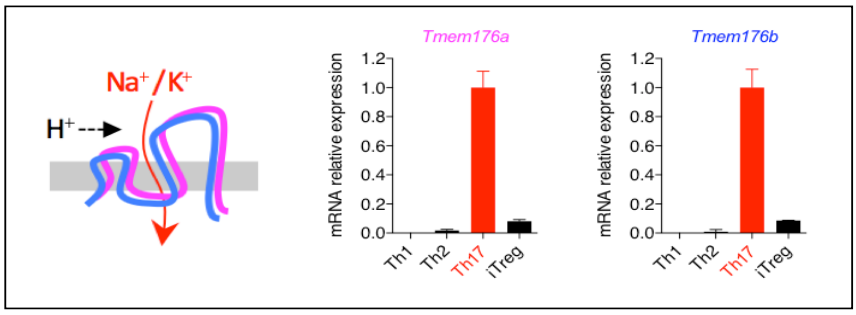

Figure 1. Schematic representation of the four-span transmembrane proteins TMEM176A and TMEM176B acting as non-selective cation channels activated by acidity and strongly expressed in Th17 cells.

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Characterization of tumor infiltrating Foxp3+ Tregs-specific channelome by whole-genome transcriptional profiling

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Tumor infiltrating Foxp3+ regulatory T lymphocytes (TI Foxp3+Tregs) are potent suppressors or CD4+ and CD8+ effector T cells-meditated anti-tumor immune response. High infiltration of tumor by Foxp3+ Tregs correlates with poor prognosis in cancer patients. TI Foxp3+Tregs are targets of new anti-cancer immune checkpoints therapies and their depletion within tumor site has been reported to increase anti-tumor specific immune responses and reduce tumor burden However, only some patients achieve durable benefit and long-term survival. Identification of new strategies selectively targeting TI Foxp3+Tregs without affecting conventional T cell-mediated response remains to be discovered. The goal of this study was to compare transcriptional profiles of mouse TI Foxp3+ Tregs with their lymph node naïve and activated counterparts and conventional CD4+ T cells by whole-genome transcriptional profiling. By using *GeneChip*® Mouse Transcriptome Array we demonstrate highly dynamic channelome signature in all analysed T cell populations and identify ~70 transcripts containing specific channelome signature of TI Foxp3+ Tregs. Our findings provide insights into the molecular characteristics of TI Foxp3+ Tregs and define potential new targets for tumor immunotherapy.

nfP2X$_7$ A CANCER SPECIFIC THERAPEUTIC TARGET

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P2X$_7$ is an ATP-gated receptor, involved in inflammation, cell death, proliferation and cell migration. Alterations in these cell processes are associated with development of cancer and recent publications have linked P2X7 directly to development of a number of malignancies including Prostate, Lung and Skin cancers. Upon rapid ATP stimulation, P2X$_7$ induces a fast inward non-selective cationic current. In contrast, a prolonged ATP activation triggers the opening of a large molecular weight pore, which is associated with membrane depolarisation and cell death. Biosceptre has identified a cancer specific conformational variant of P2X$_7$ in which a normally hidden epitope (E200) is exposed. This variant, termed non-functional P2X$_7$ (nfP2X$_7$) is unable to form a large transmembrane pore in response to activation by ATP, a critical adaption to facilitate cell survival in the tumour microenvironment where extracellular ATP concentrations are known to be very high. Here we will review the body of evidence supporting the role of nfP2X$_7$ as a cancer specific target and present Biosceptre's therapeutic approach against nfP2X$_7$.

This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

Neuronal overexpression of STIM1 in mouse brain shapes hippocampal synaptic plasticity and animal behavior

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STIM1 is an endoplasmic reticulum calcium sensor that is involved in several processes in neurons, including store-operated calcium entry. STIM1 also inhibits voltage-gated calcium channels, such as $Ca_v1.2$ and $Ca_v3.1$, and is thus considered a multifunctional protein. The aim of this work was to investigate the ways in which transgenic neuronal overexpression of STIM1 in FVB/NJ mice affects animal behavior and the electrophysiological properties of neurons in acute hippocampal slices. Our data indicate that STIM1 overexpression in neurons in the brain perturbs metabotropic glutamate receptor signaling, leading to impairments in long-term depression in hippocampus, however, further molecular biology studies are needed to identify the exact mechanism of this phenomenon. The behavioral analyzes revealed a decrease in anxiety-like behavior in parallel with improvements in contextual learning and no changes in locomotor performance. Altogether, the presented results point to novel functions of STIM1 in the regulation of synaptic plasticity in the mouse hippocampus that could influence the animal behavior. The data also provides additional evidence of the role of STIM1 outside the canonical SOCE pathway.

P2X7 receptor associates with mitochondrial dysfunction in monocytes during severe sepsis

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Sepsis remains the leading cause of death in critical-care units. Sepsis initiate with a hyperinflammatory response damaging different tissues and organs, which is then followed by an acute immunoparalysis due in part to a lack of aerobic metabolism in monocytes after the infection. In this work, we aim to characterize the molecular mechanisms responsible of the aerobic metabolism paralysis in monocytes from human sepsis patients of abdominal origin. We found that the cell surface expression of the ion channel P2X7 receptor increased in septic monocytes when compared with a control group of patients undergoing abdominal surgery but not developing sepsis. Despite this increase in P2X7 receptor, ATP failed to induce ASC aggregation and IL-1beta release in a fraction of septic patients. In these patients P2X7 receptor expression correlated with a lack of mitochondrial membrane potential ($P<0.05$; $r=0.738$). Also, this group of patients presented a high mortality during the septic episode. P2X7 receptor stimulation in human blood monocytes before LPS-priming, induced a decrease of mitochondrial membrane potential and impaired the respond of the monocytes to LPS and the engagement of the NLRP3 inflammasome. Our results suggest that during human sepsis P2X7 receptor expression increases in monocytes and could induce immunoparalysis in monocytes by damaging mitochondria.

This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

P2X receptors and lysosome function

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Lysosomes are an important source of Ca^{2+} contributing to localized cytosolic Ca^{2+} signals that control lysosome fusion with late endosomes, lysosome secretion and autophagy. The dysregulation of these processes is associated with cancer, lysosome storage diseases and neurodegenerative diseases. The P2X4 receptor is one of a few different Ca^{2+} channels targeted to the limiting membrane of lysosomes where it is regulated by luminal ATP and luminal pH. ATP levels are high but the acidity of the lysosomes inhibits P2X4 receptor activation. Alkalinisation of the lysosome activates P2X4 and the resultant cytosolic Ca^{2+} signal triggers lysosome homotypic fusion in a calmodulin-dependent manner[1]. We are interested in identifying physiological triggers of lysosome alkalinisation that can recruit P2X4 activity and have identified P2X7 and GPCRs that couples to the $G_{\alpha}q$ papthway as regulators of lysosome P2X4 receptors. Functional cross talk between these receptors promotes lysosome homotypic fusion and lysosome exocytosis. It also inhibits autophagic flux. In cancer cells this cross talk between P2X4 and P2X7 contributes to the secretion of enzymes that promotes cell invasiveness.

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This abstract fits best into: WG1 (cells), WG2 (diseases), WG3 (drugs)

RELEVANCE OF SEXUAL BIAS IN PATHOGENESIS OF AUTOIMMUNE NEUROINFLAMMATION FOR IMMUNOMUDULATORY/ANTI-OXIDANT AGENT ACTION: LESSONS FROM DIMETHYL FUMARATE ADMINISTRATION

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Abstract

The study revealed sex-specific differences in efficacy of dimethyl fumarate (DMF) to moderate the clinical severity of experimental autoimmune encephalomyelitis (EAE) in Dark Agouti rats, and indicated differences in sensitivity of several DMF cellular/molecular targets relevant for this specificity. In rats of both sexes, DMF administration from the day of immunization attenuated EAE severity, but to a greater extent in males, leading to loss of the sexual dimorphism observed in vehicle-administered controls. Consistently, DMF was more efficient in diminishing the number of highly pathogenic IFN-γ+GM-CSF+ IL-17+ T cells infiltrating male than female rat spinal cord (SC). Additionally, DMF-induced increase in the expression of nuclear factor E2-related factor 2 and heme oxigenase-1 in SC. Consequently, the frequency of CD163+ cells among CD11b+CD45+ SC cells and the expression of arginase-1, the marker of anti-inflammatory myeloid cells, was more prominent in male rat SC. Accordingly, DMF impaired CD4+ T lymphocytes reactivation to a greater extent in male compared with female SC, most likely due to a more prominent upregulation of the regulatory CD83 molecule on myeloid cells from male rats. Consistently, DMF diminished NO production in SC cell cultures, $O_2^{\bullet -}$ SC tissue level and SC oxidative damage in males compared with females.

STIM1 promotes phagosomal maturation and antigen cross- presentation in dendritic cells.

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Antigen cross-presentation by dendritic cells (DCs) is a key immune mechanism for the activation of cytotoxic T cells critical for the defence against viruses, intracellular pathogens and cancer. Antigens acquired through phagocytosis in DCs produce potent T cell responses, but the intracellular signalling and trafficking pathways regulating cross-presentation of phagocytically derived antigens are not well understood. Calcium signalling regulates a variety of processes in other immune cells, including secretion, migration and phagocytosis, yet the role of molecular regulators of calcium signalling during cross-presentation has not been explored. In the present study, we show that ablation of the ER-resident calcium regulator STIM1 in myeolid cells in LysM-Cre;Stim1$^{fl/fl}$ mice impaired cross-presentation in vivo. This effect was reproduced in vitro using primary bone-marrow derived DCs, as well as a CD8^{+} murine DC cell line stably expressing shSTIM1. Although store-operated calcium entry was defective, STIM1 was neither required for the upregulation of differentiation marker CD11c, maturation markers CD40, CD80, CD86 and MHC-CII in response to microbial products LPS or CpG, nor for robust phagocytic ingestion, but significantly reduced phagosomal proteolysis and phago-lysosome fusion. Interestingly, these defects in phagosomal maturation correlated with a reduced number of periphagosomal calcium hotspots as well as reduced ER-phagosome membrane contact sites. Together these data suggest that STIM1-dependent calcium signalling enhances cross-presentation in DCs by facilitating fusion of endomembranes that deliver the required cohort of enzymes and transporters to phagosomes.

This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

TRPV1 channels of sensory nerves, tumour cells and immune cells: Do all work against breast cancer?

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Majority of the TRPV1 channels are found in sensory nerve ending which are sensitive to capsaicin, a pungent ingredient in hot chili peppers. We previously documented that inhibition of TRPV1 containing (capsaicin sensitive) sensory nerve fibres markedly increased lung and heart metastasis of 4T1 breast carcinoma. Similarly activation of TRPV1 channels with low dose capsaicin decreases the number of lung metastasis. Using a high throughput screening assay, we also found that inactivating TRPV1 containing sensory nerve fibres decreases the expression of possible tumour suppressive genes (1,2). Further studied demonstrated that Substance P released upon activation of TRPV1 channels is involved in anti-tumoral effects (3).

Recently we found that TRPV1 channels are also found in 4T1 breast carcinoma cell as well as in the ones metastasized to liver and brain. Treatment of these cells with several TRPV1 agonists markedly suppressed growth of metastatic breast carcinoma cells concentration-dependently. Surprisingly TRPV1 antagonists (MSK-195; capsazepine, AMG-9810) also suppressed cell proliferation with varying degrees demonstrating that these agents are more of a regulator of the channel in tumour cells rather than simple agonists or antagonists. .

TRPV1 channels are mostly expressed in red-pulp of the spleen in both control and tumour-bearing animals suggesting that myeloid cells express TRPV1 channels more. Western blot analysis demonstrated that splenocytes express different isoforms of TRPV1 compared to brain tissue which further differed in splenocytes of tumour-bearing animals. We determine changes in LPS and Con-A-induced cytokine response of mix leukocyte culture obtained from spleen and lymph nodes from control and tumour bearing mice. TRPV1 agonists capsaicin markedly increased IFN-g response to both LPS and Con-A in control mice. Massive systemic inflammatory response was observed in tumour-bearing mice. Although there were exceptions, capsaicin in general suppressed cytokine release from mix leukocyte culture obtained from tumour bearing mice.

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Ca^{2+}calmodulin-dependent protein kinase II (CaMKII) : an enzyme in the crossroads of T cell activation and anergy.

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Calcium 2+ (Ca) plays an essential role in lymphocyte activation and differentiation by affecting signaling pathways leading to cytokine production. Among the enzymes responding to calcium increase, CaMKII has been involved in anergy with a still poorly characterized role. IL-10 produced by different T lymphocyte subpopulations is critical mediator of tolerance. We tested the hypothesis that CaMKII may be involved in IL-10 production. We report that CaMKII upregulates IL-10 production by primary human T lymphocytes stimulated through the antigen receptor or bypassing that.

Overexpression of constitutively active mutant forms of Calcineurin or CaMKII specifically increased IL-10 transcription and secretion in T lymphocytes. Constitutively active CaMKII specifically activated IL-10 promoter activity, whereas it inhibited IL-2 and IL-4 promoter(1). This effect was mediated by the first 500 bp fragment, carrying binding sites for Myocyte Enhancer Factor-2 (MEF2). The same fragment was sensitive to cAMP mediated inhibition of IL-10 promoter activity(2). Constitutively active CaMKII activated MEF2 mediated transcription, in T lymphocytes stimulated through TCR. CAMKII inhibitor KN-62 and cAMP elevating agents inhibited MEF2 binding in cell lysates of the same cells. Moreover, overexpression of MEF2 enhanced IL-10 promoter activity. Our data suggest a distinct role of CaMKII in the induction of anergy in T lymphocytes, by differential regulation of IL-10 and IL-2 gene transcription. They also identify MEF2 as a molecular target which can integrate calcium and cAMP signals.

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This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

Therapeutic targeting of ion channels in immune cells

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The last decade brought about breakthrough discoveries in therapeutic targeting of immune cell ion channels: 1, molecular identification of enigmatic ion channels in immune cells, e.g., the description of the STIM-Orai1 complex being responsible for the CRAC current (1). 2, The recognition of immune cell subtype-specific expression of ion channels e.g. the Kv1.3 vs. KCa3.1 dominance of T cell subsets (2). 3, understanding the pathophysiological role of various immune cell subtypes in human diseases with special emphasis on the effector memory T cells in a subset of autoimmune diseases (multiple sclerosis, rheumatoid arthritis, (3)). 4, Medicinal chemistry of small-molecule inhibitors of ion channels resulted in the development of PAP-1 and TRAM-34, high affinity and highly selective inhibitors of Kv1.3 and KCa3.1 K^+ channels of lymphocytes, respectively (4). 5, One of the largest development in the field of targeting ion channels was the identification and engineering of high affinity and selectivity peptide inhibitors of Kv1.3. Picomolar affinity drug candidates (e.g. ShK analogues (3) and Vm24 (5)) are either in clinical trials or in the preclinical phase backed with successful animal experiments in the treatment of psoriasis and MS. The talk aims to summarize the recent advancements and highlight potential drug candidates.

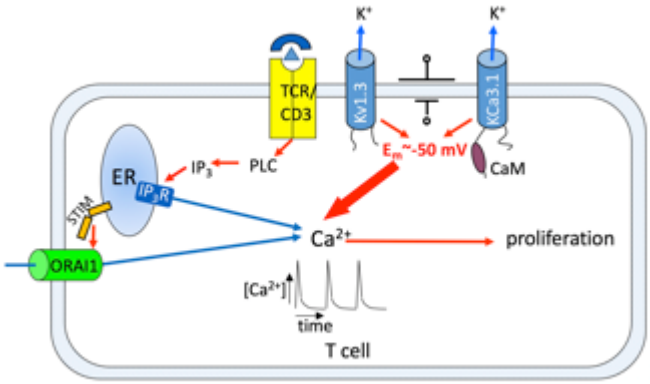

Fig 1.: Role of cation channels in T cell activation. Key to the figure: TCR/CD3: T cell receptor complex; PLC: phospholipase Cγ; IP_3 and IP_3R: inositol 1,4,5 triphosphate and its receptor in the membrane of the endoplasmic reticulum (ER); STIM and ORAI: components of the CRAC channel; Kv1.3 and KCa3.1: the voltage-gated K^+ and intermediate conductance Ca^{2+} -activated K^+ channel of T cells, respectively; CaM: calmodulin, Em: membrane potential.

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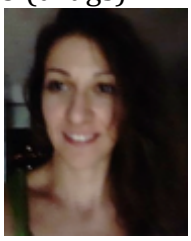

Potassium inwardly rectifying channel Kir4.1 in microglial cells in the rat model of ALS

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting upper and lower motor neurons. Astrocytes and microglia have a prominent role in disease development. Potassium inwardly rectifying channel, Kir4.1, expressed in astrocytes has the major role in potassium homeostasis in the central nervous system (CNS). Its expression is reduced in astrocytes of the motor cortex and brainstem of end-phase hSOD^{G93A} ALS rat model (1). Studies on the cervical and lumbar spinal cord, indicated a general reduction in Kir4.1 expression. Interestingly, in the ventral horn gray matter and white matter of end phase ALS animals, islets of Kir4.1 demonstrating immunoreactivity similar to those shown in control animals were observed. Although literature does not show expression of Kir4.1 in CNS microglia, utilizing microglial markers, Iba1, Cd11b and Cd68, Kir4.1-positive islets were identified as clusters of microglia. Colocalization of immunofluorescence of microglial markers and Kir4.1, quantified by Pearson and Manders coefficients, showed positive signal colocalization, both in cervical and lumbar spinal cord of ALS rats, in contrast to control animals. Previous studies indicated microglia activation in the ALS model (2) and the presented results could indicate a K-channel – based change in physiology of reactive microglia as a specific pathological process.

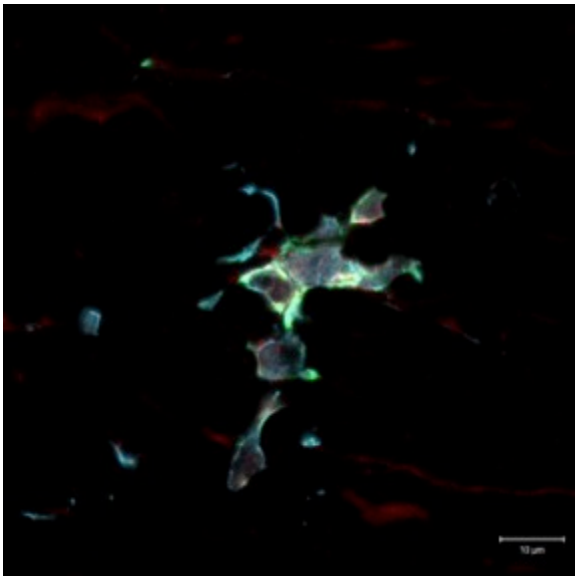

Fig. 1 Kir4.1 positive cluster of microglial cells in lumbar spinal cord white matter in ALS rat model. Cd68 (green), Kir4.1 (red), Iba1 (blue).

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Cathepsin X Involvement in Neuroinflammation-Induced Neurodegeneration

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Neuroinflammation is closely implicated in the pathogenesis of neurodegenerative disorders, such as Parkinson's disease (PD), where the hallmark of neuroinflammation is activated microglia. Microglia-derived lysosomal cathepsins, including cathepsin X, are increasingly recognized as important mediators of the inflammation-induced neruodegeneration. Recent study revealed that up-regulated expression and activity of microglial cathepsin X as wells as increased release of cathepsin X after lipopolysaccharide (LPS) stimulation leads to microglia activation-mediated neurodegeneration. Cathepsin X inhibitor caused neuroprotection via its suppression of microglia activation. Moreover, the immunomodulatory role of cathepsin X has been also shown in microglial co-activation, where cathepsin X inhibition proved to diminish increased neuroinflammation by LPS and poly(IC) co-stimulation. Our recent study revealed that LPS also induced the expression and upregulated enzymatic activity of cathepsin X in brain regions observed in *in vivo* models of PD, with a preference cathepsin X upregulation in microglia cells and astrocytes in lesioned striatum. Taken together, these findings propose the potential function of microglial cathepsin X in inflammation-induced neurodegeneration. Knowing the involvement of cathepsin X in the neurodegenerative processes represent a step towards the development of new molecules for the treatment of neurodegenerative diseases.

This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

Aquaporins are modulated by oxidative stress in cancer cells

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Aquaporins facilitate water and glycerol permeation through membranes. Additionally, AQP3 was reported to transport H_2O_2. Since AQP3 and AQP5 are strongly expressed in cancer tissues, we cloned AQP5 in yeast and demonstrated its ability to transport H_2O_2. This feature was correlated with cell sensitivity to acute oxidative stress and improved cell survival and resistance in chronic conditions, suggesting a mechanism for its involvement in tumorigenesis [1]. These results prompted us to investigate if aquaporins could contribute to growth stimulation in cancer cells, especially in cancer stem cells.
Oxidative modifications of extracellular matrix were introduced to simulate tumor microenvironment. Breast cancer stem cells were grown on different surfaces and stress conditions to examine changes in cellular responses in growth and antioxidative status. By measuring GSH levels and Nfr2 expression, we found that cells adapt to changes in microenvironment under oxidative stress. To evaluate if possible changes in response to oxidative stress are related to changes in aquaporin expression, thus contributing to activation of cancer stem cells in respect to malignancy, AQP gene expression was assessed on different breast cancer cell lines subsequently to a H_2O_2 stimulus. We found a differential aquaporin expression pattern depending on tumor aggressiveness that was modulated by H_2O_2, suggesting a potential role in tumorigenesis.

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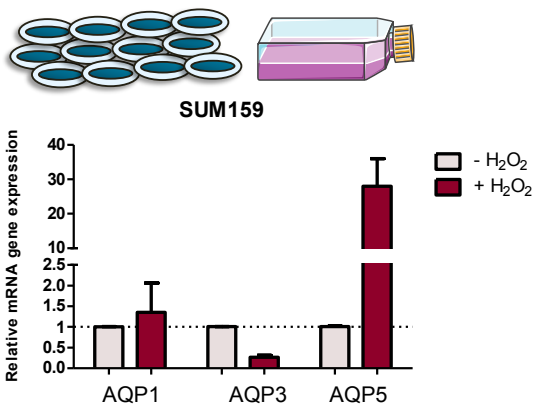

TRP ion channels in thermosensation, thermoregulation and metabolism

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Obesity and diabetes type 2 are well established global epidemics and contributors to a number of different metabolic and cardiovascular complications. The prevalence rates of these diseases are every increasing among countries resulting in a corresponding public health burden. One of the most appealing target for the treatment of obesity is the possibility to activate brown adipose tissue (BAT) which presence has been demonstrated in adult human. Given its high metabolic energy-expending activity producing heat, it has been recently explored as a possible target to be activated in order to dissipate energy and thus to reduce fat depots. Furthermore, the same BAT activators have been shown to induce a transdifferentiation of white adipocytes or to activate a third type of adipocyte, named beige adipocyte, that upon appropriate stimuli can acquire functional characteristics close to those of brown adipocytes. Expansion or activation of BAT prevents obesity and diabetes and it is well known that chronic cold exposure enhances thermogenesis in BAT through uncoupling protein 1 (UCP1) expression and activation triggered via β-adrenergic pathway. Different members of the TRP receptor family are functionally expressed in human and their activation in adipose tissue and muscle up-regulates gene expression leading to energetic substrates utilization, oxygen consumption and heat production. These effects somehow mimic those induced by chronic cold exposure as for the activation of TRPM8 receptor in mouse in vivo that has been shown to prevent diet-induced obesity and glucose metabolism alterations.
These observations disclose a previously unknown role for TRP receptors, suggesting that their stimulation in adipose tissue and muscle could represent a novel and promising way to treat obesity and its metabolic complications.

This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

High levels of secreted ATP are responsible for pro-inflammatory cytokine secretion by stressed inflammatory cell

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Cell stress contributes to generate inflammation. We have shown that monocytes subjected to strong inflammatory stimuli undergo stress, with generation of high amounts of ROS that drive secretion of huge levels of ATP. Secreted ATP triggers P2X7R, leading to enhanced inflammasome activation and IL-1β secretion. Later, cells under stress slow-down protein translation and fail to secrete anti-inflammatory cytokines like IL-1receptor antagonist, thus failing to dampen inflammation (1). Many hereditary diseases have an inflammatory component. To investigate whether the presence of a mutant protein may cause cell stress and inflammation *per se*, we studied various hereditary diseases and observed that, when a proteotoxic mutant is expressed by professional inflammatory cells, stress occurs and through increased ATP release activates an explosive inflammatory response. This mechanism is operative in autoinflammatory diseases, either when the causative gene is involved in IL-1β production or regulation (e.g. CAPS syndrome, linked to NLRP3 mutations) (2), or when it is unrelated to IL-1 (e.g. TRAPS syndrome, linked to TNFα receptor mutations). Differently, when expressed in non-immune cells (e.g. cystic fibrosis), the stress caused by the proteotoxic leads to release of transcellular signals that recruit healthy, non stressed inflammatory cells: inflammation occurs, but generates a milder symptomatology.

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Transcriptional activation induced by cancer database derived Orai1 mutants (WG1/WG2)

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The Orai1 channel forms the Ca^{2+} selective pore for essential immune cell responses that requires endoplasmic reticulum store-depletion and STIM1 interaction for its activation. However, patho-physiological Orai1 Ca^{2+} signaling has been linked to several immune diseases, tubular myopathy and cancer. Here, we systematically screened for Orai1 mutants derived from large-scale cancer genomics data sets. We identified five Orai1 mutations from cancer patients that resulted in constitutive active gating and transcriptional activation. Two of these Orai1 mutations are located at a TM connectivity segment, similar as a previously determined Orai1 myophathy single point mutation. These Orai1 mutants resulted in activation of the transcription factors NFAT and MITF. Thus we here identify a critical Orai1 segment that switches the channel into an open conformation to induce Ca^{2+} flow and transcription. This work was supported by FWF projects P26067 and P28701 (to R.S.), as well as COST action BM 1406.

This abstract fits best into : **WG1 (cells)**, WG2 (diseases), WG3 (drugs)

The regulation of the store operated calcium entry (SOCE) process by Sigma-1R

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Sigma1R is a 223 amino acid long chaperone protein targeted to the membrane of the endoplasmic reticulum (ER). It is primarily located at mitochondria-associated ER membranes (MAM), but can also translocate to the periphery of the cell and localize to ER-plasma membrane (PM) junctions. As a chaperone, Sigma1R regulates oxidative stress and cell survival and has important pro-survival effects in neurons in the CNS. Loss of activity of Sigma1R is associated with Alzheimer's disease, Parkinson's disease and Amyotrophic Lateral Sclerosis (ALS). Sigma1R is a regulator of Ca2+ signalling at the MAM and at the ER-PM junction. At the MAM it is thought to promote Ca2+ transfer to mitochondria and at ER-PM junctions we recently showed that it inhibits SOCE. Increased expression of Sigma1R in HEK293 cells via baculoviral infection profoundly inhibited SOCE mediated via STIM1 and Orai1. It also slowed the rate of translocation of NFAT-GFP to the nucleus. Over-expression of STIM1 in HEK293 cells caused a profound increase in SOCE in HEK293 cells which could be blocked by expression of the dominant negative Orai mutant (R91W). Similarly STIM2 overexpression profoundly increased SOCE in an Orai1 dependent manner. Interestingly STIM1 could reverse the inhibition of SOCE by Sigma1R whereas STIM2 could not. We are currently investigating the underlying mechanisms.

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This abstract fits best into (please underline): WG1 (cells), WG2 (diseases), WG3 (drugs)

T cell cation channels in the immunological synapse

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Ca^{2+}-dependent activation of T cells requires the concerted interplay of ion channels: the efflux of K^+ through Kv1.3 and KCa3.1 maintains the driving force for the Ca^{2+} influx via CRAC/Orai1. The sustained/elevated Ca^{2+}-level in of SLE T cells can contribute their hyperactivity, which might occur due to the reduced residence time of Kv1.3 in the immunological synapse (IS). The knock-down of actin-binding proteins (HS1/WASp/WAVE2), which may be responsible for the immobilization of ion channels, hampers Ca^{2+}-signaling and IS formation of T lymphocytes. Hence, the immobilization of T cell ion channels by actin-binding proteins can be a key to understanding autoimmunity. We could show that Kv1.3 co-localizes with and is in the proximity of the HS1 in the IS of T cells. Also its analog, cortactin could anchor and restrict diffusion of Kv1.3 channels to the actin cytoskeleton upon F-actin polymerization, which is a hallmark of IS formation in T cells [1]. Furthermore, we could show that HS1 has a physical interaction with Orai1 N-terminus, which predicts a functional interaction of these proteins. Based on these, we suppose that the anchoring of cation channels in the IS have physiological role. We need further experiments to clarify the role Orai1-HS1 interaction.

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Expression of genes involved in SOCE in *Danio rerio*

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Store-Operated Calcium Entry (SOCE) is a major calcium influx mechanism in non-excitable cells, however, there is increasing evidence indicating its significance in neurons (1). Dysregulated calcium homeostasis is a feature of many neurodegenerative disorders (2, 3, 4). Since zebrafish is a promising model of a number of human diseases we analyze its SOCE components to make models of some pathologies. Using RT-PCR we estimated the level of expression of main SOCE players – *stim*s and *orai*s in zebrafish larvae and in various tissues obtained from adult fish. Similarly to what was observed in rodents, *orai2* dominates in brain (5), nevertheless there are some differences between mammalian and zebrafish expression pattern regarding Stim proteins. It was shown that *Stim2* is mainly expressed in the mouse brain (5, 6), while in zebrafish both *stim2* isoforms dominate in muscles, however their level in brain is still relatively high. Using CRSPR/Cas9 technology was generated *stim2b* knock-out and found a decrease in the level of *orai1a* and *orai2* transcripts. However, expression of other genes involved in calcium homeostasis (*stims* and *trp* channels) was not changed. We will analyze calcium homeostasis in neurons of *stim2b* knock-out by crossing it with GCaMP5g line expressing calcium probe in neurons.

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