The

5th Polish Zebrafish Society Workshop

Abstract Book

Lublin

29.06 - 1.07.2022

ORGANIZING COMMITTEE:

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LIST OF CONTENTS:

Introduction

Welcome to the "**5th Polish Zebrafish Society Workshop**" organized by the **Polish Zebrafish Society** and held **under the patronage of the Medical University of Lublin Vice-Chancellor of Science prof. dr. hab. n. med. Andrzeja Stypulaka**.

The training is intended **for all researchers interested in using zebrafish** (*Danio rerio*) **as a model organism**.

At the first day of Workshop, **we invite everyone for open lectures** (no registration needed), regarding **investigating human diseases with the use of zebrafish as a model organism** (1:00 p.m. - 5:30 p.m., 29.06.2022). Stay with us after lectures to enjoy get together at the **Poster session**!

The lectures will be held at the Collegium Pathologicum, Medical University of Lublin, Dr K. Jaczewskiego 8B, 20-090 Lublin.

During the next two days (30.06 and 1.07.2022), the **practical training** will be provided to familiarize participants with zebrafish as a model organism: breeding, maintaining, basic techniques, tools and possible use in research will be discussed. The topics will cover toxicology, application of the CRISPR-Cas9 gene editing technology, genotyping, behavioral analysis, microinjection (including cancer cells microinjections to show zebrafish as a xenograft model), microscopy imaging (i.e., Light Sheet, phenotypic analysis) and more.

The workshop will be held at the Center for Experimental Medicine (Ośrodek Medycyny Doświadczalnej) of the Medical University of Lublin Dr K. Jaczewskiego 8d.

Program of the 5th Polish Zebrafish Society Workshop

Day I
LECTURES and poster session (open to public)

The lectures will be held at the Collegium Pathologicum, Medical University of Lublin, Dr K. Jaczewskiego 8B, 20-090 Lublin.

13.00-17.00

1. "The zebrafish: a model for human diseases" - **Marta Migocka-Patrzałek** - *University of Wroclaw*
2. "Zebrafish in behavioral studies" - **Barbara Budzyńska** - *Medical University of Lublin*
3. "Zebrafish and other model fish species in toxicology" – **Maciej Kamaszewski** - *Warsaw University of Life Sciences*
4. "Zebrafish xenograft models in preclinical oncology" - **Anna Boguszewska-Czubara** - M*edical University of Lublin*

20 min break

1. "Zebrafish developmental neurobiology owes to progress in research methodology" - **Vladimir Korzh** - *International Institute of Molecular and Cell Biology*
2. "Using the zebrafish model to understand immunity and host-pathogen interactions" - **Tomasz Prajsnar** - *Jagiellonian University*
3. "Can fish mimic a human? Zebrafish as a model in translational research" - **Anna Sarosiak** -*Institute of Physiology and Pathology of Hearing*
4. "Genetic modifications and genotyping" - **Niedharsan Pooranachandran** - *Jagiellonian University*

17.30 - 20.00 Poster session/ get together

Day II
Practical parts schedule

The workshop practical part will be held at the Center for Experimental Medicine (Ośrodek Medycyny Doświadczalnej) of the Medical University of Lublin Dr K. Jaczewskiego 8d.

8.00-8:30 Registration + welcome

Time	Group I	Group II	Group III	Group IV
8.30-10.30	Toxicity screening using zebrafish embryos (LiCl)-Part I (Marta Migocka-Patrzałek) + Dissection of organs from adult	Epilepsy (Kinga Gaweł) + Light sheet Anna Sarosiak	Microinjections (basics) (Przemko Tylzanowski) +Zebrafish xenograft model – (Anna Boguszewska-Czubara)	Behavior – (Barbara Budzyńska)+ Visit in the zebrafish facility (Agnieszka Jakubaszek)

	zebrafish, finclip (Piotr Podlasz)			
10.30-12.30	Behavior – (Barbara Budzyńska) +Visit in the zebrafish facility (Agnieszka Jakubaszek)	Toxicity screening using zebrafish embryos (LiCl)- Part I (Marta Migocka-Patrzałek) + Dissection of organs from adult zebrafish, finclip (Piotr Podlasz)	Epilepsy (Kinga Gaweł) + Light sheet Anna Sarosiak	Microiniections (basics) (Przemko Tylzanowski) +Zebrafish xenograft model – (Anna Boguszewska-Czubara)

12.30-14.00	Lunch & Discussion	Lunch & Discussion	Lunch & Discussion	Lunch & Discussion

14.00-16.00	Microiniections (basics) (Przemko Tylzanowski) +Zebrafish xenograft model – (Anna Boguszewska-Czubara)	Behavior – (Barbara Budzyńska) +Visit in the zebrafish facility (Agnieszka Jakubaszek)	Toxicity screening using zebrafish embryos (LiCl)- Part I (Marta Migocka-Patrzałek) + Dissection of organs from adult zebrafish, finclip (Piotr Podlasz)	Epilepsy (Kinga Gaweł) + Light sheet Anna Sarosiak
16.00-18.00	Epilepsy (Kinga Gaweł) + Light sheet Anna Sarosiak	Microiniections (basics) (Przemko Tylzanowski) +Zebrafish xenograft model – (Anna Boguszewska-Czubara)	Behavior – (Barbara Budzyńska) +Visit in the zebrafish facility (Agnieszka Jakubaszek)	Toxicity screening using zebrafish embryos (LiCl)- Part I (Marta Migocka-Patrzałek) + Dissection of organs from adult zebrafish, finclip (Piotr Podlasz)

18.30-20.00/21.00 Social event

DAY III

Time	Group I	Group II	Group III	Group IV
9.00-10.00	Toxicity screening using zebrafish embryos (LiCl)-Part II (Marta Migocka-Patrzałek)	Leica Thunder + Light sheet (Anna Sarosiak, Tomasz Prajsnar)	Visualization of xenografts, transgenic ZF (Piotr Podlasz) Nikon confocal	CRISPR (Niedharsan Pooranachandran)
10.00-11.00	CRISPR (Niedharsan Pooranachandran)	Toxicity screening using zebrafish embryos (LiCl)-Part II (Marta Migocka-Patrzałek)	Leica Thunder + Light sheet (Anna Sarosiak, Tomasz Prajsnar)	Visualization of xenografts, transgenic ZF (Piotr Podlasz) Light sheet luxendo
11.00-12.00	Visualization of xenografts, transgenic ZF (Piotr Podlasz) Light sheet luxendo	CRISPR (Niedharsan Pooranachandran)	Toxicity screening using zebrafish embryos (LiCl)-Part II (Marta Migocka-Patrzałek)	Leica Thunder + Light sheet (Anna Sarosiak, Tomasz Prajsnar)
12.00-13.00	Leica Thunder + Light sheet (Anna Sarosiak, Tomasz Prajsnar)	Visualization of xenografts, transgenic ZF (Piotr Podlasz) Light sheet luxendo	CRISPR (Niedharsan Pooranachandran)	Toxicity screening using zebrafish embryos (LiCl)-Part II (Marta Migocka-Patrzałek)
13.00-14.30	Lunch & Discussion	Lunch & Discussion	Lunch & Discussion	Lunch & Discussion

Abstracts

Lectures

1. The zebrafish: a model for human diseases – Marta Migocka-Patrzalek

Marta Migocka-Patrzałek

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Many different animal species has important roles as experimental model organisms in advance biomedical research. The significant biological similarities between humans and animals are reason why researchers can investigate novel drugs and therapies in animals before applying their discoveries to humans. Zebrafish is one of the most popular research models because of its numerous advantages. The number of research publications with the use of zebrafish has been significantly increasing year by year reaching almost 5000 records in 2021. Such wide use of this small fish comes from its features, such as external fertilization, rapid development, transparent nature of embryo, and high quantity of offspring. The expanding range of molecular and bioinformatic tools and growing network of scientific teams working on zebrafish leads to its increasing popularity. It is also worth mentioning that more than 70% of human genes have their counterparts in zebrafish. Therefore, zebrafish mutants could be an effective model for human disorders and genetic diseases. Such models enable the investigation of disease pathomorphology and the search for novel, effective therapies. The small size and large quantity of the zebrafish embryos allows also for high-throughput screening of potentially therapeutical compounds. In fact, the emergence of the zebrafish as an alternative vertebrate model has transformed the scale of drug screening due to lower costs. Although the zebrafish model has its limitations, such as being different from the human respiratory and reproductive system, it is an important biomedical model in (almost) every aspect of biology.

Research project partly supported by program „Excellence initiative – research university" for years 2020-2026 for University of Wrocław.

Keywords: model organism, zebrafish, animal model of human diseases

2. Zebrafish in behavioral studies - Barbara Budzyńska

Independent Laboratory of Behavioral Research
Medical University of Lublin

In recent years, the model of the Danio rerio (zebrafish) has become particularly useful in neurobiological research and pharmacogenetics, due to the high degree of morphological, physiological, and genetic similarity between the zebrafish and humans. Developing outside the female's body, the embryo, due to its transparency and rapid development, can be used for toxicological studies. Furthermore, the embryonic period ends 72 hours after fertilization with the larvae hatching, then 30 days last the juvenile period. From the first days, the larvae swim freely, respond to stimuli and exhibit full behavioral complexity, which contributes to the use of both adult fish and larvae in behavioral studies.

Despite the differences in the anatomical structure of the nervous system of these fish and mammals, the Danio rerio has most of the structures found in the mammalian brain, essential neurotransmitters, receptors, transporters, and enzymes responsible for the synthesis and metabolism of mediators. Dysfunction of these systems is responsible for diseases of the central nervous system, thus the zebrafish can be successfully used to study the diseases associated with disorders of this system.

Some behaviors of the Danio rerio are analogous to those observed in rodents. Among other things, the larvae of the zebrafish exhibit a photomotor response or anxiety-like behavior, assessed as thigmotaxis. Their behavioral repertoire can play a role in many research fields, such as high-throughput studies on the toxicology and pharmacological activity of new synthetic or naturally derived compounds. Such a broad application of the Danio rerio model in preliminary studies may result in a reduction in the number of rodents used in testing new drugs, both in terms of evaluating pharmacological activity and safety of use. Many other advantages of the model can also be mentioned, including the relatively small amounts of the test compound required, or the use of automated systems to assess the behavior of both larvae and adults. However, despite these numerous advantages, the question arises whether the use of the Danio rerio model can replace rodents in preclinical studies?

3. Zebrafish and other model fish species in toxicology – Maciej Kamaszewski

Department of Ichthyology and Biotechnology in Aquaculture, Institute of Animal Sciences, Warsaw University of Life Sciences (WULS–SGGW), 02-787 Warsaw, Poland

4. Zebrafish xenograft models in preclinical oncology - Anna Boguszewska-Czubara

Department of Medical Chemistry, Medical University of Lublin

As cancer is among the leading causes of death worldwide and cancer rates are highest in countries whose populations have the highest life expectancy, education level, and standard of living there is a great need to search for new anti-tumor drugs or innovative therapeutic approaches. For that purpose xenograft animal models are developed to provide more accurate, efficient and effective results of preclinical studies. The first use of human cell xenograft in zebrafish was reported in 2005, when green fluorescent protein (GFP)-labeled human cell lines were transplanted into blastula stage embryos, and since then, this model has been improved, adapted to new needs and developed for innovative imaging and visualization methods.

Zebrafish xenograft model offers a number of advantages over other xenograft models, like minimal maintenance and care requirements relative in comparison to mammalian models, quick development and high fecundity to ensure fast experiments with high number of repeats at low costs, a complement of orthotopic organs and tissues such as the brain, heart, and liver as well as a functioning circulatory system in 2-day post fertilization larva. And the most important among all: zebrafish possess lack an adaptive immune system in early life what ensure xenografts of human cancer cells without immune rejection, requires several times less cells for xenotransplantation and provide a model where cancer progression can be evaluated non-invasively within the host due to the transparent nature of embryo-larva zebrafish.The zebrafish xenograft model is not without limitations, including problems with dosing drugs via immersion method, problems with ADME evaluation and certain biological characteristics like greater capacity to regenerate multiple tissue types or some differences in gene function.

However, zebrafish xenograft model can be exciting tool to provide new and important data concerning tumor growth, proliferation, metastatic behaviours if the experiment is planned with attention to every detail and properly executed by an experienced researcher. The critical points of the experiment are to determine the optimal maintenance temperature for fish and cells in the same time, plan the course of the experiment, choose the place of cells implantation as well as their quantity and to select appropriate method of therapy dosage and administration. Thorough rethinking and planning of these factors depending on the needs of the experiment affects the course of the study and the quality of the obtained results. And so, zebrafish can provide a more authentic view to investigate human cancers as “avatars” for rapidly evaluating potential patient-personalized therapies.

5. Zebrafish developmental neurobiology owes to progress in research methodology - Vladimir Korzh

International Institute of Molecular and Cell Biology in Warsaw

Sydney Brenner, a Nobel Prize winner in 2000, once famously said: “Progress in science depends on new techniques, new discoveries and new ideas, probably in that order.” The zebrafish research model has its own specifics in terms of development and application of research methodology. Therefore, I will only add, to what Sydney Brenner said, that success will come when researchers have a very good understanding of the zebrafish model.

Over the 40 years of history of zebrafish biology, which started from studies in developmental neurobiology, a large number of research methods were developed for analysis of whole mounted fixed and live zebrafish embryos and larvae. Some examples illustrating this path will be discussed in this presentation, which will demonstrate, hopefully, how new methodologies may help to develop further an understanding of the old problems.

6. Using the zebrafish model to understand immunity and host-pathogen interactions - Tomasz Prajsnar

Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Faculty of Biology, Jagiellonian University

For the last two decades, zebrafish have proven to be a great model system for studying immune response and host-pathogen interactions. This is mainly due to the optical transparency of their larval forms and amenability to genetic manipulations such as CRISPR/Cas9-mediated mutagenesis and transgenesis.

In this lecture, I will introduce you to the zebrafish immune system and subsequently to the state-of-the-art tools useful in studying inflammation and infection (both viral and bacterial) in zebrafish.

In addition, I will present the recent zebrafish-related achievements of the Department of Evolutionary Immunology at the Jagiellonian University in the field of Infection and Immunity.

7. Can fish mimic a human? Zebrafish as a model in translational research - Anna Sarosiak

Sarosiak A.[1], Jędrychowska J.[2], Oziębło D. [1], Leja M. [1], Gan N. [1], Bałdyga N.[1], Skarżyński H[3]., Korzh V. [2], Ołdak M.[1]

[1]*Department of Genetics, Institute of Physiology and Pathology of Hearing, Warsaw, Poland*

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Zebrafish is a well-recognized animal model for disease and translational research. It is widely used for studying the pathogenicity and function of human genetic variants, dissecting mechanisms of infection, inflammation and cancer, developing models of metabolic and other diseases, and creating and testing of new therapeutic approaches, including pharmaceutical drug screenings and toxicity studies. Zebrafish model combines high throughput abilities of invertebrates and a significant genetic similarity to humans with approximately 70% of human genes having at least one obvious zebrafish orthologue.

In this review, we highlight recent successful researches in the field of translational medicine and present the results of our latest research on *TBC1D24* gene that is involved in the development of autosomal dominant hearing loss (ADHL). In this study we use zebrafish model for deciphering the pathogenicity of novel *TBC1D24* variants. So far, our group published four novel ADHL-causative *TBC1D24* probably pathogenic variants by performing high-throughput genetic testing in families with ADHL. We show that the knock-out of *tbc1d24* leads to impaired mechanotransduction in hair cells and results in reduction in the number of hair cells and neuromasts in zebrafish We also present our preliminary behavioral data supporting the evidence on pathogenicity of a novel p.Asp185Asn variant in *TBC1D24* gene, opening the gate for further studies on pathogenicity of other *TBC1D24* ADHL-causative variants using the zebrafish model.

8. Genetic modifications and genotyping - Niedharsan Pooranachandran

Jagiellonian University, Kraków

Zebrafish has become an excellent model for studying molecular mechanisms thanks to its high degree of evolutionary conservation at both the genetic level and development. The increasing number of molecular tools to manipulate the zebrafish genome has equipped researchers with the ability to study almost any gene of interest. CRISPR/CAS9 is the most recent and predominant gene editing tool used in Zebrafish. In this talk, an overview of the CRISPR/CAS9 technology will be covered from a technical standpoint, along with a brief intro to the various transgenesis approaches, and how CRISPR can be integrated with transgenesis.

Posters

1. **Using zebrafish model to verify pathogenicity of novel genetic variants causative for hearing loss - Nina Gan**

Sarosiak A.[1], Jędrychowska J.[2], Gan N.[1], Oziębło D.[1], Leja M.[1], , Bałdyga N.[1], Skarżyński H. [3], Korzh V. [2], Ołdak M.[1]

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Hearing loss (HL) is one of the most common disability of human senses, affecting 1-2 in 1000 live births. Around half of confirmed HL cases have a genetic background. To date, 124 genes have been identified as involved in the development of non-syndromic HL. Such genetic heterogeneity of HL makes next generation sequencing (NGS) a prominent tool for the genetic diagnosis of HL. NGS generates a vast amount of data, identifying thousands of variants for each patient – creating a need for differentiation between the benign and the disease-causing variants.

Two novel variants in two HL genes – p.Asp714His in *WFS1* and p.Asp185Asn in *TBC1D24* were selected for the study. Each of them was identified in HL patients of Genetic Outpatient Clinic of Institute of Physiology and Pathology of Hearing, using different NGS-based approaches. Variants segregated with HL in patients' families showing an autosomal dominant inheritance pattern and have been selected to verify their pathogenic potential using the zebrafish model.

Expression of both orthologue genes, *wfs1b* and *tbc1d24*, have been studied using the whole mount in-situ hybridization (WISH) and quantitative PCR (qPCR). Subsequently, a knock-out model using CRISPR/Cas9 method was created and morphology and function of crispants' hair cells and neuromasts were assessed using selected immunofluorescent stainings. Preliminary behavioral studies were performed using rescue model of p.Asp185Asn *tbc1d24* morphants.

Both genes were expressed at the early neurodevelopmental stage. The *tbc1d24* was expressed in clusters of primary neurons and in sensory cranial ganglia. The expression of *wfs1b* localized to the brain area and was diffused. Knock-out of *tbc1d24* lead to impaired mechanotransduction and resulted in reduction of hair cells and neuromast number. The performed behavioral studies suggested pathogenic character of p.Asp185Asn in *TBC1D24* gene. Further studies are needed

to understand the role of *wfs1* and *tbc1d24* in the zebrafish ear and nervous system and to decipher the pathogenicity of other *WFS1* and *TBC1D24* HL-causative variants.

This work was supported by grant awarded by National Science Center Sonata BIS 6 no. 2016/22/E/NZ5/00470: „Integrating whole genome sequencing and zebrafish functional studies to uncover new molecular basis of autosomal dominant hearing loss".

2. Zebrafish as a memory loss model - Justyna Grymuza

Biomedical Department of Medical University in Lublin, Poland

Zebrafish has numerous experimental advantages, which can be beneficial in research corelated to nervous system diseases and neurodegenerative diseases such as Alzheimer. From a structural perspective, the zebrafish brain is similarly aligned as the mammalian brain and possesses high homogenicity hippocampus-like and amygdala-like structure, which are main areas responsible for memory in human brain. The primary neurotransmiter system in zebrafish, including the noradrenergic, serotonergic, dopaminergic and histaminergic systems, show many similarities to the mammalian system. The homogeneous zebrafish dopamine and acetylcholine systems can be used in understanding the course and finding potential treatment of Alzheimer's disease (AD). The currently approved therapy for AD includes acetylcholinesterase inhibitors (AChEI) and a N-methyl D-aspartate receptor antagonist (NMDA), which are thought to preserve cholinergic neurotransmission.

Keywords: zebrafish, memory loss, Alzheimer's disease, cholinergic, dopaminergic, Acetylcholinesterase inhibitors

3. Inhibition of CXCR1-2 chemokine receptors affects stress response and stress-induced neutrophil redistribution in fish - Katarzyna Kłak

Katarzyna Klak, Magdalena Maciuszek, Magdalena Marcinkowska, Magdalena Chadzinska

Department of Evolutionary Immunology, Institute of Zoology and Biomedical Research, Jagiellonian University, 30-387 Kraków, Poland

In fish, stress causes the activation of the hypothalamus-pituitary–head kidney (HPI) stress axis, manifested by the release of CRH, ACTH and cortisol as well as redistribution of neutrophilic granulocytes from the lymphatic organs e.g., head kidney into the blood circulation.

Physiologic responses to stressors enable to overcome potential threat but also form an evolutionary tradeoff between the protective glucocorticoid effects considered compensatory and/or adaptive, versus the maladaptive and detrimental consequences of prolonged, excessive glucocorticoid secretion. Therefore, it must be tightly regulated. Apart from the essential hormonal feedback regulation, evidence accrues that cytokines, e.g., proinflammatory IL-1β, also play an important regulatory role in the stress axis.

Here we aimed to study the role of CXCR1 and 2 and their ligands - CXCL8 chemokines in the regulation of stress axis activity and stress-induced neutrophil redistribution in common carp (*Cyprinus carpio* L.).

Young fish were injected with reparixin (REP, non-competitive allosteric inhibitor of chemokine CXCR1 and CXCR2 receptors), SB225002 (SB, nonpeptide CXCR2 receptor inhibitor) or with vehicle (control fish) and after 1 h were stressed for 11 h by netting. The level of blood cortisol, number of neutrophils in the head kidney and circulation as well as expression of stress-related and chemokine genes were measured. We found that CXCR1-2 inhibition decreased stress-induced cortisol synthesis. Moreover, REP and SB injection prevented stress-induced upregulation of CRH gene expression in the hypothalamus while SB injection upregulated the expression of *11β-hsd2* (encoding enzyme involved in cortisol conversion to inactive cortisone) in the head kidney. Furthermore, in the head kidney and peripheral blood leukocytes, REP and SB-treatment down-regulated expression of genes encoding CXCL8 chemokines. Finally, CXCR1-CXCR2 inhibition prevented the stress-induced redistribution of neutrophils into the circulation.

Our data indicate that activation of the stress axis and stress-induced neutrophil redistribution are regulated by interaction between CXCL8 chemokines and their receptors.

4. Functional studies of hereditary human mutations leading to craniofacial malformations – Paulina Krzesińska

Paulina Krzesińska[1], Anna Jaruga[1], Krystian Kuźniarz[2], Przemko Tylzanowski[1,3]

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Orofacial clefts are the most commonly diagnosed face birth defects during pregnancy. They can be either syndromic or non-syndromic. We can classify facial clefting depending on affected structures: cleft lip (CL), cleft palate (CP), or both cleft lip and palate (CLP). The etiology of CLP is not fully understood, although it has been shown that genetic abnormalities can lead to incorrect palate development. Our research focused on searching for the genetic basis of the hereditary NCLP in a Polish family with three affected members. We found the mutations in *MASP1*, *EHHADH*, and *ACTN2* genes. *MASP1* gene is likely responsible for directing the migration of neural crest cells during embryonic development. Disorders in the *EHHADH* gene are associated with Zellweger syndrome, one of the symptoms of which is a craniofacial deformity. Mutations in the *ACTN2* gene are associated with the myopathy and cardiomyopathy. It is probably also related to the occurrence of deep bite defects. Despite the large amount of data on NCLP, the molecular mechanisms of this malformation are not well understood. This knowledge is necessary to understand the consequences of the hereditary non-syndromic occurrence of developmental defects as well as to explore pharmacological solutions to the problem. Our research will generate data that will get us a step closer to understanding the intricate connection between genome, gene transcription, and tissue patterning.

Key words: craniofacial malformations, Orofacial clefts, compound inheritance, *EHHADH*, *MASP1*.

5. MDMA modulates anxiety and social behaviours in zebrafish - Monika Maciag

Monika Maciag[1,2], Barbara Budzynska[1]

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Mental disorders are an umbrella term that covers psychiatric, behavioural or emotional dysfunctions. It is estimated that in 2021, mental disorders affected nearly 1 billion people worldwide, i.e., 13% of the global population. Although new classes of psychiatric medicines were introduced over the last decades, the same neurotransmission continued to represent their main targets.

3,4-Methylenedioxymethamphetamine (MDMA, 'Ecstasy') is one of the amphetamine derivatives, which belongs to psychostimulants with additional euphoric, prosocial, empathogenic, anxiolytic and hallucinogenic properties. Most recently, MDMA passed a big clinical trial for the treatment of post-traumatic stress disorder (PTSD).

At the moment, the possible explanations for how MDMA produces its effects on anxiety-related and social behaviours remain speculative. Therefore, we assessed here the acute effects of MDMA on 21 days post fertilisation (dpf) zebrafish behaviours. Next, we evaluated the involvement of the oxytocin system in the MDMA-induced effects.

Fish were exposed to the tested compounds one hour prior to experiments. An anxiety behaviour was tested based on a thigmotactic response – the tendency to move close to the boundaries and to avoid the centre of the open arena. The procedure was enriched with alteration in light and dark conditions. To assess social preference for conspecifics, the three-chamber social test was performed.

MDMA elicited anxiolytic activity in fish, observed by an increase in the distance moved and time spent in the centre of the arena as well as an increase in crossings to the inner zone. It was associated with prosocial behaviour, and the oxytocin system was implicated in these effects. The obtained results may suggest a therapeutic value of psychedelics drugs, such as MDMA to treat mental illness.

The authors acknowledge financial support from the Medical University of Lublin (grant no. PBmb211).

6. Analysis of bee venom components for their anti-glioma activity in a cell and fish model (Danio rerio) - Agata Małek

Department of Medical Chemistry, Medical University of Lublin, Lublin, Poland

Background: One of the substances of natural origin with potential anticancer properties is bee venom (BV), also known as apitoxin. Previous studies have suggested the anticancer properties of bee venom (BV) and/or its major components against prostate, ovarian, breast, lung and several other cancers. However, literature data on the effect of bee venom on glioma cells are scarce. The main components of apitoxin have the ability to penetrate the blood-brain barier, which effectively limits the penetration of many therapeutic substances into the Central Nervous System. The mechanism of anticancer properties of bee venom is not fully understood and, significantly, seems to depend on the type of cancer cells. Matrix metalloproteinases (MMPs) play a crucial role in the invasion and metastasis of various cancers. Inhibition of MMPs activity may be a potential target for anticancer therapy. Some natural compounds, including BV, exhibit the property of inhibiting the activity of MMPs.

Aim of the study: The project aims to evaluate the effect of BV and its individual fractions on glioblastoma cell viability in cell culture conditions and with the use of a fish model on zebrafish embryos. The aim of the study is also to investigate the influence of BV and its main components on MMP-2 and MMP-9 activity in glioblastoma cell lines in comparison to physiological glial cells.

Methods: The venom will be fractionated by liquid chromatography. With the use of cell cultures, the influence of BV on cancer cells viability (MTT colorimetric method) and their ability to synthesize tumor-promoting MMPs by gelatin zymography will be analyzed. The most active fraction will be used to evaluate their anti-tumor activity in a zebrafish embryo model into which human glioblastoma cells will be transplanted.

Conclusions: Preliminary studies have indicated that BV has both cytotoxic and inhibitory effects on MMP-2 and MMP-9 secretion in selected glioblastoma cell lines, suggesting antitumor properties of apitoxin. Future conclusions from the planned study will determine whether BV or its components may be a candidate for further research to develop effective treatments for glioblastoma.

Supervisor: Prof. Jacek Kurzepa, MD, PhD

Auxillary supervisor: Anna Boguszewska-Czubara, PhD

Keywords: bee venom; glioblastoma; anticancer potential; MMP-2; MMP-9

7. Time of infection and light regime affect the antiviral response and clock genes expression in zebrafish – Mikołaj Mazur

Mikolaj Mazur[1], Mikolaj Adamek[2], Krzysztof Rakus[1], Magdalena Chadzinska[1], Lukasz Pijanowski[1]

[1]Department of Evolutionary Immunology, Jagiellonian University, Gronostajowa 9, PL 30-387 Krakow, Poland, [2] Fish Disease Research Unit, University of Veterinary Medicine Hannover Foundation, Hannover, Germany

Circadian oscillations are crucial for a proper functioning of an organism as they affect both physiology as well as the behavior of animals. It is well known that virtually all processes within an organism are controlled by the biological clock, even the immune response, but the amount of data regarding this kind of interactions in fish is limited. Therefore, in the present study, we aimed to study the effects of time of infection and light regime on the expression of clock genes and antiviral response in the late phase of viral infection with Tilapia Lake Virus infection in zebrafish.

Adult zebrafish were i.p. infected with medium containing TiLV (1×10^7 TCID50/ml) or with L15 medium (mock-infected) at two different time points of the day (ZT/CT2 and ZT/CT14) and under two different light regimes: 12 h light: 12 h dark (LD) and constant darkness (DD). Gene expression of clock and antiviral proteins as well as viral load was measured in the kidney on 14 dpi with RT-qPCR.

We observed that the differences in the expression of clock genes in the kidney were present only under the DD regime. Moreover, the expression of genes encoding the positive part of a circadian feedback loop (clock and arntl) was higher at the CT2 (morning) than at CT14, while expression of genes encoding negative part of clock (per and cry) was higher at the CT14 (night) than at CT2. We did not observe differences in the expression of clock genes between mock- and TiLV-infected fish.

Interestingly, in fish kept under different light regimes statistically significant differences in the expression of gene encoding IFN type I – crucial antiviral cytokine was found. Furthermore, we found that the expression of several genes involved in the antiviral response depended on whether the infection occurred in the morning or in the evening, however the virus load was similar at all tested time points.

Our results suggest that antiviral immune response in zebrafish is, at least partially, regulated by biological clock and depends on the light regime and time of the day when fish are infected.

8. Development of a high-throughput translation platform for the preclinical screening of the new chemical substances with a potential therapeutic effect in disorders of the central nervous system – Małgorzata Mierzejewska

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The aim of my PhD studies is to develop a set of research methods enabling high-throughput screening of new substances with a potential therapeutic effect in disorders of the central nervous system, including neurodegenerative diseases, congenital defects, hereditary diseases, disorders of the immune system and epilepsy. The model organism used in the first stage of screening will be an embryo or zebrafish (Danio rerio) larvae. The development of this research platform will enable a quick, low-cost, and at the same time "screening" of substances with a potential therapeutic effect that does not require the use of higher organisms. The substances selected in this way can be used for further research with the use of higher organisms, including laboratory rodents. Special attention will be paid to diseases such as epilepsy, including Dravet syndrome, anxiety and depression. So far, I have conducted research in the field of behaviour analysis of 5 dpf larvae by using Danio Vision (Noldus) and dedicated behavioural analysis programs such as Ethovision (Noldus) and DanioScope(Noldus). **Conclusions:** The Dark-Light Transition test and Thigmotaxis test seem to be appropriate for testing anxiolytic substances in zebrafish larvae. 10 µM of diazepam shows the strong anxiolytic efficacy and therefore can be used as a reference substance in further studies related to anxiety-like behaviour.

Keywords: Danio rerio, Transpharmation Poland, Dravet Syndrome, Anxiety, Epilepsy, Screening, NCS, Noldus

9. Study of the function of the galanin during regeneration of the nervous system - **Shiho Okitsu-Sakurayama**

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[Background] Galanin is a neuropeptide that is conserved among different species and widely expressed in vertebrates' central and peripheral nervous systems. Previous studies suggested that increased galanin concentration might have a trophic influence on nerve repair. Moreover, Our preliminary data indicate that in zebrafish gal -/- mutant regeneration of the spinal cord is impaired. Adult zebrafish retain the capacity of robust axonal regeneration and can morphologically and functionally recover from the optic nerve and spinal cord injuries. An acute inflammatory response occurs after CNS injury in zebrafish, which has recently been suggested to positively contribute to the regenerative process. In the present study, we examined the possibility that galanin is involved in regeneration of nervous system by using spinal cord lesions injury model.

[Result] First, to assess whether ends of transected spinal cords structurally re-connect after a lesion, we transected the spinal cord at 3 dpf of the zebrafish. We used 3 types of zebrafish for the experiment. First one with the normal level of galanin as a control. Second with inducible overexpression of galanin, with galanin under control of heat shock protein promotor (Gal HS). The third one created with CRISPR-cas9 technology in our lab line with a lack of expression of galanin (Gal -/-). All of them expressed RPF in the motor neurons. In each group, spinal cord regeneration could be observed 48 hours after transection. Next, we investigated functional recovery through spinal cord regeneration using the DanioVision system (Noldus). Gal -/- group recovered function more quickly than the other groups. On the other hand, Gal HS showed a long-term recovery of function. Final, we examined the effects of galanin on changing gene expression. IL-10, TNFa, and IL-6 showed a similar gene expression pattern. IL-1b was rapidly increased by injury in the control group and Gal -/- group at 3 dpf. IRG1L2 was most abundantly expressed in the control group.

[Conclusion] We observed spinal cord regeneration after 48 hours of transection. We found differences between the respective groups in spinal cord regeneration, functional recovery and

changes in gene expression. However, further experiments are needed to examine the involvement of galanin in spinal cord regeneration.

10. Effect of selected natural cytotoxic substances on embryonal rhabdomyosarcoma cells (ERMS) *in vitro* and *in vivo* - Radzka Justyna

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Rhabdomyosarcomas (RMSs) are a heterogeneous group of malignant myogenic tumors. These tumors originate from undifferentiated primary mesenchymal cells with the potential to differentiate into skeletal muscles. The most frequent histological types of RMSs in children are ERMS (Embryonal RMS, approximately 75%) and ARMS (Alveoral RMS, 25% of patients). A common method to detect the early stages of RMSs is immunodetection of MyoD1 and myogenin (MyoG), representatives of MRFs (Myogenic Regulatory Factors) in tumors. Clinically RMS is treated with a combination of surgery, chemotherapy, and radiation therapy (RT) adjusted to the stage of the disease. It has been shown that chemotherapy and RT in RMSs treatment remain fundamental. However, the RMSs chemotherapy has many disadvantages such as induction of multidrug resistance proteins activity (MDR) and the appearance of toxic side effects. It is also noteworthy that almost all anti-cancer agents affect not only cancer but also healthy cells. Moreover, the majority of cytostatic compounds are only approved for the treatment of adults but not for pediatric cancers. The RT application is also very problematic for young children and infants due to its high toxicity.

The aim of the project is an assessment of the therapeutic, anti-cancer potential of selected natural substances in the treatment of embryonal rhabdomyosarcoma (ERMS) in cellular and zebrafish models. In our studies, we will analyze biochanin A, caffeic acid phenethyl ester as novel, natural, poorly known in ERMS therapy, and anti-cancer agents. Commonly used chemotherapeutic agents, vinorelbine and daunorubicin will be used as a positive control. Assessment of the therapeutic potential will be carried out using two research models. *In vitro* tests will be performed on the commercially available ERMS cell line (RD) and L6 line (as a control). Contrary to mouse models, the ease and relative cost-effectiveness of performing mentioned screens in zebrafish can be adapted to embryos transplanted with human cancer cells

without the need for immunosuppression because, for the first month of life, the zebrafish larvae have not developed an adaptive immune response. In our project, *in vivo* tests will be conducted on xenotransplantation of the human GFP-labeled ERMS (RD) cells

into zebrafish embryos within the frame of this project. We plan to use numerous techniques in the research: transcriptome analysis (RNA-Seq), flow cytometry, immunocytochemistry, TEM, and numerous microscopic analysis. This will allow us to obtain valuable and more reliable data, which will establish the basis for the development of therapy for patients suffering from ERMS.

Our research confirmed cytotoxicity of daunorubicin and vinorelbine. Based on available data we established concentrations of biochanin A and caffeic acid phenethyl ester. So far, the *in vitro* cytotoxicity of selected agents (biochanin A and caffeic acid phenethyl ester) was analyzed after 24h/48h and tested with MTT test. Our results lead to conclusion, that application of used cytostatics significantly decreased the viability of RD cells compared with L6 cells after both 24-hour and 48-hour incubation with cytostatics. Obtained results will be "starting point" for further *in vitro* and *in vivo* studies. Based on our results we established IC50 (the half maximal inhibitory concentration) which will be used in *in vivo* studies. For RD lines IC50: 73,785 [μM] (caffeic acid phenethyl ester), 53,308 [μM] (biochanin A), 0,201 [mg/ml] (vinorelbine), 6,77 [μM] (daunorubicin). For L6 lines IC50: 85,812 [μM] (caffeic acid phenethyl ester), 42,383 [μM] (biochanin A), 0,154 [mg/ml] (vinorelbine), 6,93 [μM] (daunorubicin).

The research proposed in the project is an innovative approach to commonly used methods in anticancer therapies. Our goal is to gain new knowledge about the basic processes and phenomena occurring in cancer cells. Improving knowledge of the mechanism standard chemotherapy, new natural chemotherapeutic agents and identification of cellular response to therapy has the potential to revolutionize and improve the planning of cancer strategies.

11. Functional studies of mutations responsible for the hereditary human limb malformations - Ramanujam Akshaya

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Genetic and epigenetic factors are the basis of many diseases, but the aetiology of many of them is poorly understood. This project aims at the genetic identification of candidate families with hereditary skeletal malformations such as clubfoot (talipes equinovarus, TEV), Pallister-Hall syndrome (PHS) or Greig cephalopolysyndactyly syndrome (GCPS). Next, we will identify the mutations linked to the disorders and carry out functional studies to correlate the genotype and phenotype. Until now, we identified a novel missense mutation c.301C>T p.Arg101Trp in the TPM2 gene linked to familial isolated TEV. Additionally, we aim to characterize five GLI3 mutations (c.2255C>G p.Ser752*, c.2017delC p.Gly674Valfs*19, c.2686G>A p.Asp896Asn, c.2721C>G p.Ser907Arg, and c.3018C>A p.Ser1006Arg) causing GCPS or PHS. Thus the next step of our project will examine the consequences of mutation with gain of functional studies by overexpression of the mutated protein and CRISPR/Cas9 approaches in vivo using zebrafish as model system to demonstrate that they are indeed linked to patient phenotype. Our project results will have implications on the clinical aspects of skeletal development,that may provide a platform to develop pharmacological interventions for some of the inborn skeletal malformation.

Key words: Skeletal malformation, idiopathic mutations, TPM2, genotype– phenotype correlation, GLI3.

12. Effect of UVB radiation and tryptophan-derived AhR ligands on AhR expression in melanocytes and melanoma cells – Karolina Szalast

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Melanoma, representing only 5% of all skin cancers, is responsible for majority of skin cancer related deaths due to its propensity to metastasize. The environmental studies revealed that UVB radiation is the major environmental factor inducing melanoma. In addition, human skin is exposed every day to various biologically active compounds found in skin care products, food or endogenously synthesized by skin cells. One of the latter compounds are tryptophan-derived Aryl hydrocarbon receptor (AhR) ligands, synthesized from tryptophan via enzymatic pathway (i.e. L-kynurenine, kynurenic acid) or as a stable photoproduct 6-formylindolo[3,2-b]carbazole (FICZ). Tryptophan-derived AhR ligands have unknown biological activity on UVB-induced melanoma promotion and progression.

The aim of the study was to determine the effect of tryptophan-derived AhR ligands (L-kynurenine, kynurenic acid, FICZ) on the expression of the AhR receptor in melanocytes and melanoma cells and their proliferation. The role of UVB radiation on biological activity of ligands was also studied.

The study was conducted on human adult primary epithelial melanocytes and human metastatic melanoma cells (SK-MEL-3). Expression of the AhR receptor was determined by Western blot. Proliferation was measured using the BrdU assay. A dose of UVB radiation of 20 mJ /cm^2 was used to for irradiation of cells.

The results show that tryptophan-derivatived AhR ligands in high concentrations decreased the expression of the AhR receptor in melanocytes and melanoma cells. AhR ligands inhibited the proliferation of melanocytes and melanoma cells. The most potent inhibitory effect was observed in melanoma cells exposed to L-kynurenine. UVB radiation sensitized cells to the antiproliferative effects of substances.

Tryptophan-derivatived AhR ligands in high concentrations may cause a reduction in AhR expression in both normal and cancer cells, suggesting potential non-receptor-dependent mechanisms effects of these substances on cells.

13. Zebrafish as a model to study human diseases - Anna Tomańska

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Zebrafish (Danio rerio) is one of the key model organisms used in translational medicine, bringing the development of methods and means of human therapy. The growing interest in this species in recent decades, along with the potential of innovative optical methods and the susceptibility of this animal to manipulation by the researcher, make it a key player in many biomedical research. In highly developed countries, it has also become an animal used for drug screening and investigating the disease mechanism details in personalized medicine. There are many reasons for scientists to use zebrafish in research, and there are many opportunities for the development in them. Methodologically, it is possible to indicate the assignment of the research topic for the use of zebrafish in terms of organs, systems, and tissues. But they also show great potential in combinatorial research for polygenic diseases. Research systematics, therefore, enters the molecular realm when we can analyze zebrafish studies considering the size of the structure. The features of zebrafish as a model organism predispose it to projects exploring even diseases rarely appearing in the human population. The scientifically important features of zebrafish are high genetic and physiological similarity to humans, with a well-understood genome, the possibility to maintain constant parameters of breeding, external fertilization, and a large number of embryos. The Zebrafish model is less controversial in the face of the ethical challenges of animal participation in research. Maintenance and use in research is cheaper compared to laboratory mice, and require less space and labor. Recent studies include neurological research, sensory and conduction, and the development of the nervous system. Also oncological, pediatric, dental, endocrinological, and diabetic research, in the field of transplantology and immunology. Study of pain reactions, the physiology of stress and anxiety, aggression. Much attention has also been paid to retinal, and metabolic diseases and regenerative medicine. Researchers also conducted research with the use of zebrafish on heart rhythm disturbances, arrhythmia, the functions of the digestive tract, organ development studies, especially the kidney, gene defects and polygenic diseases, behavioral genetics, alcoholism and the mechanisms of addiction, and molecular psychiatry. As well as toxicity studies, environmental and indicator research, other viral, bacterial, fungal, and prion diseases, host-pathogen interactions and leukemia, skin cancer, and sarcoma. **Research project supported by** program „Excellence initiative – research university" for years 2020-2026 for University of Wrocław

14. Evaluation of the potential and mechanisms of anticancer activity of newly synthesized chemical compounds on selected cell lines and in a zebrafish model - Łucja Justyna Walczak-Nowicka

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Cancers are the most common cause of death worldwide. Current anti-cancer therapies are associated with side effects for the patient and ussually do not allow for a cure. This is mainly due to the fact that these compounds act non-selectively towards cancer cells. New thiosemicarbazide derivatives have a broad spectrum of activity against various cancers and combine different mechanisms of action useful in the treatment of cancers. The aim of the planned study is to evaluate the anticancer potential and elucidate the molecular mechanisms of action of newly synthesized derivatives with thiosemicarbazide structure in *in vitro* and in a *zebrafish* model.The planned study is related to my project implemented in the Doctoral School of Pharmaceutical Sciences at the Medical University of Lublin. The planned study consists of two parts: *in vitro* and *in vivo*. In the first phase antiproliferative potential of the new derivatives will be evaluated on various cell lines. Inhibitory concentrations will be determined: IC10, IC25, IC50, IC90. Moreover, their influence on apoptosis/necrosis process and cell cycle will also be examined. The antiangiogenetic potential of the tested derivatives will be checked in the microtubule formation assay, as well as by evaluation of gene expression. In the second stage, only the most promising compounds will be analyzed for anticancer properties. Acute toxicity (FET) will be assessed according to OECD procedure in *zebrafish* (wild type). Subsequently, the in *zebrafish* Tg(fli1:EGFP) antiangiogenetic potential of the tested compounds will be re-evaluated to confirm/exclude it.. In the future, the results of the conducted experiments will be the basis for applying to the National Science Center for funding of further stages of research aimed at precise evaluation of the mechanisms of the most promising compounds. In conclusion: The results of the conducted research will contribute to finding new cytostatic drug candidates and developing new therapeutic strategies for cancer, which will significantly impact the development of the scientific discipline.

15. Zebrafish as a model to study neuroinflammation driven by an oral pathogen *Porphyromonas gingivalis* – Magdalena Widziołek-Pooranachandran

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Accumulating evidence highlights the importance of chronic, often subclinical inflammation (e.g. periodontitis) as the underlying condition for the development of many pathologies such as cancer, cardiovascular disease, and neurodegeneration.

In this project, we examined the role of the key-stone pathogen of periodontal disease, a nonmotile, Gram-negative, obligately anaerobic bacterium *Porphyromonas gingivalis* (*Pg*) and its predominant virulence factors (cysteine proteases - gingipains) in the pathogenesis of neuroinflammation.

We employed zebrafish larval model to determine: (i) if and how systemic *Pg* infection and gingipains affect the Blood-Brain-Barrier (BBB) and whether *Pg* induce neuroinflammation, (ii) the impact of systemic *Pg* infection and gingipains on phenotypic complexity of microglia in the context of neuroinflammation.

16. *Carlina acaulis* as a source of biologically active substances - Wnorowska Sylwia

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Carlina acaulis is a monocarpic herb from the Asteraceae family occurring in many European countries including Poland. Various species of *Carlina* have been used in folk medicine to treat many skin disorders like a cancer. Current research indicates that the extract from leaves of *Carlica acaulis* inhibits proliferation of human melanoma cells *in vitro* in a dose-dependent fashion. Extracts obtained from the roots of *Carlina acaulis* showed opposite biological activity - melanoma cells treated with root extracts grew faster than control cells. However, the substances responsible for the observed biological activity towards melanoma cells remain unknown. Thus, the general aim of my research is to isolate a substance responsible

for antiproliferative activity of *Carlina acaulis* leaves extract. The next step will be to determine the chemical structure of active ingredient and to identify additional properties of Carlina acaulis constituents.

The plants were obtained from the Botanical Garden of Maria Curie Skłodowska University in Lublin. Roots and leaves will be dried and pulverized. Then, the plant material was subjected to ultrasonic energy-assisted extraction, successively with hexane, dichloromethane, methanol and water. This way, an exhaustive extraction of the metabolites of the *Carlina plants* was carried out. The initial cytotoxicity assessment was conducted by MTT assay in human fibroblasts and keratinocytes as well as in developing zebrafish embryos. Raw extract displayed limited toxicity under assay conditions. Further experiments will be conducted using human cancer cell lines. After the assessment of biological activity, the most active extract will be fractionated using extraction techniques such as liquid-liquid extraction and solid phase extraction. The obtained fractions will be further assessed for pharmacological activity and the most active ones will be separated into individual compounds using a high-performance, semi-preparative liquid chromatograph.