The Polish Zebrafish Society

4th workshop of the Polish Zebrafish Society
"Zebrafish and Human diseases"

Wrocław
14-15.02.2019

Edited by Arnold Garbiec

Organizers:

The Polish Zebrafish Society

Department of Animal Developmental Biology
Faculty of Biological Sciences
University of Wroclaw

Przemko Tylżanowski (KU Leuven, Belgium and Medical University in Lublin, Poland)

Marta Migocka-Patrzałek (University of Wroclaw, Poland)

Piotr Podlasz (University of Warmia and Mazury in Olsztyn, Poland)

Małgorzata Daczewska (University of Wroclaw, Poland)

Magda Dubińska-Magiera (University of Wroclaw, Poland)

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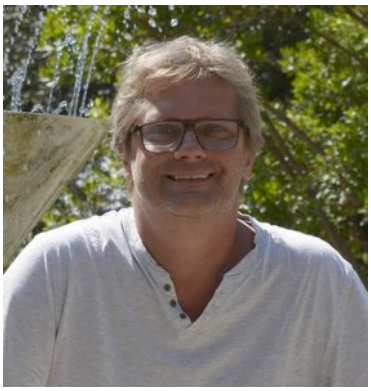
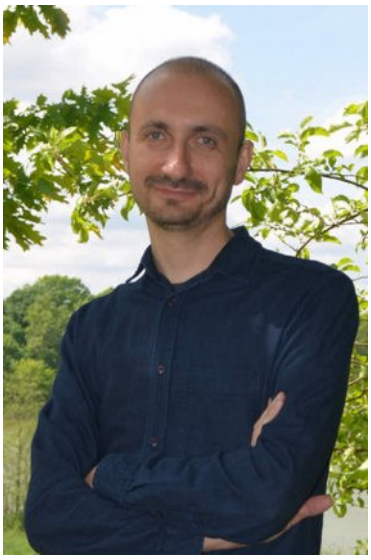


Przemko Tylżanowski

My research group is located in two places: KU Leuven, Leuven, Belgium and Medical University in Lublin, Poland. My research focuses on two aspects of joint and skeletal biology. We investigate the molecular and cellular events governing the formation of the vertebrate limb using developmental models. We also investigate in vivo function of genes that, when mutated, lead to skeletal malformations in humans. To address these issues, we use three developmental models based on mouse, chick and zebrafish.



Piotr Podlasz

Piotr Podlasz, DVM, PhD works at the Laboratory of Genomics and Transcryptomics at the Department of Pathophysiology, Forensic Veterinary and Administration, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, Poland. His main field of interest is study of the function of neuropeptides in the physiological and pathological processes. In his research, he uses mainly zebrafish (*Danio rerio*), a model organism that has recently become very popular in biomedical research. He gained knowledge about the use of this model organism during many stays in zebrafish labs, including at the University of Helsinki, Finland and the Max Planck Institute in Tübingen, Germany. For over 12 years, he has using zebrafish in research at the Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, which makes him first scientist in Poland who uses this organism for scientific research. He is also one of the main co-creators and Member of the Board of the Polish Zebrafish Society. He is a co-author of about 40 scientific publications. He was/is supervisor of several master's and PhD students.
http://www.uwm.edu.pl/zebrafish/

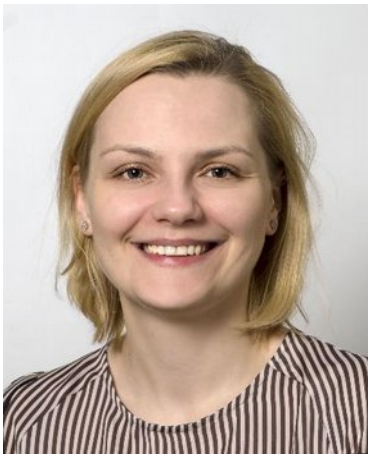
Magdalena Oroń

I completed master studies in biotechnology on Intercollegiate Faculty of Biotechnology, University of Gdańsk and Medical University of Gdańsk. The research for PhD thesis I performed in the International Institute of Molecular and Cell Biology in Warsaw in laboratory of prof. Maciej Żylicz. I studied the role of gain-of-function p53 mutants in regulation of alternative splicing in breast cancer. Currently I continue my career in cancer biology field. I work in the Mossakowski Medical Research Centre in the Laboratory of Human Disease Multiomics established by dr. Dawid Walerych. Our aim is to understand molecular mechanisms of cancer development by using molecular large-scale analyses. In particular my research is focused on molecular programs of cellular proteasome machinery leading to human neoplasias.

Tomasz Prajsnar

I am an infection biologist studying bacterial infections using zebrafish and mouse models. I have worked at University of Sheffield (UK) and Leiden University (NL). I am especially interested in roles of professional phagocytes - neutrophils a macrophages in diseases caused by intracellular pathogens.

Kinga Gaweł



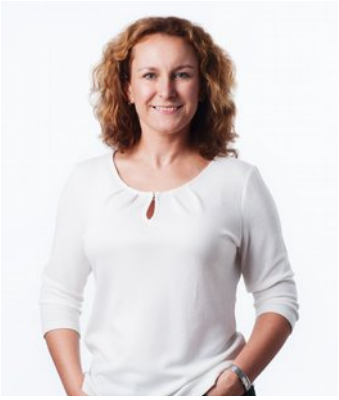
Kinga Gaweł obtained her PhD at the Faculty of Pharmacy, Medical University of Lublin. She is working as a research assistant at the Department of Experimental and Clinical Pharmacology, Faculty of Medicine, Medical University of Lublin. She did her internships at the Uppsala University, Sweden (financed by ETIUDA3 programme, National Science Centre, Poland) and University of Oslo, Norway („Mobilność Plus V” programme, financed by Polish Ministry of Science and Higher Education). Her project „GEMZ-Genetic Epilepsy Models in Zebrafish” has been awarded within a Marie Curie Individual Fellowships programme. This project, supervised by Professor Camila V. Esguerra is conducted at the University of Oslo (01.01.2019-31.12.2020).

Wietske van der Ent

I performed my PhD research in the Netherlands at the Leiden University Medical Center, where I developed xenograft and transgenic cancer models in zebrafish for Ewing sarcoma. With these models, I investigated cancer cell dissemination, blood vessel growth, and involvement of the innate immune system in these processes. After completing my PhD, I went to Institut Curie in Paris, where I continued researching Ewing sarcoma. I am currently working on my second post-doc project, to develop a disease model in zebrafish for epilepsy, with the aims to use these models in a large-scale drug screen.

Małgorzata Daczewska

Prof. Małgorzata Daczewska is the head of Department Animal Developmental Biology since 2011. Her scientific interests focus on animal developmental biology of invertebrates and vertebrates. The main projects in her Department are involved in studies of neuromuscular and muscles metabolic human diseases conducted in two model organisms: the fruit fly and zebrafish. The Department comprises a Zebrafish Laboratory. The Laboratory is equipped in zebrafish facility with zebrafish WT and transgenic lines and provides material used in diploma and PhD projects.



Marta Migocka-Patrzałek

Assistant Professor in Department of Animal Developmental Biology, Institute of Experimental Biology, Faculty of Biological Sciences, University of Wroclaw, Poland
My research interests are focused on the animal development, especially muscle development and myopathies in zebrafish with particular emphasis on McArdle disease. This human disorder is caused by deficiency of muscle glycogen phosphorylase which leads to fatigue and muscle pain during exercise. I am also involved in the academic life, teaching and popularization of science. Among other activities I am co-author of the educational project "e-danio. Dive for knowledge" and “Danio Adventure. Science and Discovery”.

https://www.researchgate.net/profile/Marta_Migocka-Patrzalek

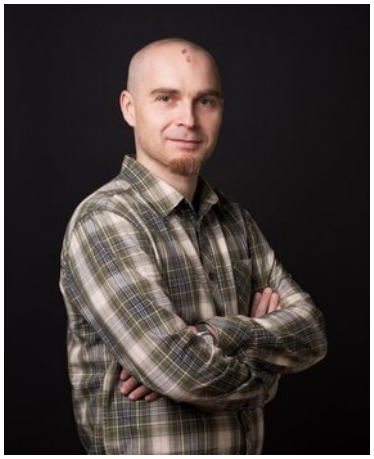
Magda Dubińska-Magiera

Assistant Professor in Department of Animal Developmental Biology, Institute of Experimental Biology, Faculty of Biological Sciences, University of Wroclaw, Poland
As a researcher I am focused on animal development, especially in the field of vertebrate and invertebrate muscle development. The aim of my research is to understand the function of small heat shock proteins in the development and functioning of muscles and nerves. I also teach undergraduate and master's students (conducting classes and lectures) in the field of developmental biology, histological techniques, the biology of the cell and biochemistry. Besides research and teaching I also take part in the popularization of science conducting laboratory workshops and projects.
www.researchgate.net/profile/Magda_Dubinska-Magiera

Joanna Niedbalska-Tarnowska

Laboratory of Molecular and Cellular Immunology, Department of Tumor Immunology, Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences
Department of Animal Developmental Biology, Institute of Experimental Biology, University of Wroclaw
My scientific interests concern the development of animals, especially the role of small heat shock protein HSPB8 in the development of the muscles of *Danio rerio*. Recently I am focusing on understanding the role of IM: 7145024 protein in the development of cilia present in the hair cells in the inner ear and the lateral line. I am, also the main contractor in the Danio Adventure project, which aims to assess the protective effects of L-carnitine and coenzyme Q10 on post-statine myopathy in *Danio rerio*.

Arnold Garbiec



I work in the Department of Animal Developmental Biology, Institute of Experimental Biology, Faculty of Biological Sciences, University of Wroclaw.
I am focused on animal development, especially in the field of vertebrate muscle development.

Online resources

www.zfin.org This is the Zebrafish Information Network site, run by the University of Oregon – find information about mutants, transgenics, protocols, genomics, expression patterns, etc.

www.ensembl.org/Danio_rerio/ This is the Zebrafish Genome Project website. You can look for your favorite gene in zebrafish using the SSAHA search program.

www.zebrafish.org.pl -website of Polish Zebrafish Society

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10.20-10.40	Coffee break	
10.40-12.00	**Cancer cells injections**, conducted by Magdalena Oroń	**Epilepsy**, conducted by Kinga Gaweł and Wietske van der Ent
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13.30-15.30	**Lectures** (open to public) Session I (chairperson Marta Migocka-Patrzałek) 13.30 - opening 13.45 - What's at the end of the fishing line…. - Przemko Tylżanowski 14.20 - Zebrafish as a model for human cancer - Magdalena Oroń 14.55 - Cancer xenotransplantation models in zebrafish - Wietske van der Ent	
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10.20-10.35	Coffee brake		
10.35-12.25	**VAST BioImager - whole zebrafish imaging** (VAST BioImager™, Union Biometrica - Francis Smet), Arnold Garbiec	**Zebrafish behavior** (Noldus -Albert Willemsen), Joanna Niedbalska-Tarnowska	**Zebrafish morphology** (Spinning Disk Confocal Super Resolution Microscope, Olympus - Wojciech Brutkowski), Piotr Podlasz
12.25-12.40	Coffee and sandwich		
12.40-14.30	**Zebrafish morphology** (Spinning Disk Confocal Super Resolution Microscope, Olympus - Wojciech Brutkowski), Piotr Podlasz	**VAST BioImager - whole zebrafish imaging** (VAST BioImager™, Union Biometrica - Francis Smet), Arnold Garbiec	**Zebrafish behavior** (Noldus -Albert Willemsen), Joanna Niedbalska-Tarnowska
14.30	Closing remarks and Lunch		

Practical parts

EEG recordings in larval zebrafish

Prepare low melting point agarose stock

1) Dissolve agarose powder in E3 medium to a final concentration of 2%
2) Heat up a mixture in a microwave w/o boiling it
3) Distribute 1-1.5 ml of aliquots in 2 ml reaction tubes
4) Keep the aliquots in the freezer -20˚C

Prepare agarose for mounting

1) Make aliquots of agarose in a heat-block at 70-80˚C
2) Vortex the aliquots
3) Keep them in heating-block at 37˚C (Note: low melting point agarose quickly hardens when cooled). Do NOT exceed temperature- it may harm the fish.

Mounting

1) Transfer a larva on petri-dish lid and suck up the medium, then cover it by two drops of agar and using plastic capillair suck it up into the capillary. Then, mount it on the slide and let it cool down and harden. Give two more drops of agar on two sides of larva to make it more stable.
2) Using a scalpel, remove gently a piece of agar from the top of larva's head.
3) Insert the needle on the optic tectum (or forebrain) and apply enough amount of ACSF solution (ACSF).

Recipe for artifical cerebrospinal fluid (ACF)

NOTE:
- Make separate stocks of ACSF-A and ACSF-B, to store at -20 degrees.
- Add the compounds in order, one after the other, making sure each is dissolved before adding the next.
- Keep the aliquots apart, and mix in a ratio of 1:1 immediately before the experiment (Remember: new solution should be used each time, because when mixed, substances easily precipitate and it may increase the resistance)

For ACSF-A, to 200mL MQ water, add:

Compound	Concentration in 1x solution	Grams needed for 200mL 2x stock solution
NaCl	124 mM	2.876
KCl	2.5 mM	0.074
MgSO4	2.0 mM	0.096
KH2PO4	1.25 mM	0.068
CaCl2▪ 2 H2O*	2.5 mM	0.148

(*Or use anhydrous, but then recalculate the amount needed)

For ACSF-B, to 200mL MQ water, add:

Compound	Concentration in 1x solution	Grams needed for 200mL 2x stock solution
NaHCO3	26 mM	0.88
Glucose	10 mM	0.72
Sucrose	4 mM	0.5472

For the detailed description of parameters of EEG recordings see: Afrikanova et al.: *Validation of the zebrafish pentylenetetrazol seizure model: locomotor versus electrographic responses to antiepileptic drugs*. PLoS One. 2013, 8(1):e54166.

Poster session

Inherited retinal dystrophies – the selected results and the need to use the zebrafish model

Skorczyk-Werner Anna[1], Wawrocka Anna[1], Wicher Katarzyna[1], Chiang Wei-Chieh[2], Lin Jonathan H.[2], Jarmuż-Szymczak Małgorzata[3], Kostrzewska-Poczekaj Magdalena[3], Jamsheer Aleksander[1], Płoski Rafał[4], Rydzanicz Małgorzata[4], Kohl Susanne[5], Weisschuh Nicole[5], Wissinger Bernd[5], Krawczyński Maciej R. [1, 6]

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[4] *Department of Medical Genetics, Warsaw Medical University, 3c, Pawinskiego St, Warsaw, Poland*
[5] *Institute for Ophthalmic Research, University of Tubingen, Tubingen, Germany*
[6] *Center for Medical Genetics Genesis, 4, Grudzieniec St, Poznań, Poland*

Keywords: Inherited retinal dystrophies (IRD); Cone-rod dystrophy (CRD); achromatopsia (ACHM); ATF6 (activating transcription factor 6A)

Inherited retinal dystrophies (IRD) constitute a highly genetically heterogeneous group of disorders characterized by progressive dysfunction of photoreceptors and retinal pigment epithelium (RPE) cells. Whole Exome Sequencing (WES) and Whole Genome Sequencing (WGS) give the opportunity to discover novel genes associated with IRD. Functional analyses are indispensable in cases with mutations in novel genes.
Here we report two siblings affected by an IRD phenotype and a novel pathogenic *ATF6* (activating transcription factor 6A) mutation identified by WES. Cone-rod dystrophy (CRD) and achromatopsia (ACHM) belong to inherited retinal dystrophies. ATF6 is a key regulator of the Unfolded Protein Response (UPR) pathway. Recently, the *ATF6* mutations have been identified as a novel cause of achromatopsia. Clinical findings in the siblings included decreased visual acuity, severe photophobia and reduced color discrimination. The results of the full ophthalmological examination suggest rather cone-rod dystrophy than achromatopsia. NGS retina panel, and finally WES were performed. WES analysis revealed a novel homozygous *ATF6* gene mutation. The pathogenicity of the mutation was confirmed by functional analyses on patients' fibroblasts and the recombinant protein. The *ATF6* mutants show impaired endoplasmic reticulum (ER) to Golgi apparatus trafficking and diminished regulated intramembrane proteolysis and transcriptional activity in response to ER stress. This is the first case of CRD caused by the *ATF6* mutation. As the *ATF6* gene is expressed in almost all tissues the functional analyses with the use of patients' fibroblasts was possible, but large part of retinal disease-associated genes are expressed exclusively in the eye, which requires the necessity of using the animal model in studies on the potential pathogenicity of variants of novel genes. Zebrafish can be a useful model as the retina of this species is more similar to the human retina than the retina of the mouse.

Establishing the models of autism in Zebrafish to test significance of kynurenine pathway in ASD

Iwona Żarnowska, Krystyna Mitosek-Szewczyk, Waldemar Andrzej Turski

Medical University, Lublin, Poland

Autism spectrum disorders (ASD) present a significant and increasing burden on society. Converging evidence supports the role of complex interaction between genetic factors, enviromental pollutants and the innate immune system reactions in the alterations of early brain development and the pathogenesis of the ASD relevant behavioral symptoms (Persico AM, 2013). There is growing evidence to support the role of alterations in the kynurenine pathway (KP) of tryptofan degradation in pathogenesis of ASD and related neurodevelopmental disorders (Schwarcz R, 2014, Lim et al., 2016). Recent work has highlighted the neuroprotective potential of inhibiting two critical regulatory enzymes in KP—kynurenine-3-monooxygenase (KMO) and tryptophan-2,3-dioxygenase (TDO) (Breda et al., 2016).

Zebrafish models of ASD, possessing high physiological and genetic homology to humans and reproducing rapidly, are particularly useful for studying the development and genetics of vertebrates, including the effects of environmental pollutants on early embryonic development. (Kalnaff et al., 2014, Lee et al.2016)

We are going to study significance of KP perturbation in early brain development in ASD:

1. Generate and validate mutant lines of zebrafish to model ASD (using CRISPR)
2. In order to model enviromental influence in ASD, exposure to valproic acid in embrionic and larval zebrafish will be investigated
3. Using comercially available TDO blokers, it is planned to test for ability to suppress or revert abnormal phenotypes to baseline

Key words: Autism spectrum disorders, Models, Kynurenine pathway, Zebrafish

Erythropoietin inteisifies proapoptotic actvity of LFM-13 in colon cancer cells

Justyna Magdalena Hermanowicz[1,2], Beata Sieklucka[2], Tomasz Domaniewski[3], Krystyna Pawlak[3], Dariusz Pawlak[1]

[1] Department of Pharmacodynamics, [2]Department of Clinical Pharmacy,[3]Department of Monitored Pharmacotherapy, [4] Medical University of Bialystok, Mickiewicza 2C, 15-222 Bialystok, Poland

Background: Btk is non-receptor tyrosine kinases involved in the activation of signaling pathways responsible for maturation and viability of the cells. It plays an important role in the development of B-cell tumors, activating antiapoptotic pathways. Btk has previously been reported to be overexpressed in prostate cancer which correlated with cancer grades. Colorectal cancer is among the five most frequent causes for cancer-related deaths in Europe. This kind of cancer often accompanied by anemia which is treated with erythropoietin supplement. The aim of this study was to assess the effects of combination therapy with erythropoiethin beta (Epo) and LFM-A13 (Btk inhibitor) on colorectal carcinoma cells both in *in vitro* and in animal models.
Methods: DLD-1 and HT-29 human colon adenocarcinoma cells were cutured in medium with Epo and LFM-A13. Cell proliferation was measured with an automated cell counter. Expression of BTK by Western Blotting and AKT mRNA by RT-PCR, and its protein was assessed and confocal microscopy, respectively. *Analysis of apoptosis* by *flow-cytometry* was also carried out. Nude mice were inoculated with adenocarcinoma cells and treated with Epo and LFM-A13.
Results: Herein, we found that simultaneous use of Epo and LFM-A13 exert an additive inhibitory effect on colon cancer cell growth. Featured therapeutic scheme resulted in effective cell killing, accompanied by attenuation of BTK, AKT signaling pathway and increased of apoptosis. This combination is also effective *in vivo* studies, where the combined administration of Epo and LFM-A13 significantly reduces the rate of growth of tumor cells and leads to the complete regression of the colorectal cancer cells.
Conclusions: Results of this study show that adding Epo significantly enhances the antitumor activity of LFM-A13. The results of our study indicate the potential use of a combination of Epo and LFM- A13 as an effective therapeutic approach for colorectal cancer. Further we plan to evaluate the antiproliferative activity of simultaneous use of Epo and LFM-A13 on metastasis in an experimental model of *Danio rerio* xenografts.

Keywords: apoptosis; Bruton's tyrosine kinase; colon cancer; erythropoietin; LFM-A13

4-Thiazolidinone based derivatives as potential anticancer and anticonvulsant agents.

Nazar Trotsko, Agata Paneth, Monika Wujec

Department of Organic Chemistry, Faculty of Pharmacy with Medical Analytics Division, Medical University of Lublin, 4A Chodźki, 20-093 Lublin, Poland

Keywords: 4-thiazolidinone based derivatives; anticancer activity; anticonvulsant activity

4-Thiazolidinone derivatives and their narrow group – thiazolidine-2,4-diones (TZDs) - are the object of special scientific studies. This attention is due to not only the wide possibilities for chemical modification of these derivatives, but also to a diverse spectrum of pharmacological properties and affinity for various biological targets. Consequently, TZD derivatives are the object of great interest as sources of novel drug candidates with anti-inflammatory, antimicrobial, antidiabetic, anticancer activity.

The new TZD derivatives were synthesized and modified in position 5 of this system by our research group. These derivatives in their structure contained a fragment of chlorobenzhydrazide or 4-substituted thiosemicarbazide. Some of these compounds showed anti-proliferative activity against the human lung carcinoma (A549), human breast adenocarcinoma (MCF-7) and human hepatocellular carcinoma (HepG2) cell lines based on *in vitro* tests.

The second group of TZD derivatives was modified at position 5 by azoles (imidazolidine, rhodanine, TZD and 2-thiohydantoin) and piperazine derivatives. The newly obtained substance was tested for antibacterial activity. These compounds showed antibacterial activity. The SAR analysis was performed. Out of these, leading structures with antibacterial potential were selected.

Besides, this group of derivatives showing the required pharmacophoric elements according to Unverferth K. et al. (Unverferth K. et al.// J. Med. Chem. 1998, 41, 63-73) as anticonvulsant agents.

The third group of compounds contained 4-thiazolidinone derivatives was modified in position 2, 3 and 5 of thiazolidine ring. Such group of compounds showed anti-*Toxoplasma gondii* activity.

In view of demonstrated anti-proliferative activity and potential anticonvulsant activity it seems to be purposeful carried out tests for lead compounds *in vivo*. Especially that *Danio rerio* is considered an excellent model for studying the potential biological activity of new molecules.

Zebrafish as a toxicological and behavioural model in preclinical studies – can MEK/ERK pathway be a drug discovery target in neuropsychiatric disorders?

Agnieszka Michalak

Chair and Department of Pharmacology and Pharmacodynamics, Medical University of Lublin, Poland

Activation of MEK/ERK pathway can play multiple roles in the regulation of neuronal function. In the nervous system, ERK regulates brain development, neuroinflammation, neural death and synaptic plasticity. Mounting evidence indicates that MEK/ERK signaling is implicated in the pathogenesis of neurodegenerative diseases, drug addiction and depression, among others. Moreover, MEK/ERK pathway has been also reported to be involved in anxiety-related as well as epileptiform behaviours in rodents.

My current ongoing research projects focus on the role of the MEK/ERK pathway in anxiety-related responses in animal models including mice and zebrafish larvae. Zebrafish is a highly promising pharmacological model in investigating neurological disorders, i.e. anxiety or epilepsy. Therefore, zebrafish would be an invaluable tool in investigating possible anxiolytic and antiepileptic properties of substances inhibiting MEK/ERK pathway. Moreover, far-reaching plans include using adult zebrafish in behavioural testing, including e.g. appetitive, shoaling and tank diving assays.

The second area of my interest is using zebrafish as a toxicological model in accordance with the OECD Principles of Good Laboratory Practice (GLP). For this purpose, I am interested in Fish Embryo Acute Toxicity (FET) test with the zebrafish to determine acute toxicity of chemicals on embryonic stages of fish.

Keywords: MEK/ERK pathway, neuropsychiatric disorders, anxiety, FET, GLP

Embryonic zebrafish xenograft as a new model for cancer drug discovery

Marcin Łukasik

Department of Applied Toxicology, Medical University of Warsaw

Over the last decade, we have performed a large number of research programs addressing to new anticancer drug candidates in preclinical studies using cellular (in vitro) and murine in vivo models such as mice and rats. Because zebrafish and mammals share common molecular pathways of tumor progression, we are going to use zebrafish embryos as a new animal model in our studies on cancer. Using zebrafish in cancer research provides several advantages over traditional approaches including external fertilization, rapid development, high fertility and small size of the adult animal. Therefore, these advantages have inspired a group of enthusiastic peoples at the Department of Applied Toxicology to use this organism in our studies. We are going to use zebrafish xenografts injecting cancer cells into the zebrafish embryos. To date, we consider to use some labeled cancer cells and genetic biomarkers which allows us to distinguish human cancer cells in real-time due to treatments. The major goal of the research is addressed to study a number of new drug candidates including novel bioengineered nanomaterials. To data, we will mainly focus on metastasis and angiogenesis in zebrafish xenografts. From our perspective, zebrafish xenografts have become a versatile and reliable tool that may have a huge impact on cancer drug discovery process in the near future.

Keywords: zebrafish, cancer, embryos, xenograft

Integrating whole genome sequencing and zebrafish functional studies to uncover new molecular basisof autosomal dominant hearing loss

Anna Sarosiak [1,2], Agnieszka Madejska [1], Dominika Oziębło [1,2], Marcin Leja [1,2], Przemko Tylżanowski [3,4], Monika Ołdak [1]

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2. Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland

3. Department of Development and Regeneration, University of Leuven, Leuven, Belgium

4. Medical University of Lublin, Lublin, Poland

The aim of this presentation is to introduce the objectives of our project. Autosomal dominant hearing loss (ADHL) is the second most common form of hereditary hearing loss (HL) and detection rate of causative mutation reaches approximately 65%. This suggests that performing extensive genetic testing, based on highthroughput DNA sequencing on 100 families with ADHL may lead to a discovery of novel disease genes.

To determine the function of the newly identified genes/alleles in the development of the auditory organ we will use a zebrafish model system, which is a valuable animal model for studying development and function of the vertebrate inner ear. We will analyze hearing defects by (i) investigating the morphology of the auditory system, (ii) obtaining a zebrafish knock-down, knock-out and/or knock-in models for the newly identified genes and alleles, (iii) performing rescue studies with human mRNA to verify pathogenicity of the detected novel alleles and (iv) using of behavioral studies testing hearing responses to asses function of the hearing apparatus.

Thus, the main purpose of the presented project is to detect novel molecular basis of ADHL and to implement the zebrafish animal model to dissect the underlying mechanism of action of the newly discovered genes and alleles. We assume that we will discover new ADHL genes and thus identify new signaling pathways responsible for the development and functioning of the organ of hearing and balance.

Funding was received by NCN Research Grant no. 2016/22/E/NZ5/00470 SONATA BIS6.

Erythropoietin inteisifies proapoptotic actvity of LFM-13 in colon cancer cells

Justyna Magdalena Hermanowicz[1,2], Beata Sieklucka[1,3], Tomasz Domaniewski[3], Krystyna Pawlak[3], Dariusz Pawlak[1]

[1] Department of Pharmacodynamics, [2]Department of Clinical Pharmacy,[3]Department of Monitored Pharmacotherapy, [4] Medical University of Bialystok, Mickiewicza 2C, 15-222 Bialystok, Poland

Background: Btk is non-receptor tyrosine kinases involved in the activation of signaling pathways responsible for maturation and viability of the cells. It plays an important role in the development of B-cell tumors, activating antiapoptotic pathways. Btk has previously been reported to be overexpressed in prostate cancer which correlated with cancer grades. Colorectal cancer is among the five most frequent causes for cancer-related deaths in Europe. This kind of cancer often accompanied by anemia which is treated with erythropoietin supplement. The aim of this study was to assess the effects of combination therapy with erythropoiethin beta (Epo) and LFM-A13 (Btk inhibitor) on colorectal carcinoma cells both *in vitro* and in animal models.
Methods: DLD-1 and HT-29 human colon adenocarcinoma cells were cutured in medium with Epo and LFM-A13. Cell proliferation was measured with an automated cell counter. Expression of BTK by Western Blotting and AKT mRNA by RT-PCR, and its protein was assessed and confocal microscopy, respectively. *Analysis of apoptosis* by *flow-cytometry* was also carried out. Nude mice were inoculated with adenocarcinoma cells and treated with Epo and LFM-A13.
Results: Herein, we found that simultaneous use of Epo and LFM-A13 exert an additive inhibitory effect on colon cancer cell growth. Featured therapeutic scheme resulted in effective cell killing, accompanied by attenuation of BTK, AKT signaling pathway and increased of apoptosis. This combination is also effective *in vivo* studies, where the combined administration of Epo and LFM-A13 significantly reduces the rate of growth of tumor cells and leads to the complete regression of the colorectal cancer cells.
Conclusions: Results of this study show that that adding Epo significantly enhances the antitumor activity of LFM-A13. The results of our study indicate the potential use of a combination of Epo and LFM- A13 as an effective therapeutic approach for colorectal cancer. Further we plan to evaluate the antiproliferative activity of simultaneous use of Epo and LFM-A13 on metastasis in an experimental model of *Danio rerio* xenografts.

Effect of microplastics in feed on indicators of animal health status in *Danio rerio* model

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Keywords: zebrafish, microplastic, *Tenebrio molitor,* gene expression, qPCR

Despite the growing amount of data on the impact of microplastics pollution in marine ecosystems, very little research focuses on these pollutants in freshwater ecosystems. Microplastic is considered to be one of the main sources of pollution in the environment, however, up to now, we do not have full knowledge of their movement and reactions in organisms. Microplastic in the industry is deliberately added to home and cosmetic products. Under the influence of weather conditions, they can undergo gradation from larger pieces of plastic. As a result, there is a wide range of physicochemical properties of microplastics. One of the most commonly produced and used plastics is polystyrene (PS), for instance in Europe demand for PS in 2014 was about 3.5 million tons. Until now, the molecular reactions induced by plastic particles in aquatic vertebrate species have not been fully researched, although there is an urgent need to develop these pathways for important groups of environmental compounds, such as microplastics.

Research conducted at our University showed that mealworms (the larvae of *Tenebrio molitor*) chew and eat Styrofoam, a common polystyrene product and it is efficiently degraded in the larval gut. Moreover, these insects have gained more and more attention in recent years as a potential source of protein for animal feed. The purpose of the experiment is to produce feed with the addition of microplastic and to estimate impact on zebrafish health status. For four weeks, the test group was fed with the addition of microplastics. The liver, intestines, spleen and kidney were then extracted and RNA isolation was performed. Finally, the analysis of gene expression in the direction of markers of inflammation was performed. In the above-mentioned organs in the research group an increase in the level of expression of markers of inflammation was observed. In the case of TNFα, the expression is almost three times higher in comparison to the control. The expression of IL-10 and IL-8 is on equal level, which the increase in expression is estimated to be more than twice that of the control group. Lower increases in expression were noted in the case of TNFβ, IL-6. For IL-1β, the increase in expression is minimal. We intend to perform a full histopathological analysis to confirm the obtained results.

Zebrafish as an animal model for assessing the toxicity of new drug candidates and nanoparticles

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Danio rerio (zebrafish) is used as a preclinical model and is increasingly applied for assessing the toxicity and efficacy of new drug candidates and nanomaterials. To date, a number of studies confirm that mammalian and zebrafish models are quite comparable and used in predictive toxicities. This convenient, predictive animal model confirmed its usefulness in the field of many different preclinical studies. It has also been used for a long time in ecotoxicological studies [1, 2] and can be used in variety of innovative research such as new drug development, neurodevelopmental and neurodegenerative disorders, oncology and nanotoxicology. The main role of the zebrafish model is reflected in the complementing of missing information between cell and mammalian studies. Although in vitro assay using cultured cells are commonly used to evaluate potential drug effect, they are frequent not predictive of the complex metabolism that affect drug efficacy and causes toxicity in animals. Therefore, many compounds that appear effective in vitro failed during costly animal trials [3].

The zebrafish embryo has become an important vertebrate model for assessing drug effects in preclinical studies. It exhibits unique characteristics, including ease of maintenance and drug administration, short reproductive cycle and transparency that permits visual assessment of developing cells and organs. Using zebrafish it is possible to obtain results quickly at lower costs. Recently, zebrafish has been shown to be a useful animal for assessing compound-induced neurotoxicity, hepatotoxicity, cardiotoxicity, important for developmental toxicity testing.

Zebrafish offer a range of advantages for the study of neurodegenerative diseases, particularly for testing of potential therapeutic candidates. Zebrafish are a particularly useful tool because large numbers of zebrafish can be housed in a small space, at relatively low cost, and bred rapidly to provide large sample sizes. Zebrafish also possess many physiological and anatomical similarities to humans, are transparent during development, and can be genetically modified. Finally, zebrafish can absorb compounds added to water that can be applied in new pharmacokinetic assays. They develop movement within 30 hours postfertilization (hpf) providing a simple and rapid readout of therapeutic efficacy. For these reasons, zebrafish have been used extensively in recent years to study neurodevelopmental and neurodegenerative disorders such as Parkinson’s disease,

Huntington's disease, spinocerebellar ataxia, hereditary spastic paraplegia, Amyotrophic lateral sclerosis, and spinal muscular atrophy [4].

The zebrafish has a number of features that make it excellent model for the toxicological evaluation of innovative nanomaterials, which translates into the tremendous amount of conducted research. In general acute toxicity studies are carry out, although the zebrafish model also has considerable potential for organ toxicity testing.

We used the *Danio rerio* embryonic model for the toxicity assessment of engineered nanomaterials, which, among others, may serve as drug delivery systems used for diagnostic and therapeutic purposes, including neurodegenerative diseases and other neurological disorders.

We evaluated the embryotoxic potential of hydrogen peroxide (H_2O_2) used as oxidative stress inductor in graphene-metal type nanoparticles (GMNs) coated with and without human albumin (protein corona). The acute embryotoxic effect of hydrogen peroxide was observed in embryos treated with and without GMNs. Interestingly, GMNs decorated with albumin mitigated embryotoxic effects due to H_2O_2 treatments in both dose- and time-related fashions. The protein corona placed on graphene-metal type nanoparticles plays a modulatory role for the acute oxidative stress in zebrafish embryos.

Although many qualitative and end point for predicting toxicity in mammals can be assessed in zebrafish embryos, direct comparison requires further validation. Several significant challenges remain in order to establish zebrafish as a predictive animal model for neurotoxicity, hepatotoxicity and cardiotoxicity. In the future, I'm going to investigate the specific molecular biomarkers unable to elucidate the underlying mechanisms of organ toxicities due to novel nanoparticle exposures.

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The comparison of new reversal agents for anticoagulants in nonclinical safety studies.

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Anticoagulants such as unfractionated heparin (UFH), low-molecular-weight heparins (LMWHs) and fondaparinux are widely used in prevention and treatment of thromboembolic disorders. Protamine, the only registered antidote of UFH, may cause unacceptable toxicity. We developed heparin binding copolymer (HBC), a new synthetic agent directly binding UFH, LMWHs and fondaparinux, and neutralizing their anticoagulant effect in rats (Kalaska et al., 2016). However, it is necessary to exclude the potential toxicity of HBC before first use in humans. Our aim is to evaluate the safety profile of HBC, and compare it with the other antidotes registered or currently in the development: protamine, andexanet alfa and ciraparantag in two different animal models. We developed a new method enabling the simultaneous monitoring of main cardiovascular and respiratory parameters in one animal. Our method is based on the combination of two devices: Plugsys with arterial blood pressure unit (Transonics System, USA) and PhysioSuite (Kent Scientific, USA), which allow to register 7 circulatory and respiratory parameters. The possible acute toxicity of HBC (6, 20, 40 mg/kg) and protamine (10, 15, 20 mg/kg) was assessed during one-hour monitoring of mean blood pressure, heart rate, body temperature, oxygen saturation, perfusion, peak CO_2 and respiratory rate in male Wistar rats. All experiments involving animals were approved by Local Ethical Committees. The preliminary safety data indicates that HBC was safer from protamine and could be a novel antidote for all parenteral anticoagulants in patients who suffer a major bleeding or require emergency surgery. Additionally we plan to evaluate and compare the effects of various doses of HBC, protamine, andexanet alfa and ciraparantag on the morphological alterations, cardiac rhythm, lethality rate and biodistribution in Zebrafish model (*Danio rerio*).

Keywords: anticoagulants, antidotes, hemorrhage, thrombosis, safe study

Protective effects of propolis extract on zebrafish during oxidative stress and inflammatory reaction;

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Key words: propolis, zebrafish larvae, angiogenesis, oxidative stress, inflammation;

Propolis is a bee product produced from tree buds exudatives and used in hive as a defensive substance and building material. It is used in medicine from the centuries. Nowadays its pharmacological properties are broadly tested in many different ways.

There are investigations identify the propolis as an powerful antioxidative factor. Active compounds of propolis extract, especially caffeic acid phenethyl ester (CAPE), blocks ROS production in several systems. Additionally, propolis inhibits peroxidation of LDL and nitration of proteins.

The main goal of planned assay is to investigate the protective influence of propolis on organisms and estimate, whether in the future it can be used in new fields of medicine.

To investigate the effect of propolis on the angiogenesis in the oxidative stress the transgenic zebrafish line Tg(Fli1:EGFP) will be used. The effect will be studied qualitatively and quantitatively by using the confocal microscope. The expression of genes related to angiogenesis and oxidative stress will be evaluated by using RT-qPCR.

There is broadly known, that exposition to oxidative stress damage hair cells located in the inner ear and lateral line neuromasts. Protective effect of propolis on this cells will be evaluated using Tg(BRN3C:GFP) zebrafish line. Changes in the number of hair cells will be observed by using the confocal microscope. Additionally, there will be investigated if propolis changing the expression level of genes related to oxidative stress using RT-qPCR.

Anti-inflammatory properties of propolis will be tested using Tg(mpx:GFP) line, by evaluating neutrophil migration and measuring pro- and anti-inflammatory cytokines genes expression level, using RT-qPCR. In addition, behavioral responses exhibited by fish when exposed to various factors will be examined in each part of the experiment.

Zebrafish-based evaluation of anxiolytic assay on extracts and compound from *Boswelia spp*.

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Keywords : zebrafish, anxiety, phytotherapy, ethnobotanic

Purpose

In Middle East countries, frankincense is used for its analgesic, relaxant and anti-inflammatory properties [1] . In this work, we investigated the anxiolytic activity of extracts from *Boswellia serrata* and *Boswellia sacra* species with a zebrafish anxiety model. Our results showed that only some extracts of *Boswellia spp* (Burseraceae) demonstrated a significant anxiolytic activity.

Research methods

In this study we examined 6 extracts with different polarities as well as one essential oil and one major compound isolated from *Boswelia sacra*. The extracts, the essential oil and the isolated compound were evaluated using a zebrafish model for anxiety based on a light/dark preference thygmotaxis

Results

The hexane extract of *Boswellia sacra* showed the highest anxiolytic activity of all the extracts compared to the control and the diazepam (10 µM). This extract reduced (at the level of 93%, 59,5% and 30,6% respectively) thigmotaxis behaviour both in light and dark environment at all tested concentrations (75 µM, 50 µM and 25 µM). The pure compound from hexane extract were also tested and also showed significant activity, however less than extract. The other extracts (methanol, ethanol, DCM) showed very little reduction/no significant activity. Those results demonstrate that only the hexane extract have anxiolytic properties.

Conclusions

Previous studies reported the anticonvulsant activity of *Boswellia* extracts and this led us to investigate another biological activity of this resin. The extracts and the pure compound showed significant anxiolytic activity which give us prospects for further study on frankincense. The next step will be to find other compounds responsible for this bioactivity.

Reference

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Synthesis of new triazole derivatives with potential biological activity

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In spite of the large variety of available drugs, central nervous system (CNS) illnesses remain a huge issue in the healthcare system. Depression is more and more often categorized as a disease of nowadays. It is estimated that by 2020 it will has become the second most common cause of health-related disability around the world. A chronic pain may constitute the cause of depression. A pain accompanies many different diseases, and directly affects the mood and therefore one's standard of living. We have a wide range of analgesics, but most of them come with side effects. As a result of the tolerance effect they eventually become ineffective.

The search for new drugs affecting the CNS remains a challenge for scientists. We are looking for new, more effective and at the same time less harmful treatment methods.
In medicinal chemistry, while searching for the structures of new drugs, it is a common procedure to replace particle fragments or functional groups in compounds of known properties with bioisosteric groups.

At the Department of Organic Chemistry, the research concerning synthesizing compounds which affect the CNS has been carried out for many years. The presented study concerns the synthesis of s-triazole derivatives, including the compounds substituted at N4- or S-position with potential antinociceptive and anticonvulsant properties.

Most of synthetized compound were already tested using zebrafish model for epilepsy. The seizures were induced by pentylenetetrazole (PTZ) that blocks ɣ-aminobutyric acid (GABA)-mediated transmission. For some compound we find significant decrease in seizures (even 55% compared to control). The most promising derivative is TK3 – 4-(3-chlorophenyl)-5-(4-fluorophenyl)-3-pentylthio-2,4-dihydro-3H-1,2,4-triazole.

The preventive effect of L-carnitine or CoQ10 application in zebrafish post-statin myopathies

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For years, developed societies struggling with civilization diseases, among which one of the most important is the hypercholesterolemia and its aftereffects especially cardiovascular system diseases.

For this reason cholesterol-lowering drugs - statins and fibrates are the most commonly used drugs. The statins action is based on inhibition of the 3-Hydroxy-3-methylglutaryl-CoA reductase (HMGCR) pathway results in myopathic side effects in up to 7% of treated patients[1]. Also, studies have shown, that muscle damage after statins treatment occurs in rats[2], mouse muscle cells *in vitro*[3], and zebrafish larvae[4]. The most common defect observed in post-statin myopathies is the deficiency of CoQ10, an essential cofactor participates in the electron transport chain in mitochondria, prevents oxidative stress, and regenerates active antioxidant properties of vitamins C and E. The deficiency of L-carnitine is the second defect found in muscle tissue of patients with post-statins myopathies. The listed clinical observations and the fact that zebrafish is a good model for toxicological research[5], have led us to the following research hypothesis: supplementation with CoQ10 or L-carnitine reduces zebrafish muscle damage induced by statins application.

The first set of experiments enabled estimation of the minimum effective dose (MED) of lovastatin, CoQ10, L-carnitine.

The second set of experiments was conducted to understand the role of CoQ10 or L-carnitine in lovastatin-induced myopathy.

The results suggest that L-carnitine has higher protective activity against post-statin myopathy.

The experimental data included in this work was obtained during scientific workshops for high school students (DANiO) which were founded by the City Council of Wroclaw and organized by the Academy of Young Scholars and Artists and the Department of Animal Developmental Biology, University of Wrocław.

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The Ten Commandments of zebrafish facility- KEEP FISH HAPPY!

1. **A facility MUST have a designated manager who coordinates the work of the facility.**

2. **Wash your hands before handling ANYTHING in the fish facility and then put disposable gloves on. Gloves, dedicated shoes or shoe protectors and a lab coat must be worn at ALL times**

3. **Limit the access to the main facility to authorized and trained personnel (LEGAL REQUIREMENT) only and limit the human traffic not to stress the fish. Nonessential visitors should not be allowed in the facility**

4. **Enter the facility only during the light cycle. Do not disturb photoperiod nor fish while they sleep.**

5. **Avoid glass at all cost. Be aware that glass can be easily broken (danger to the fish and personnel) and might bind detergents or other chemicals that can affect fish.**

6. **For eggs/larvae manipulations use ONLY disposables (e.g. plates, pipettes) and NEVER reuse them.**

7. **Whatever falls on the floor cannot have contact with fish or go into the tanks. If fish would end up on the floor, you must euthanize it and dispose it correctly (biohazard, waste category I). If it is a net, lid, or other equipment, sterilizes it when possible before using it again or discard.**

8. **All fish lines MUST HAVE a certificate of origin and a health certificate from the sending facility and MUST be placed in quarantine (even if bleached eggs have been imported). NO EXCEPTIONS.**

9. **Every six months send sentinel fish for pathogen testing and once a year carry out a thorough testing including histopathological examination, with an accredited company.**

10. **Ensure that your aquatic system works well and biological filtration is sufficient to keep water parameters at its optimum. Use feeds from sources offering safe and good quality products. Fish must be visually inspected daily (during feeding for instance). Report fish that look or behave abnormal.**

If you will keep fish happy, they will keep you happy.

Notes

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