Abstract book of the

Polish Zebrafish Society

Virtual meeting

23rd of October 2020

Organizers:
The Polish Zebrafish Society

Organizing comitee:

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 byMarta Migocka-Patrzałek

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Coffee break (11.00 - 11.10)	-	-
Part III (11.10 – 12.00)	Members' presentations	5. Justyna Magdalena Hermanowicz 6. Katerina Makarova 7. Barbara Budźyńska
12.00 - 13.00	Closing remarks	

*General discussion **agenda**:

1- Apologies to IIMCB, and kind request to provide contact person from the Institute.

Proposal for a "plan B" in our communication ways (web page, FB, and maybe more…)

2- Please send us list of grants;

3- Please send us also list of zebrafish strains;

4- Please send us list of instruments as well - PLEASE!!!!!!!!!!

5- What do you think about a list of skills? – for discussion

7- Next Workshop

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9- SURPRISE!!!!

10- Varia

Abstracts

1. Reactive oxygen species and autophagy in *Staphylococcus aureus* infection – double-edged swords? - Tomasz Prajsnar

Staphylococcus aureus is an important human pathogen responsible for a wide range of pathologies, with no vaccine available and common antibiotic resistance. Accumulating evidence indicates that *S. aureus* intracellular infection of professional phagocytes is an important step in the disease progression. However, the mechanism of *S. aureus* phagocyte parasitism is unknown. We have recently shown that upon *S. aureus* internalization, neutrophils undergo autophagy-related LC3-associated phagocytosis (LAP) leading to formation of intraphagocyte niche and subsequent bacterial dissemination. The intraneutrophil LAPosomes are non-acidified and that inhibition of production of reactive oxygen species (ROS) leads to abrogation of LAP and formation of acidified phagosomes what results in better disease outcome. However the mechanisms governing ROS production, the subsequent autophagic response and phagosomal acidification still remain elusive.

The present project, funded by the National Science Centre (NCN), takes an interdisciplinary approach to understand how the intracellular infection is initiated and what intraphagocyte pathways are involved. We will verify whether *S. aureus* subverts host innate immune response by exploiting autophagic response and explore the role of ROS production and phagosomal acidification in *S. aureus*-infected phagocytes. Studies will be performed on *in vivo* zebrafish and mouse models of *S. aureus* infection and *ex vivo* studies of human phagocytes using combination of candidate gene and unbiased approaches. We will generate tools in zebrafish (CRISPR/Cas9-mediated knockouts and Tol2-mediated fluorescence reporter transgenic lines) which will allow us to study the role of selected genes in ROS generation, phagosomal acidification and autophagic response to *S. aureus* infection *in vivo*.

2. Type I interferon-dependent response of zebrafish larvae during tilapia lake virus (TiLV) infection - Magdalena Widziolek

Magdalena Widziolek[1], Klaudia Janik[1], Miriam Mojzesz[1], Niedharsan Pooranachandran[1], Mikolaj Adamek[2], Anna Pecio[3], Win Surachetpong[4], Jean-Pierre Levraud[5], Pierre Boudinot[6], Magdalena Chadzinska[1], Krzysztof Rakus[1,*]

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Tilapia lake virus (TiLV; genus: *Tilapinevirus*, family: *Amnoonviridae*) is a recently characterised enveloped virus with a linear, negative-sense single-stranded RNA genome. This virus is responsible for causing high mortality in tilapia species since 2009. In the present study, we demonstrated that zebrafish (*Danio rerio*) larvae are susceptible to TiLV infection upon systemic injection into Duct of Cuvier. TiLV replicated in zebrafish larvae and caused their high mortality (of about 70 %) within 5 days post-infection. Histopathological examination revealed that TiLV infection caused pathological abnormalities in zebrafish larvae that were well visible within the brain and gastrointestinal track. Moreover, gene expression analysis revealed that TiLV infection induced up-regulation of the expression of the immune-related genes encoding pathogen recognition receptors involved in sensing of viral RNA (*rig-I (ddx58), tlr3, tlr22*), transcription factors (*irf3, irf7*), type I interferon (*infφ1*), antiviral protein (*mxa*), and pro-inflammatory cytokine (*il-1β*). In addition, we demonstrated the protective role of the recombinant zebrafish IFNφ1 on the survival of zebrafish larvae during TiLV infection. Our results show the importance of type I IFN response during TiLV infection in zebrafish larvae and demonstrate that zebrafish is a good model organism to study interactions between, a newly emerging in aquaculture virus, TiLV and fish host.

3. Phenotypic characterization of new zebrafish model of Dravet syndrome - Kinga Gaweł

Dravet syndrome (DS), developmental and epileptic encephalopathy, is characterized by recurrent, intractable seizures. In 80% of cases, the disease is caused by *de novo* mutations in *SCN1A* gene, encoding sodium channels $Na_v1.1$. Currently approved drugs i.e. cannabidiol nad stiripentol do not modify disease, but ameliorate seizures. Thus, searching for new therapeutic treamtent options as well as pathological mechanisms leading to DS is crucial. In our latest papar, we described a new zebrafish model of DS. *scn1lab*$^{-/-}$ larvae exhibited spontaneous seizures. Using transgenic reporter line, we were able to prove 40% decrease in GABA-ergic neurons arborization in the optic tectum of 6 days old *scn1lab*$^{-/-}$ larvae. It was accompanied by increase in neurons proliferation in the same structure. Next, we assessed the activity of fenfluramine, a potent serotonin releaser, to counteract the abovementiond changes in DS mutants. Here, we observed that chronic fenfluramine treatment not only decreased number of seizures, but also reversed the changes in GABA-ergic neurons arborization, as well as hyperproliferation. In conclusion, our study provide new evidence for (1) DS-linked epileptogenesis mechanisms, and (2) disease-modyfing effects of fenfluramine in DS.

4. Zebrafish model as an in vivo platform for evaluation of cardiovascular drug safety and efficacy - Monika Maciąg

Monika Maciąg[1,2], Artur Wnorowski[1], Anita Płazińska[1]

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Cardiovascular diseases are a leading cause of mortality globally. Therefore, searching efficacious new therapies is an important and intense area of research. Recently, the use of zebrafish model in preclinical studies has greatly increased. The time- and cost- effective assays make larval zebrafish as a perfect platform for high-throughput *in vivo* study of complex processes.

So far, a few cardiotoxic drugs have been proposed as zebrafish-based heart failure models. Doxorubicin, β-adrenergic agonists, and terfenadine are characterized by well-documented cardiotoxicity. Therefore, the aim of study was to uncover how zebrafish heart responds to cardiotoxic treatment and then to determine whether human medications are able to manage heart dysfunction in zebrafish.

Zebrafish were exposed to both cardiotoxic and cardioprotective drugs for 96 hours post fertilization. The compounds were compared with respect to the elicited changes in heart rate, blood flow, and diameter of vessels using DanioScope software (Noldus). All of the tested cardiotoxic compounds display concentration-dependent heart rate inhibition, however in the different range of concentrations. Whereas, only epinephrine-induced cardiotoxicity was attenuated by medication.

Here, we try to answer one of the most fundamental question about whether zebrafish heart responds to cardiotoxic and cardioprotective drugs in the same as humans. And whether it may and should therefore be applied for investigation of novel therapeutic strategy to treat cardiac diseases.

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5. Combination of Bruton's tyrosine kinase inhibitor with erythropoietin as a new option for breast cancer therapy - Justyna Magdalena Hermanowicz

Justyna Magdalena Hermanowicz[1,2], Anna Tankiewicz-Kwedlo [3], Beata Sieklucka [1], Krystyna Pawlak [3]and Dariusz Pawlak[1]*

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Abstract

Bruton's tyrosine kinase (BTK) is a major apoptosis regulator that activates multiple antiapoptotic signaling molecules and networks. The therapeutic potential of BTK inhibition by LFM-A13, besides the inhibition of DNA synthesis, the reduction of proliferation, survival and cell migration, also promotes apoptosis. The aim of the present study was to evaluate the effect of simultaneous use of erythropoietin (Epo) and LFM-A13 on the viability and tumor development of breast cancer cells. We assessed apoptosis using several biochemical markers (phosphatidylserine (PS) externalization, loss of mitochondrial membrane potential (MMP), and activation of caspase 6). We also explored the effects of LFM-A13 alone and in combination with Epo on cell cycle progression and BTK and cyclin D1 expression. **Results:** The results demonstrated that Epo, significant intensifies the anticancer activity of LFM-A13 in MCF-7 and MDA-MB-231. The featured therapeutic scheme efficiently blocked the tumor development in zebrafish experimental cancer model. Epo and LFM-A13 administered together resulted in effective cell killing, accompanied by attenuation of the BTK signaling pathways, loss of MMP, accumulation of apoptotic breast cancer cells with externalized PS, a slight increase in phase G0/G1, and a reduction in cyclin D1 expression. **Conclusions**: This therapeutic scheme may be useful in the treatment of breast cancer patients with high BTK expression. Simultaneous use of Epo and LFM-A13 should be considered as a combination therapy for breast cancer.

6. In vivo melanin radical detection in Danio rerio embryos with X-band EPR spectroscopy - Katerina Makarova

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Melanin radicals formation is associated with the mechanism of UV- induced skin photocarcinogenesis and new treatments of melanoma. However, there is no effective way to study melanin radical *in vivo* in a model as zebrafish, which allow high-throughput screens. This study proposes detecting melanin radical *in vivo* in wild-type zebrafish embryos with Electron Paramagnetic Resonance (EPR). We have used a benchtop EPR X-band spectrometer (Magnettech/Adani, MS200) with a multi-harmonic analyzer eSpect+ by Novilet. Additionally, we have developed a special flat EPR capillaryfor the *Danio rerio* embryos which allow measuring embryos *in vivo* for several subsequent days.

Melanin radical gives a single line in the EPR spectrum. Thus, zebrafish embryos after developing pigmentation (stages >27 hpf) give a single peak in EPR spectrum, which intensity is related to the amount of melanin radical. This signal is missing in pigment-free albino line. The specificity of the EPR signal was confirmed, as the intensity of the signal detected in the *D. rerio* embryos changed after application of dugs inhibiting or accelerating the synthesis of melanin (e.g., hydroquinone, sesamol, PTU). Thus, EPR spectroscopy could be used to access the level and monitor changes in the melanin radical in living zebrafish embryos. This method opens new possibilities for melanoma studies

7. Zebrafish larvae in behavioural studies - can we replace rodents? - Barbara Budzyńska

Barbara Budzyńska

Samodzielna Pracownia Badań Behawioralnych

Rodents are the basis for research in many areas, but Danio rerio model is gaining popularity. It was shown that the pharmacological responses of the zebrafish were similar to those seen in rodents. After quick maturation of the sensory and motor system, zebrafish larvae are able to show robust and complex behaviours in the first week of development. Zebrafish larvae display e.g., the photomotor response, visual motor response, and light/dark avoidance response. The thier behavioural repertoire can play a role in many research fields such as drug discovery and high-throughput screening, testing the behavioural effects of chemicals and toxins from a safety pharmacology perspective. Zebrafish displayed also anxiety-like behaviours including dark-avoidance in the *light/dark box*, and thigmotaxis in the *open field* test. This use can result in reduce the numbers of rodents used in the drug discovery and compound screening process. Thus, zebrafish can provide a complement to rodent models, especially in the fields of neurobehavioural research and drug discovery.

Remember about our webpage:

www.zebrafish.org.pl