Abstract book of the

Polish Zebrafish Society

2nd virtual meeting

15 September 2021

Organizers:
The Polish Zebrafish Society

Organizing committee:

Przemko Tylżanowski, Department of Development and Regeneration, University of Leuven

Marta Migocka-Patrzałek, The Department of Animal Developmental Biology, Institute of Experimental Biology, University of Wroclaw

Piotr Podlasz, Department of Pathophysiology, Forensic Veterinary and Administration, Faculty of Veterinary Medicine, University of Warmia and Mazury

Edited by Marta Migocka-Patrzałek

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Abstracts

1. Optimising two-photon microscopy for investigating neural activity in the larval zebrafish eye and brain - Filip Janiak

Filip Janiak[1], Phillip Bartel[1], Michael Bale[1], Takeshi Yoshimatsu[1], Mingyi Zhou[1], Emilia Komulainen[1], Kevin Staras[1], Lucia Prieto-Godino[2], Thomas Euler[3,4], Miguel Maravall[1], Tom Baden[1,3]
1: Sussex Neuroscience, School of Life Sciences, University of Sussex, UK; 2: The Francis Crick Institute, London, UK; 3: Institute of Ophthalmic Research, University of Tuebingen, Germany; 4: Centre for Integrative Neuroscience, University of Tuebingen, Germany.

In vertebrate vision, signals are pre-processed in the retina and projected to diverse brain areas to ultimately drive behaviour. However, how different brain areas integrate different streams of visual information, and how they are connected, remains an area of active research. For example, many retinal output axons do not simply innervate a single brain target, but instead bifurcate to simultaneously drive two or more distinct areas based on the same original spike train. Is this bifurcation simply a duplication of original message, or might there be additional, asymmetric modification of the message in each output branch? To understand these types of questions, we would ideally like to simultaneously monitor neuronal activity in the eye and multiple brain areas.

We present our progress towards achieving this imaging capability using scanning 2-photon scanning microscopy which allows imaging inside the live retina without strongly exciting photoreceptors. Our ultra-low-cost solution only requires optical modifications of a typical commercial microscopes' optical pathway and the addition of an electrical tunable lens.

By simplifying and tuning a standard Sutter-MOM 2-photon setup into a noncollimated design, we can extend our x-y field of view up to 3.5 mm diameter using a standard x20 objective. In hand, our z-range covers 0.6 mm within 2 ms without strongly distorting the excitation volume or efficiency. Our design also provides for millisecond excitation power adjustment via a Pockels cell, as required. Finally, we achieve arbitrary spectrum optical/optogenetic stimulation by synchronizing our light sources with the scan retrace. Taken together, we can create arbitrary scan-paths with a maximal travel time of 2 ms during arbitrary spectrum optical stimulation. Our approach can be easy adapted to experiments on different animal models, like mouse, rats and drosophila.

2. Spin probe and spin trap toxicity for zebrafish (danio rerio) embryos - Katerina Makarova

Katerina Makarova[1], Katarzyna Zawada[1], Malgorzata Wiweger[2]

[1] Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Warsaw

[2] Laboratory of Neurodegeneration, International Institute of Molecular and Cell Biology in Warsaw

Electron Paramagnetic Resonance (EPR) is a technique that allows direct detection of free radicals. In most cases, probes and spin traps are used to determine the redox status with the EPR technique [1]. Spin probes such as TEMPO, TEMPOL, 5-DSA, OX063 are paramagnetic chemical compounds that can be directly observed by EPR spectroscopy and give information concerning the physicochemical parameters of microenvironment. On the other hand, spin traps (e.g. DMPO, PBN, POBN) are compounds that only after capturing free radicals give a specific EPR signal. Thus, it is possible to perform a qualitative and quantitative analysis of individual free radicals which is important e.g. in the studies of oxidative stress.

Models based on zebrafish embryos are used in oxidative stress studies [2]. Additionally, the zebrafish is one of the few laboratory animals whose size allows to perform analyzes using standard EPR resonators. In *in vivo* studies, both probes and spin traps should be administered to the animals before the start of the actual experiment, at concentrations high enough to obtain an EPR signal and safe for the animal at the same time.

This study investigated the toxicity of TEMPO, TEMPOL, and 3-carbamoyl-PROXYL spin probes and PBN, POBN, and DMPO spin traps for wild-type *D. rerio* embryos at short (30 minutes) and long (2 days) exposure. The aim was to determine the concentration range allowing the evaluation of oxidative stress with the EPR technique in the *in vivo D. rerio* embryo model.

Acknowledgement: The research was performed under grant “Innovator incubator 2.0” from Technology Transfer Office, Medical University of Warsaw.

3. The Good, the Bad and the Ugly – histopathology in zebrafish - Karolina Naumowicz

Department of Pathophysiology, Forensic Veterinary Medicine and Administration,
Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland

Histopathology in fish, especially in the zebrafish field, is often troublesome and its results can be more than confusing. It is considered a key endpoint in animal studies, the importance of which is often overlooked by research teams. This is explained by the common but erroneous statement that the basic techniques of pathology are completely standardized compared to modern molecular, biochemical, genetic and "omic" methods. In fact, many variables can influence the outcome of even "routine" pathological techniques, and failure to define these factors makes it impossible to properly evaluate the results.

The main traps of histopathology in zebrafish are inadequate experiment design, improper histological processing, inadequate data analysis, misinterpretation of the diagnosis, peer review by persons with insufficient specialist knowledge of the test species, model system or methods used, non-compliance with existing guidelines, and last but not least increasing pressure to publish quickly.

To increase the reliability of the histopathological assessment widely available guidelines were created. The international standards include guidelines for research planning "Animal Research: Reporting In Vivo Experiments" (ARRIVE), the "Gold Standard of the Publication Checklist" (GSPC), the Minimum Information for Publication of Experimental Pathology Data (MINPEPA), the global open Registry Nomenclature Information System (goRENI), the International Harmonization of Nomenclature and Diagnostic Criteria for Lesions (INHAND), the Registry of Industrial Toxicology Animal-data (RITA). These guidelines comprehensively cover study planning, publication elements and detailed technical aspects of the histopathological assessment.

4. Voltage-gated potassium channels as developmental regulators of inner ear - Justyna Jedrychowska

International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland

Voltage-gated potassium channels selectively regulate transport of K^+ along electrochemical gradient in plasma membrane. They are involved in a variety of biological processes including mechanotransduction, protein transport, neuronal impulse, etc. The zebrafish Kcnb1 encodes the electrically-active subunit of the slow-inactivating delayed rectifier (IK) Kv2.1, a member of the Kv2 subfamily of Kv channels expressed in the mammalian, *Xenopus'* and zebrafish's inner ear. Moreover, it is known that of all bodily fluids, a fluid of the inner ear – endolymph, has the highest concentration of K^+. Kcnb1 is expressed in cells lining the cavity of developing inner ear of zebrafish (Shen et al., Development, 2016). Hence, Kv2.1 could be important for ear development, where it may be required for proper hearing and spatial orientation. Using the loss-of-function *kcnb1* mutant, a role of this gene was studied during zebrafish development and a link between deficiency of Kcnb1 and abnormal development and functioning of the inner ear has been shown. Ear of developing zebrafish *kcnb1*$^{-/-}$ and its hearing stones, otoliths are small. The orientation of kinocilia of mutants' mechanosensory cells is affected. All these phenomena taken together could be responsible for behavioral defects in hearing and balance detected in Kcnb1 morphants and mutants. Kcnb1 plays an important role during development and function of zebrafish ear.

5. Role of antisense oligonucleotide-induced intronic miRNA on axonal morphogenesis - Savani Anbalagan

Institute of Molecular Biology & Biotechnology, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland. savanb@amu.edu.pl

Monogenic diseases due to haploinsufficiency are caused by reduced expression or deficient proteins. A promising approach to restore gene expression is via antisense oligonucleotides (ASO) targeting non-productive splice sites that can increase targeted gene expression. There are 1246 disease-associated genes whose expression can be potentially modulated via ASO's targeting non-productive splice sites. Although this is a straightforward approach, a major issue arises when the targeted gene is embedded with intronic miRNA. Whether ASO's targeting host genes embedded with intronic miRNA affect miRNA expression and function is unknown. While studying how glial cells regulate axonal morphogenesis in larval zebrafish, we made a serenpeditous observation that ASO targeting host gene splice site disrupt developmental axonal morphogenesis and the phenotype can be rescued only by intronic miRNA knockdown. To the best of our knowledge, this is the first time an indirect effect of ASO-induced intronic miRNA dysregulation is observed. Our results call for a careful evaluation of ASO's when targeting host genes embedded with intronic miRNA.

Key words: Intronic miRNA, antisense oligonucleotides, axonal morphogenesis

6. Knockdown of tubgcp2 results in small head and cell cycle disruption of brain neural progenitor cells in zebrafish embryos - Dawidziuk Mateusz

Dawidziuk Mateusz[1], Kasica Natalia[2], Bartoszewski Slawomir[3], Podlasz Piotr[4], Winata Cecilia Lanny[5], Wiszniewski Wojciech[6], Gawlinski Pawel[1]

[1] Department of Medical Genetics, Institute of Mother and Child, Warsaw, Poland.
[2] Department of Animal Anatomy, University of Warmia and Mazury, Olsztyn, Poland.
[3] Department of Biology, University of Rzeszow, Rzeszow, Poland.
[4] Department of Pathophysiology, Forensic Veterinary Medicine and Administration, Faculty of Veterinary Medicine, University of Warmia and Mazury, Olsztyn, Poland.
[5] Laboratory of Zebrafish Developmental Genomics, International Institute of Molecular and Cell Biology in Warsaw, Warsaw, Poland.
[6] Department of Molecular and Medical Genetics, Oregon Health and Science University, Portland, USA.

Nine year old boy with microcephaly and delayed psychomotor development was admitted to the Genetic Counselling of the Department of Medical Genetics at IMC. Exome sequencing revealed bi-allelic variants in TUBGCP2 gene encoding a protein that is part of a multiprotein complex responsible for microtubules nucleation. However, the involvement of this gene in the development of the central nervous system is yet unknown. Using GeneMatcher we found three additional families carrying possibly pathogenic variants in TUBGCP2 with similar phenotypes. This allowed for delineation of previously undescribed neurodevelopmental disorder. To investigate molecular mechanisms responsible for disruption of proper growth of the human brain caused by mutations in this gene, we investigated homologs of human TUBGCP2 in model organisms, Danio rerio and Drosophila melanogaster. Knockdown of tubgcp2 by morpholino revealed significant head reduction in 3 days post fertilization zebrafish embryos. This phenotype was rescued in more than 30% of embryos by coinjection of corresponding mRNA. Additionally, pH3 staining, similar to TUBGCP3 published data, revealed higher number of dividing cells in the brain of morphants in comparison to the wild type that suggests block of the cell cycle in the M phase. Also, developmental aberrations including larval brain were observed in Drosophila melanogaster mutant for either hypomorphic or amorphic alleles of Grip84, a homolog of TUBGCP2. Our data confirms that TUBGCP2 and its homologs, are necessary for proper brain development in human as well as zebrafish and fly, and that disruption of mitotic cell division is the likely cause of smaller head phenotype.

7. Neuroinflammation and sickness behavior in zebrafish infected with Tilapia lake virus - Miriam Mojzesz

Miriam Mojzesz[1], Magdalena Widziolek[1], Mikolaj Adamek[2], Urszula Orzechowska[1], Piotr Podlasz[3], Tomasz Prajsnar[1], Niedharsan Pooranachandran[1], Anna Pecio[4], Anna Michalik[5], Win Surachetpong[6], Magdalena Chadzinska[1], Krzysztof Rakus[1*]

[1]Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland
[2]Fish Disease Research Unit, Institute for Parasitology, University of Veterinary Medicine, Buenteweg 17, 30559 Hannover, Germany
[3]Department of Pathophysiology, Forensic Veterinary and Administration, Faculty of Veterinary Medicine, University of Warmia and Mazury, Michała Oczapowskiego 13, 10-719 Olsztyn, Poland
[4]Department of Comparative Anatomy, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland
[5]Department of Invertebrate Development and Morphology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland
[6]Department of Veterinary Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, 50 Ngam Wong Wan Road, Ladyao, Chatuchak, 10900 Bangkok, Thailand
*Corresponding author: Krzysztof Rakus, Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University, Gronostajowa 9, 30-387 Krakow, Poland. E-mail: krzysztof.rakus@uj.edu.pl

In mammals, relationship between immune system and behavior is widely studied. In fish, the knowledge about the brain immune response and behavioral changes during viral infection in the brain is very limited. To investigate this subject, we used, developed in our laboratory, model of tilapia lake virus (TiLV) infection of larvae and adult zebrafish (Danio rerio). Our results demonstrated that zebrafish brain in both developmental stages is susceptible to TiLV infection. Moreover, we demonstrated that TiLV persist in the brain of adult zebrafish for at least 90 days, even when the virus is not detectable in other peripheral organs such as spleen, kidney and liver and induces pathological changes in the brain morphology. The gene

expression study demonstrated that TiLV induces strong anti-viral immune and inflammatory response and up-regulation of microglia/macrophages markers in the brain. Furthermore using zebrafish larvae and in vivo microscopy, we showed the activation of microglia during TiLV infection. Finally, we found that during TiLV infection zebrafish show clear sickness behavior: decreased locomotor activity and food intake and tend to stay in the bottom zone of aquaria. This is the first study presenting a comprehensive analysis of the brain immune response connected with microglia activation and subsequent sickness behavior during systemic viral infection of zebrafish. We believe that the extended knowledge of evolutionary conservation of important ligands and receptors involved in the regulation of the immune response within nervous system and in inducing behavioral changes will support hypothesis about the functional importance of these.

This work was supported by the National Science Centre of Poland under Sonata Bis 5 project (Grant number UMO-2015/18/E/NZ6/00516).

8. Modelling chemotherapy-associated cardiotoxicity in zebrafish: Pharmacological evaluation of doxorubicin-induced heart failure - Monika Maciag

Monika Maciag[1,2], Artur Wnorowski[1], Anita Plazinska[1]

[1]Department of Biopharmacy, Medical University of Lublin, Lublin, Poland

[2]Laboratory of Behavioural Studies, Medical University of Lublin, Lublin, Poland

Although doxorubicin belongs to one of the most effective anticancer drugs, its use leads to life-threatening heart failure. Cardiotoxic properties of doxorubicin have been thoroughly studied in both rodent models and human patients. However, it remains unknown how the zebrafish heart responds to this well-known cardiotoxic compound. Modelling cardiac diseases in zebrafish has many advantages compared to the traditional murine model. Optical transparency and rapid development allow for visualisation of the circulatory system within individual animals even at the embryonic stages. Validation of drug-dependent cardiotoxicity profile is essential for further translation of zebrafish data to humans.

To determine whether both existing and novel therapies attenuate the doxorubicin-induced cardiotoxicity in zebrafish, we investigated the protective effects of β-blockers (metoprolol and carvedilol), angiotensin receptor blocker (valsartan), and dexrazoxane (the only clinically approved cardioprotective agent used in anthracycline-based anticancer therapy) against doxorubicin.

Zebrafish were exposed to both cardiotoxic and cardioprotective drugs from 24 to 96 hours post fertilisation. The compounds were compared regarding the elicited changes in cardiovascular parameters and morphological alterations using DanioScope (Noldus).

The zebrafish model reflects key aspects of doxorubicin-induced cardiotoxicity, i.e. reduction in heart rate, blood flow and vasoconstriction. The functional dysfunctions are accompanied by reactive oxygen species overproduction. Additionally, we show that the cardiotoxicity is mitigated by dexrazoxane and pharmacological inhibition of the angiotensin system, and to a lesser extent, the β-adrenergic system. In conclusion, our results support the value of the zebrafish model for studying cardiovascular disease, including in the cardio-oncology field.

The authors acknowledge financial support from the National Science Centre, Poland (Grant 2017/25/B/NZ7/02654).

9. Screening of endosulfan and its degradation products toxicity using the Danio rerio embryos – Patrycja Jesionkowska

Patrycja Jesionkowska, Katerina Makarova, Katarzyna Zawada, Paweł Siudem

Department of Physical Chemistry, Faculty of Pharmacy, Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland

Endosulfan, an organochlorine insecticide, is an environmentally persistent pesticide. It poses health problems for people and aquatic animals. Endosulfan is still used in many countries, it is applied to crops like cashew or cotton in India and China despite the official ban.

In the present study *Danio rerio* (wild type, ABxTL) embryos were used as a model to compare xenoestrogenic properties of endosulfan alpha and beta, endosulfan sulfate, lactone, ether, alcohol, 1-hexene and methylcyclohexane (concentration from 1 to 100 μg/L). Embryos were exposed to the tested compounds from 24 to 72 hours post-fertilization. Endosulfan lactone 2,5 μg/l and 5 μg/l caused the biggest mortality, endosulfan beta 10 μg/l led to the biggest delay in the hatching rate. Endosulfan and its metabolites did not lead to significant differences in length of the 72 hpf larvae

We used molecular docking (*in silico*) to study the receptor-binding affinities of tested compounds to a number of zebrafish and human receptors (hERα, hERβ, fERα, fERRyA, fERRyB, hGABA-A and hGABA-B). It revealed relatively low binding affinity of all studied compounds to fish estrogen receptors. However, all studied compounds have an affinity to GABA receptors, with the highest binding affinity obtained for the endosulfan alcohol and 1-hexene. Molecular docking results suggest that endosulfan and its metabolites induce toxicity through a different mechanism.

Zebrafish *in vivo* tests and *in silico* works gave complementary results in the assessment of toxicity and estrogenicity of the studied compounds and could be applied for toxicological studies of other xenoestrogens.

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PTZ webpage

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