2020 Strategic Conference of Fish Investigators

Posters (Sorted by Poster Number)

Poster Session Times:

Odd numbered posters: February 5, from 7:30 – 10:00 pm

Even numbered posters: February 6, from 7:30 – 10:00 pm

Poster #1, Category: Brain and Behavior

Mutations in FAM50A cause Armfield XLID Syndrome: A Spliceosomopathy Impacting Neurodevelopment

Cheol-Hee Kim, Chungnam National University

Intellectual disability (ID) is a heterogeneous group of disorders and includes an excess of affected males who harbor variants on the X-chromosome (XLID). We previously localized Armfield XLID syndrome to Xq28, and here, we report rare FAM50A missense variants in nine affected males from five unrelated families with overlapping features. We generated a fam50a knockout (KO) zebrafish model hallmarked by abnormal neurogenesis and craniofacial patterning, and transient in vivo complementation assays indicated that the five nonsynonymous changes were hypomorphic. RNA sequencing (RNA-seq) analysis from fam50a KO zebrafish heads showed dysregulation of ~12% of the transcriptome, with augmented spliceosome mRNAs and concomitant depletion of transcripts involved in brain development and function. Zebrafish RNA-seq datasets showed a preponderance of 3' alternative splicing events in fam50a KO, suggesting a role in the spliceosome C complex. In sum, Armfield XLID syndrome is a spliceosomopathy underpinned by aberrant mRNA processing during development of neural structures.

Poster #2, Category: Cancer

Overexpression of Prolidase Counteracts Adriamycin-induced p53-dependent Apoptosis in MCF-7 Cells and Zebrafish Embryo Xenograft Model

Arkadiusz Surazynski, Medical University of Bialystok

Prolidase is a multifunctional protein of ability to bind and inactivate p53 function. This study was undertaken to establish the role of prolidase in the regulation of p53-dependent apoptosis in a model of prolidase overexpressed MCF-7 cells and zebrafish model. Adriamycin was used to induce apoptosis through p53 protein. The effectiveness of apoptosis was confirmed by analysis of phosphatidylserine, caspases 3 and 9 expressions and localization in MCF-7 cells and on the zebrafish embryo xenograft model.

MCF-7, MCF-7 PL (with a prolidase cDNA expression plasmid) and the zebrafish embryo (inoculated with labelled cancer cells) were incubated with different concentrations of Adriamycin for 24h.

Adriamycin decreased survival and induced apoptosis in MCF-7 cell line and zebrafish embryo xenograft model in a dose-dependent manner. Increased expression of p53, caspase 9 and 3

and their translocation to the nucleus we observed. However, the transfection of MCF-7 cells with prolidase vector (MCF-7PL) protects cells against the pro-apoptotic effects of Adriamycin.

Overexpression of prolidase in MCF-7 cells (MCF-7PL) counteracts Adriamycin – induced, p53-dependent apoptosis in these cells. It suggests, that p53 can be suppressed by forming a complex with prolidase.

This work was supported by the National Science Center (2017/25/B/NZ7/02183).

Poster #3, Category: Cancer

Erythropoietin and Bruton's Tyrosine Kinase Inhibitor as a New Therapeutic Option for Breast Cancer

Justyna Hermanowicz, Medical University of Bialystok

Bruton’s tyrosine kinase (BTK) has emerged as a new therapeutic target in many types of malignancies. LFM-A13 is a small molecule inhibitor of BTK with in vitro and in vivo anti-proliferative activity against human breast cancer. Anemia is a relatively common symptom coexisting with malignancy in turn recombinant human erythropoietin is an effective and convenient factor to treat it. We explored the effects of LFM-A13 alone and in combination with Epo on cell viability, apoptosis, cell cycle progression, BTK and cyclin D1 expression. Effectiveness of featured therapeutic combination we also examined on zebrafish embryo xenograft model. Results: The results demonstrated that Epo, significant intensifies the anticancer activity of LFM-A13 against breast cancer cells. Epo and LFM-A13 administered together has beneficial effects in cell killing, induction of apoptosis, light increase in phase G0/G1 and reduction in cyclin D1 and BTK expression. Simultaneous use Epo+LFM-A13 efficiently blocked the tumor development in zebrafish MCF-7 and MDA-MB-231 xenografts. Conclusions: Simultaneous use of Epo and LFM-A13 may be useful in the treatment of breast cancer patients with high BTK expression.

The research project was funded by grants from National Science Centre, Poland No. DEC-2017/01/X/NZ5/00362 and SUB/2/DN/19/001/2211 financed by the Medical University of Bialystok, Poland.

Poster #4, Category: Cancer

Molecular Basis of Antitumor Activity of Four Novel Transition Metal Complexes with Berenil AND Nitroimidazole

Robert Czarnomysy, Medical University of Bialystok

Imidazole ring is a known structure in many natural or synthetic drug molecules and its metal complexes can interact with DNA and do the cleavage. The biological activity of berenil seems equally interesting. According to our studies, platinum(II) complexes with berenil have comparable or greater anti-tumor activity compared to cisplatin with less drug toxicity. Therefore, the goal of this study was synthesis and anticancer activities of four novel transition metal (Pt, Pd, Au, Cu) complexes with berenil and nitroimidazole.

The complexes in which the central atom was palladium or platinum appeared to be the compounds of highest cytotoxicity in relation to neoplastic breast cancer cells MCF-7. In relation to human normal breast epithelial cell MCF-10A the new complexes were characterized with lower cytotoxicity than in case of examined neoplastic cells. Additionally our experiments revealed that these complexes inhibited the proliferation of breast cancer cells by increasing the number of apoptotic cells. In addition, we have observed that these complexes selectively concentrate in mitochondria which may explain the increased proapoptotic activity of these compounds. We have further confirmed the effectiveness of the synthesized compounds by our team on zebrafish embryo xenograft model.

This work was supported by the National Science Center (number of projects: 2018/02/X/NZ3/00734).

Poster #5, Category: Cancer
Screening of the Herb Extracts with Cisplatin-protection Effects on Normal Cells
Yun-Ju Lai, National Taiwan Normal University

Cisplatin is one of the most often used chemotherapeutics in clinical. However, it often induces renal and neural toxicity. Herbal medicine presents relatively moderate side-effects compared to western medicine. Recent studies show that it has potential to protect cells against cytotoxicity caused by drugs. Here we used hepatocellular carcinoma Hep3B and ovarian neoplasms SKOV3 cell line as cancer models, and neurocytoma SH-SY5Y as a neuron cell platform to screen the Chinese herbal extracts which act synergistically with cisplatin to inhibit survival of cancer cells, but weaken the cytotoxicity of cisplatin to neuron. Moreover, we further examined the protection effects of these herbs on the cisplatin-induced neuron toxicity by counting the number of hair cells of L1 neuromast in zebrafish lateral line. Our results found that two Chinese herbal extracts, Wolfiporia extensa and Gastrodia elata, inhibited the growth of Hep3B and SKOV3 cells but do not have cytotoxicity to retinoic acid-differentiated SH-SY5Y neuroblastoma cells. However, these two herbs do not protect hair cell damages in Zebrafish. Instead, Angelica sinensis showed slightly protection effects on the hair cells. From these results, W. extensa, G. elata and A. sinensis extracts may serve as a clinical adjuvant treatment for cisplatin in the future.

Poster #6, Category: Cancer
Zebrafish as a Model for Intrahepatic Cholangiocarcinoma
Wangta Liu, Department of Biotechnology, Kaohsiung Medical University

Intrahepatic cholangiocarcinoma (ICC) is the second most common primary liver malignancy with a poor prognosis. The molecular mechanisms underlining the pathogenesis of ICC are still elusive. We establish a ICC zebrafish model by co-expression of HBx and HCP in the liver, that are histologically and genetically similar to their human counterparts. Recent data have revealed that the transcription factor forkhead box M1 (FOXM1) was predicted to be activated in hepatitis B virus X (HBx)- and hepatitis C virus core (HCP)-induced zebrafish ICC by global

gene expression analysis. Inhibition of FOXM1 increased glycine N-methyltransferase (GNMT) expression while reducing the tumor size of HBx- and HCP-induced ICC in zebrafish. On the other hand, the involvement of FOXM1-regulated GNMT in both migration and invasion ability was confirmed by wound healing and transwell assays. The binding of FOXM1 to the FKH consensus motif (RYAAAYA) of the GNMT promoter was confirmed by promoter assay and ChIP-PCR. Taken together, FOXM1 promotes cell proliferation and inhibits migration and invasion by upregulating GNMT transcription in ICC. Our results provide new insights into targeting FOXM1 and GNMT as a potential strategy for the treatment of ICC.

Poster #7, Category: Cardiovascular Development & Function
Characterization of the Roles of Connexin32 in Zebrafish Vascular Development
Chang-Yi Wu, National Sun Yat-sen University

The process of vascular development in vertebrates are mainly divided into vasculogenesis and angiogenesis. Using zebrafish as a model organism have been intensively identified many molecules that control vascular development. However, genetic control intersegmental vessel (ISV) patterning and caudal vein plexus (CVP) formation are not fully documented. Gap junction protein Connexins32 (cx32) has been shown functions on the blood vessels in injury mice and enhances angiogenesis in cell study, however, no study about the vascular development in animal study and signal pathways related to cx32 in angiogenesis have not been clarified.

We first showed cx32 mRNA is expressed in developing vessels from 18S to 30hpf stages. Knockdown of cx32 by morpholino injection causes vascular defects in ISV and CVP, suggesting the role of cx32 in vascular growth. We showed that vascular defects likely due to the impairment of endothelial cell proliferation and migration. Consistent with vascular growth defects, loss of cx32 affects the expression of the vascular markers. Furthermore, overexpression of cx32 can rescue the loss of cx32, suggesting the specificity of morpholino knockdown. Finally, we showed the interaction between cx32 and VEGF and BMP signals. Together, we show cx32 plays an important role for vascular development in zebrafish.

Poster #8, Category: Cell Biology & Cell Migration
Genes Involved in Pigment Cell Differentiation Isolated by Gene Expression Profiling of Flounder Asymmetric Pigmentation
Hayato Yokoi, Tohoku University

Flatfish exhibit left-right asymmetric pigment pattern, providing unique system to investigate the pigment cell differentiation. Once develop as symmetric larvae, they transform their body plan to asymmetric adult form at the metamorphosis: one eye migrates to the other side and the pigmentation occurs only in the ocular side. To understand the molecular basis of the metamorphic asymmetric morphogenesis, we compared gene expression profile between ocular and blind side of the Japanese flounder (Paralichthys olivaceus). RNA-seq reads were de novo assembled and ocular side- or blind side-biased genes were selected. Candidate genes were then evaluated by RT-PCR and the tissue distribution was examined by section in situ

hybridization. Identified genes included well-characterized pigment cell markers such as gch2 and pnp4a, also included previously uncharacterized genes related to hdd and dhfr. To examine the functional role of these genes, we use medaka as a model system. Orthology was confirmed by phylogenetic and synteny analysis. Embryonic expression was compared with marker genes and seemed to be involved in the leucophore-xanthophore linage cells. Further functional analysis is under investigation via CRISPR mediated mutagenesis and GFP knock-in.

Poster #9, Category: Cell Biology & Cell Migration
Mapping the Cellular Dynamics of Liver Regeneration using Targeted Photoactivatable Cell Ablation
Elke Ober, University of Copenhagen

The liver has an intricate tissue architecture, which has the capacity to regenerate following damage from injury or disease. However, the cellular behaviours and interactions that underpin the re-establishment of liver architecture during regeneration remain unclear. Here, we report the development and application of a novel optogenetic hepatocyte ablation tool, LIVERZAP, to examine the cell behavioural dynamics of liver regeneration in vivo. Upon activation with near-infrared light, LIVERZAP induces rapid, spatiotemporally controllable ROS-mediated liver injury in zebrafish. This leads to rapid and dynamic remodelling of the biliary ductal network concomitant with dramatic hepatocyte loss. We show that both liver architecture and mass are rapidly restored through de-differentiation of biliary epithelial cells (BECs) and predominantly progenitor-cell driven regeneration. The rapid injury paradigm of LIVERZAP, only 12 min, allows for live-imaging of both injury and recovery phases. By light sheet imaging, we show that a subset of newly differentiated BECs actively migrate to rebuilt the 3D biliary network, challenging the idea that restoration of tissue architecture is predominantly passive and driven by mitotic pressure pushing neighbors into place. We propose that active migration is a critical component of liver regeneration.

Poster #10, Category: Disease Models
Oxidative Stress Response in Zebrafish
Makoto Kobayashi, University of Tsukuba

Oxidative stress plays a crucial role in the development of age-related diseases including arthritis, diabetes, dementia, cancer, and neurogenerative diseases. We have been studying regulatory mechanisms of cytoprotective responses to oxidative stress using zebrafish. The Keap1-Nrf2 pathway is the major regulator of oxidative stress response and its involvement in the aging and cancer growth has become a hot topic. In this presentation, I will address about the regulation and function of the Keap1-Nrf2 pathway in cytoprotection against a variety of stresses.

Poster #12, Category: Disease Models
Analysis of AEP(Auditory Evoked Potential) Threshold Changes & Inner Ear Hair Cell Damage

after Intraperitoneal Injection of Aminoglycoside in Adult Zebrafish

June Choi, Korea University Ansan Hospital

Purpose: The purpose of this study was to demonstrate changes of AEP and the extent of hair cell damage in the zebrafish inner ear, following systemic aminoglycoside administration.

Method: In the current study, we did intraperitoneal injection of gentamicin of 2.5,20,40 ug to adult zebrafish and observed gentamicin absorption and wash out in the inner ear. Then we counted the number of hair cells to confirm gentamicin's influence on hearing of zebrafish. In addition, we measured AEP threshold, which can measure a saccular response responding to sound

Results: In zebrafish inner ear, especially, utricle, Lagena, saccule, the number of hair cells decreased after gentamicin injection until 24 hours.In utrilce, 20 % of hair cells were damaged in region 1-4. In Lagena,20- 30% of hair cells were destructed in region 1-3. In saccule, 20-40% of hair cells decreased in percent length from rostal end of saccule (5%~90%). In AEP device, we measured adult zebrafish threshold from 800Hz to 8000Hz. Threshold was the lowest in 800Hz and 1000Hz and it increased as get higher frequency.

Conclusion: In this study, we confirmed AEP changes and inner ear hair cell damage in adult zebrafish. Furthermore, we will try to figure out drugs that can prevent hair cell damage by comparing hair cell morphology and the hearing threshold.

Poster #13, Category: Disease Models

Modeling Vitamin B Deficiency and Related Diseases with Zebrafish

Tzu-Fun Fu, National Cheng Kung University

B vitamins are a class of water-soluble vitamins and function as coenzymes in numerous intracellular biochemical reactions. Among them, vitamin B6 (pyridoxine) and B9 (folate) work closely in one-carbon metabolism and participate in nucleotide synthesis, amino acid metabolism, methyl-donor production, neural transmitter formation and redox balance, hence vital to embryogenesis and health maintenance. Using both genetic and chemical approaches, we have established B6 and/or B9 deficient zebrafish models. Both recapitulated the phenotypic hallmarks of B6/B9 deficiency observed in the patients with the corresponding vitamins deficiency. Additional but less addressed pathological characteristics, including increased vulnerability to UVB-inflicted damage and impaired melanogenesis/melanocyte function, on B6/B9 deficient larvae are also observed. Adding 5-formyltetrahydrofolate, pyridoxal-5'-phosphate or epinephrine, a melanosome transportation stimulus, rectify the anomalies appreciably. Examining the melanocyte retrograde response and the levels of precursors for epinephrine biosynthesis reveals a decreased level of dopamine. Evidence also suggests that decreased expression of Dopa decarboxylase (Ddc), the enzyme catalyzing dopamine synthesis, is at least partly responsible for the impaired melanogenesis and melanocyte malfunction. We conclude that the interruption of epinephrine biosynthetic

pathway due to the lowered Ddc expression contributes to the folate deficiency-induced impeded melanogenesis/melanocyte function.

Poster #14, Category: Disease Models
Non-autonomous Mechanism for Motor Neuron Degeneration via Oligodendrocyte Dysfunction in Amyotrophic Lateral Sclerosis
Hae-Chul Park, Korea University

Myelin is a specialized membrane that wraps around nerve fibers and is essential for normal axonal conduction in neurons. In the central nervous system, oligodendrocytes are responsible for myelin formation. Recent studies have reported pathological abnormalities in oligodendrocytes in human patients with amyotrophic lateral sclerosis (ALS) and a mouse model of ALS expressing the G93A mutation of the human superoxide dismutase 1 (mtSOD1). However, it is unclear whether oligodendrocyte pathology in ALS represents the primary dysfunction induced by mtSOD1 and how mtSOD1 contributes to oligodendrocyte degeneration and ALS pathogenesis. We analyzed GAL4-VP16-UAS transgenic zebrafish selectively expressing mtSOD1 in mature oligodendrocytes. We observed that mtSOD1 directly induced oligodendrocyte degeneration by disrupting the myelin sheath and downregulating monocarboxylate transporter 1 (MCT1), thereby causing spinal motor neuron degeneration. Pathological changes observed in this transgenic zebrafish were similar to the pathology observed in the SOD1G93A mouse model of ALS, which is characterized by expression of mtSOD1 in all cells. In addition, oligodendrocyte dysfunction induced by mtSOD1 was associated with anxiety-related behavioral abnormalities, learning impairments, and motor defects in the early symptomatic stage. We also found that treatment with potassium channel inhibitors rescued behavioral abnormalities without rescuing MCT1 expression, suggesting that myelin disruption induces behavioral abnormalities independently of MCT1. These results indicate that mtSOD1-induced dysfunction of mature oligodendrocytes is sufficient to induce motor neuron degeneration, thus informing future therapeutic strategies targeted at oligodendrocytes in ALS

Poster #15, Category: Disease Models
Targeted knockout of duox causes defects in zebrafish growth, thyroid development, and social interaction.
Jong-Su Park, Korea Institute of Toxicology

Congenital hypothyroidism (CH) is a well-known metabolic syndrome which can cause growth failure and even autism spectrum disorder (ASD). CH is caused by inadequate thyroid hormone production in infants. It can also occur due to iodine deficiency, thyroid gland defects, or thyroid hormone production error. To investigate CH, we generated a zebrafish mutant for DUOX homologue gene using CRISPR/cas9 methodology. The role of duox involves generation of H2O2 in thyroid follicles during the thyroid hormone production process. Most mamma|s have two DUOX homologs, DUOXl and DUOX2, but teleosts possess only a single duox gene. Zebrafish duox homozygous mutant larvae did not show any defects, but after the juvenile

stage, they exhibited growth retardation, failure of swim bladder separation, irregular scale formation, an absence of barbel and xanthophore formation. Given frequent comorbidity of CH and ASD, we examined adult social interaction behavior tests and confirmed that mutants exhibited decreased interest in other fish, similar to ASD patients. In this study, we confirmed that duox plays a pivotal role in thyroid hormone production and found that gene knockout leads to the formation of several morphological and physiological defects.

Poster #16, Category: Early Development
Differential Cellular Responses of Zebrafish Embryos to Ionizing Irradiation According to Developmental Stage
Yasuko Honjo, Hiroshima University

Cellular responses to ionizing radiation in early embryos are still not well-understood. Zebrafish is an excellent model for studying cellular events during early embryogenesis. Our studies are aiming to elucidate how irradiation affects each developmental stage of zebrafish embryos.

Zebrafish embryos irradiated at 1~2 hpf gave rise to the most severe phenotype in a dose-dependent manner, while those irradiated at 6 hpf (gastrulation stage) showed a little or almost no phenotypic difference even when they were irradiated with 10 Gy. We showed that cellular responses in the embryos that irradiated before gastrulation did not respond as those at later time points. P21 protein were up-regulated only at 6 hpf despite the fact that mRNA levels of p21, the downstream target of P53, were up-regulated much higher in embryos irradiated at 2 hpf than those at 6 hpf. This observation suggests that the regulation of P21 stabilization might be an indispensable component in cellular responses to DNA damages during early embryogenesis.

We also found that apoptotic cells were increased at 1 dpf in the embryos irradiated at early time points. We are currently investigating what type of cells are apoptotic and when they start to appear.

Poster #17, Category: Early Development
Functional Analysis of Zebrafish Hox Clusters
Akinori Kawamura, Saitama University

Hox genes, which specify cell identity along the anterior-posterior axis, form the organized clusters in vertebrates. The number of vertebrate Hox clusters is amplified by the whole-genome duplication events during the evolution of the vertebrates; four in mice and seven in zebrafish. Although the studies using knockout mice and compound mice of Hox genes have been accumulated, the functional roles of Hox genes in embryogenesis remain to be elucidated, largely due to the redundancy of Hox genes. In this study, we generated and characterized seven hox cluster mutants in zebrafish. The genomic regions encompassing each hox cluster were deleted by a pair of gRNAs targeted at both ends of each cluster. By examining the phenotype of each hox cluster homozygous mutant, we found abnormalities in the rhombomere patterning, the pharyngeal cartilage formation, the pectoral fins, the number of

somites, and the position of lateral lines. Micro-computed tomography scan for viable hox cluster homozygous fish revealed the malformation of axial skeletons and internal organs. Mutants of each hox cluster exhibited distinct abnormalities, suggesting that the differential role of hox clusters in the zebrafish embryogenesis.

Poster #18, Category: Early Development
Imaging of Endogenous Wnt Protein Dynamics in Zebrafish Embryos
Shinji Takada, National Institute for Basic Biology

While it has been shown that Wnt acts at a distance from its source cells, evidence also suggests that it might act locally. To investigate the action range of Wnt in tissues, we examined the distribution and dynamics of Wnt proteins in vertebrate embryos. Immunohistochemistry revealed that endogenous Wnt8 proteins are actually distributed in a gradient fashion in Xenopus embryos. Ectopically expressed Venus-tagged Wnt8 was captured by morpho-trap, a membrane-tethered form of anti-GFP nanobody, expressed apart from the producing cells by over a 10 cell-diameter, supporting a long-range transfer of Wnt proteins. We consistently, confirmed dynamic diffusion of Wnt proteins in the extracellular space by fluorescence correlation spectroscopy (FCS). In contrast, a combinatory analyses of FCS and quantitative imaging revealed that this diffusing population is small and more that 95% of Wnt proteins in the extracellular space are not mobile. For a better understanding of the dynamics of endogenous Wnt proteins, we are generating a knock-in fish line by CRISPR/Cas9-mediated genome editing, in which wnt8 protein fused with a fluorescence tag is expressed instead of an endogenous one.

Poster #19, Category: Early Development
Epo Signaling Promotes Embryonic Neural Progenitor Cell Survival and Differentiation through Upregulation of XIAP and cIAP2 Expression
Chin-Hwa Hu, Department of Bioscience & Biotechnology, National Taiwan Ocean University

Erythropoietin (EPO) was originally found as a glycopeptide cytokine required for erythrocyte production in the bone marrow. It also has functions in nonhematopoietic cells, including neural differentiation and neuroprotection. Previously we have found that hypoxia signaling plays critical roles in embryonic development. Knockdown of HIF2a suppresses Survivin and EPO expression in neural progenitor cells, suggesting there is a close relationship between Survivin and EPO genes. Although it was found that EPO signaling promotes neurogenesis, the mechanism still remains unclear. Here we have investigated the correlations between HIF2a and EPO and the possible neuroprotection mechanism of EPO in zebrafish embryos. We confirmed that EPO has critical roles in CNS neuronal development. Knockdown of EPO results in neuronal apoptosis and suppresses neural development. It was shown that transcriptions of both XIAP and cIAP2 are controlled by EPO signaling through Jak2 and NF?B pathways. Inhibition of either Jak2 or NF?B activity leads to decrease of birc4/xiap and birc3/ciap2 transcription and suppresses neurogenesis. Knockdown of birc4/xiap or birc3/ciap2 also results in suppression of neurogenesis. The aberrant neuronal differentiation could be resuced by

ectopic birc4/xiap or birc3/ciap2 mRNAs, suggesting that the EPO/Jak2-NF?B modulated XIAP and cIAP2 plays important roles in neurogenesis. Chromatin immunoprecipitation analysis shows that HIF2a binds to the regulatory region of epo gene. It suggests that both epo and survivin are regulated directly by HIF2a. In summary, here we confirm that EPO modulates birc4/xiap and birc3/ciap2 transcriptions through Jak2 and NF?B pathway. Previously, it was shown that EPO also protects injured brain cells against apoptosis through the same signaling pathway. Furthermore, we found that XIAP and cIAP2 promotes neurogenesis in the developing embryos. Our studies have shed lights on the mechanism of neural regeneration in brain-injured or neurodegenerative patients.

Poster #20, Category: Early Development
Involvement of an Oct4-related PouV gene, pou5f3/pou2, in primary neurogenesis in zebrafish embryos
Kyo Yamasu, Graduate School of Science and Engineering, Saitama University

In early vertebrate embryos, neurogenesis proceeds in proneural clusters that are generated by a gene network involving proneural genes and Notch signaling. However, what occurs between early neural induction and later initiation of neurogenesis has not been fully revealed. In the present study, we demonstrated that, during gastrulation, the expression of the Oct4-related PouV gene pou5f3, which is widely observed at earlier stages, was rapidly localized to an array of isolated spotted domains, each of which contained individual proneural clusters. Further analysis demonstrated that the anterior pou5f3 domains straddled the boundaries between rhombomere 1 (r1) and r2, whereas the posterior domains were included in r4. The effects of forced expression of an inducible negative dominant-interfering pou5f3 gene suggested that pou5f3 activated early proneural genes and soxB1. Furthermore, pou5f3 was considered to repress her4.1, a Hairy/E(spl) gene involved in lateral inhibition. Suppression of pou5f3 also altered the expression of other her genes. In vitro reporter assays in P19 cells showed that pou5f3 was repressed by neurog1, but activated by Notch signaling. These findings together demonstrate the importance of pou5f3 in neural development in vertebrate embryos.

Poster #21, Category: Genomics and Proteomics
Genetic basis underlying phenotypic diversity in domesticated goldfish (Carassius auratus)
Yoshihiro Omori, Nagahama Institute of Bioscience and Technology

The goldfish is a cyprinidae which is domesticated from a closely related species to the crucian carp in South China. Currently, at least 180 variants and 70 genetically established strains are produced and maintained all over the world. These strains possess morphological variations in body shape, coloration, scales, and morphology of fins, eyes, and hoods. Recently, we reported the assembly of the whole genome sequence of goldfish, which provides strong tools for genetic analysis of these phenotypes. In the current study, we performed whole genome re-sequencing and genome wide association study (GWAS) of the goldfish strains. We identified genetic variations establishing the population genetic structure of major goldfish strains. Our

analysis will provide progress for the identification of the proteins and their functional mechanisms responsible for the phenotypic variations in goldfish strains.

Poster #22, Category: Imaging and Technology Development
3D Imaging of Zebrafish Embryos Using the VAST BioImager
Yongwoon Kim, Union Biometrica, Inc.

3D tomographic visualizations have become a powerful approach in medical and scientific imaging. The mechanical stability of the microscope and the embryo is essential when acquiring tomographic projection datasets. We developed software and method to make 3D reconstructions of live and fixed zebrafish embryos using the VAST (Vertebrate Automated Screening Tool) BioImager, a modular, expandable platform for high throughput imaging and sorting of zebrafish larvae 2-7dpf. The system reliably and reproducibly detects, orients, and rotates the larvae to a user-defined field of view, eliminating manual manipulation. Identifying the center of rotation (COR) of the specimen is key for contrast volumetric reconstructions. We tested the efficiency of different approaches for COR detection.

The software was tested to perform tomographic reconstructions from different tissues. Here we will report volumetric reconstructions of craniofacial features, heart, and tumors and neuromast in developing zebrafish. Typical reconstructions were collected and processed in less than 5 minutes. 3D tomographic software allows to acquire and analyze morphological features at key stages of zebrafish embryonic development. In conjunction with the VAST system's high throughput positioning and orientation of zebrafish larvae, large numbers of 3D reconstructions of organs to whole fish are easily collected, enabling additional insight into morphology, structure, and phenotype.

Poster #23, Category: Organogenesis
Interlepidotrichial Joints for Locomotion
Tohru Yano, The Jikei University School of Medicine

Diarthroses (synovial joints) and synarthroses are anatomical terms for describing types of joints. In Teleostei, the fin lepidotrichia are segmented by many fibrous joints (synarthroses), though interphalangeal joints of tetrapod limbs are lubricated (diarthroses). To understand the morphological and functional features of locomotive organs, we have focused on the structure of fin-ray joints in the zebrafish fin.

We have used (1) Gal4/UAS gene-trap transgenesis to label joint-forming cells and (2) serial block-face electron microscopic imaging to reconstruct all of the cell/ECM morphology within a joint region. We have found that the fin-ray joint was paved with joint-forming cells, which covered collagenous ligaments and expressed a synovial fluid gene near a joint cavity. Our definition, the interlepidotrichial joint as a type of synovial articulation, may give a new direction to developmental, evolutional and medical studies.

Poster #24, Category: Other
Multi-well plate for the screening using small fish
Tomonori Deguchi, National Institute of Advanced Industrial Science and Technology

The drug discovery screening using zebrafish is being promoted in Europe and the United States to meet the demand for screening at an individual level with low cost and high throughput.

However, in order to carry out high-throughput screening using animals, the process of drug treatment and the construction of an evaluation system are difficult. Especially, arranging the animals in a multi-well plate in a uniform direction is a major obstacle. For this reason, several types of devices have been developed so far that can arrange zebrafish in the same direction, but the maximum number is 96 samples, and the alignment rate is not sufficiently high.

Therefore, we developed a 96-384 multi-well plate that can arrange medaka fry in a uniform direction in the well plate. As a result of making and improving prototypes many times using a 3D printer, we succeeded in developing a plate that can arrange medaka fry just after hatching in a uniform direction. The name of the present invention is “multi-well plate for model organisms (Japanese Patent Application No. 2018-172546)” and can be used not only for medaka but also for zebrafish as well as other model organisms.

Poster #25, Category: Other
Study on the effects of Natural Product Using Zebrafish and the Colloidal Stability of Emulsions for Cosmetic Formulation Applications
Boae Kim, Mokwon University

The antioxidant effect of Arthrospira platensis extract on UVB-induced ROS production were measured by DCFH-DA assay in zebrafish and HaCaT cells. To evaluate that spirulina extract can be used as a raw material for cosmetics, emulsion was made and safety evaluation experiments such as pH and viscosity measurement and particle observation were performed. As a result, zebrafishes showed LD50 values ??on UVB 50 mJ / cm2 irradiation. In spirulina treated group, ROS decreased in a concentrations dependent manner compared to the positive control group. In the emulsion preparation experiment, it was evaluated that as the concentration increased, the stability of the colloidal particles was affected. The results of this study suggest that extracts from Arthrospira platensis have industrial value as cosmetics for skin protection.

Poster #26, Category: Physiology & Metabolism
Taurine Participates in the Brain Ventricle Inflation via Volume-regulated Anion and Organic Osmolyte Channel during Zebrafish Embryogenesis
I-Hsuan Liu, National Taiwan University

The sodium osmotic gradient is necessary for the initiation of brain ventricle inflation, but a previous study predicted that organic and inorganic osmolytes play equivalently important roles in osmotic homeostasis in astrocytes. To test whether organic osmoregulation also plays a role in brain ventricle inflation, the core component for volume-regulated anion and organic

osmolyte channel (VRAC/VSOAC), lrrc8a, was investigated in zebrafish model. RT-PCR and whole-mount in situ hybridization indicated that both genes were ubiquitously expressed through 12 hpf, and around the ventricular layer of neural tubes and the cardiogenic region at 24 hpf. Knocking down either one lrrc8a paralog with morpholino oligos (MOs) resulted in abnormalities in circulation at 32 hpf. MOs or CRISPR interference against either paralog led to smaller brain ventricles at 24 hpf. Either lrrc8aa or lrrc8ab mRNA rescued the phenotypic penetrance in both lrrc8aa and lrrc8ab morphants. Supplementation of taurine in the E3 medium and overexpression csad mRNA also rescued lrrc8aa and lrrc8ab morphants. Our results indicated that the two zebrafish lrrc8a paralogs are maternal message genes and were ubiquitously expressed in early embryos. The two genes play redundant roles in the expansion of brain ventricles and the circulatory system and taurine contribute to the brain ventricle expansion via VSOAC.

Poster #27, Category: Physiology & Metabolism
Arginine Vasopressin Regulates Proliferation and Differentiation of Ionocytes
Ming-Yi Chou, Department of Life Science, National Taiwan University

Arginine vasopressin (AVP) is a conserved pleiotropic hormone that is known to regulate both water reabsorption and ion balance; however, many of the mechanisms underlying its effects remain unclear. Here, we used zebrafish embryos to investigate how AVP modulates ion and acid-base homeostasis. After incubating embryos in double-deionized water for 24 h, avp mRNA expression levels were significantly upregulated. Knockdown of AVP protein expression by an antisense morpholino oligonucleotide (MO) reduced the expression of ionocyte-related genes and downregulated whole-body Cl- content and H+ secretion, while Na+ and Ca2+ levels were not affected. Correspondingly, avp morphants showed lower sodium chloride cotransporter (NCC) and H+-ATPase rich (HR) cell numbers, but Na+/K+-ATPase rich (NaR) cell number remained unchanged. avp MO also downregulated the numbers of foxi3a- and p63-expressing cells, indicating its involvement in the differentiation and proliferation of ionocyte progenitors. Finally, the mRNA expression levels of CGRP and its receptor, CRLR1, were downregulated in avp morphants, suggesting that AVP might act through CGRP-CRLR1 signaling. Together, our results reveal a molecular/cellular pathway through which AVP regulates ion and acid-base balance, providing new insights into its function.

Poster #28, Category: Reproduction and Endocrinology
The Role of Notch Signaling in Parallel Development of Kidney and Interrenal Gland
Yi-Wen Liu, Tunghai University

The interrenal tissue in teleosts, a functional equivalent of the mammalian adrenal cortex, is the last differentiated segment of the pronephric nephron. The transcription factor network governing mammalian renal and adrenal development is largely conserved in the teleostean kidney and interrenal organogenesis. We have investigated how global and Jagged-mediated Notch pathways regulate the early organ formation of kidney and steroidogenic interrenal tissue in zebrafish. Broad inhibition of Notch signaling leads to enlarged interrenal tissue, with

the segregation between wt1- and ff1b-expressing cells being disrupted. Stage-specific activation of Notch pathway leads to inhibited interrenal steroidogenesis and yet upregulated ff1b expression. Notch ligands Jag1b and Jag2b are required for the segregation between wt1- and ff1b-expressing cells. While Wt1 is known to repress steroidogenesis, we have shown a role of Jagged-Notch for limiting wt1 expression within the steroidogenic tissue. Our study indicates the importance of Notch activity for promoting the primordial nature of interrenal cell population, and a deficiency of Notch signaling leads to premature steroidogenesis and a depletion of primordial cells. Interestingly, a knockdown of canonical Wnt corepressor LRP4 led to a kidney/interrenal phenotype resembling those in Jagged-deficient embryos, implicating a crosstalk between canonical Wnt and Notch pathways.

Poster #30, Category: Stem Cells and Regeneration
Syndecan-4 is Involved in Extracellular Matrix Remodeling during Zebrafish Heart Regeneration
Yung-Jen Chuang, National Tsing Hua University

The molecular mechanisms that regulate the halt and reversal of fibrosis during zebrafish heart regeneration are not completely deciphered yet. Previous studies have revealed that syndecan-4 (SDC4) plays a crucial role in regulating cardiac fibroblasts and inflammation following injury in higher vertebrates. Interestingly, our zebrafish heart regeneration microarray data indicated Sdc4 may associate with these processes. To investigate the role of Sdc4 in resolving scar formation and retention during zebrafish heart regeneration, we induced heart-specific Sdc4-knockdown via the siRNA approach. As expected, decreased sdc4 expression corresponded well to zebrafish heart scar retention by AFOG staining, in which collagen deposition was inhibited. Moreover, we found mmp9 and mmp2 were also up-regulated, which supports Sdc4's role in ECM remodeling. We then measured the electrocardiogram in post-heart injury zebrafish with an integrated video recording of ex vivo heart. After Sdc4 knockdown, we observed the reduced reparative capacity and abnormal ventricular contraction around the cryoinjury lesion. Electrocardiogram further showed a sustained ST-elevation in the sdc4 knockdown group, which is the marker of myocardial infarction in humans. Together, our data suggest Sdc4 could be a critical regulator of extracellular matrix remodeling during zebrafish heart regeneration.

Poster #31, Category: Stem Cells and Regeneration
Comparative Analysis of the Optic Tectum Regeneration using Zebrafish and Medaka
Yuki Shimizu, National Institute of Advanced Science and Technology

Unlike humans and other mammals, adult zebrafish have the superior capability to recover from central nervous system injury. We are focusing on adult neurogenesis and regeneration especially in optic tectum. Previously, we established a stab injury of the optic tectum and found that after the stab injury, proliferation of radial glia (RG), possessing features of neural stem cell, was induced and newborn neurons were generated from RG. Moreover, we analyze functions of Wnt, Notch, Shh signaling in proliferation and differentiation of RG after stab injury, and these molecular pathways are important for brain regeneration (Shimizu et al.,

2018, Glia and Ueda et al., 2018, J Comp. Neurol). However, mimicking molecular changes following brain injury is not enough to induce enough RG proliferation and differentiation in intact optic tectum. So recently, we performed RNA-seq analyses after stab injury and analyzed gene expression changes and also compared transcriptomic changes between brain injury model of zebrafish and mouse. We also established the same brain injury model using adult medaka to compare the regenerative capacity because of different capacity of heart regeneration in zebrafish and medaka. We observed scar like GFAP-positive glial scar in medaka optic tectum, suggesting different regenerative capacity in optic tectum.

Poster #32, Category: Toxicology
Effects of Silver Nanoparticles on Innate Immunity in Zebrafish and the Protective Effect of Pterostilbene
Ying-Jan Wang, National Taiwan University College of Medicine

Silver nanoparticles (AgNPs) pose a potential risk to ecosystems and the living organisms due to widespread use in various field, and subsequent gradual releasing into the environment. There are few studies investigating AgNPs toxicity on immunological function and the toxicity effects are not clear yet. Therefore, the purpose of this study was to investigate the toxic effects of AgNPs on innate immunity using the zebrafish embryo model and to investigate whether natural compound pterostilbene could provide protection against AgNPs-induced immune toxicity. The result indicated that the exposure of AgNPs induced developmental toxicity and death in Zebrafish. In addition, AgNPs also affect the innate immunity that the number and function of neutrophils and macrophages were affected. After embryos hatching on the third day, the immune cells were increased in AgNPs-treatment groups but decreased on the fifth day. In addition, the reverse migration ability of the immune cells was decreased in AgNPs-treatment groups. Moreover, the expression of immune-related cytokines and chemokines were also affected. The application of Pterostilbene could activate immune cells and promote the accumulation of immune cells to the injured area thereby reduce the damage caused by AgNPs. In conclusion, the treatment of AgNPs may affect the regulation of the immune system and the function of immune cells.

Poster #33, Category: Toxicology
Embryotoxicity of Sodium Hypochlorite in Zebrafish
Kyoung Ho Oh, Korea University Ansan Hospital

Object: Sodium hypochlorite(NaOCl) has wide used ranging from textile cleaning to water purification. It is potentially dangerous chemical due to its strong oxidizing properties, but it is known to be safe to use at an appropriate concentration. However, the embryotoxicity of this material has not been studied. In the current study, we examined sodium hypochlorite-induced damage of hair cells and embryotoxicity during zebrafish development. Methods: 0.1ppm, 0.625ppm, 1.25ppm concentration of sodium hypochlorite (n=each 55) The drug was continuously treated from the 4th hour of generation, and the solution was changed to a new one time a day, and morphology and survival were confirmed. On the 4th day of the

experiment, we observed neuronal toxicity and hatching rate. On the 5th day after birth, we observed heart edema, lens diameter, body length. Results: The survival rate was significantly decreased according to the concentration. ($p < 0.0001$) Hatching rate showed significant differences in 0.625ppm and 1.25 ppm compared with control group. Body length was significantly reduced according to the concentration of sodium hypochlorite. ($p<0.001$) Heart edema was not found at the concentration of 0.1ppm respectively. However, 9 out of 37 (24.32%) at 0.625ppm and 11 out of 25 (44%) at 1.25ppm caused heart edema. Lens diameter was significantly decreased in the concentration of 0.625ppm and 1.25ppm ($p < 0.0001$) Heart rate of zebrafish embryos was significantly decreased in 1.25ppm group. ($p < 0.05$) Jaw cartilage was stained with alcian blue solution. When three parameters were analyzed through the ventral image, the length at 1.25 ppm group compared to control was significantly decreased. In evaluation of neuronal toxicity, there was not TUNEL signal. The number of hair cells and thyroid follicle significantly decreased with concentration. ($p<0.001$) Conclusion: The current results suggest that sodium hypochlorite induces dose-dependent embryotoxicity in some organs, included hair cell and thyroid gland in embryos.

Poster #34, Category: Toxicology
Insecticide Imidacloprid Influences the Length and Heart Hypertrophy in the Zebrafish Embryos
Li-Wen Chen, National Taichung University of Taiwan

Neonicotinoids have become the most widely used class of insecticides world-wide. Although numerous studies have documented neonicotinoid toxicity in bees and other insects, the effects of neonicotinoid exposure in aquatic life remain largely unexplored. Thus, we assessed the effects of imidacloprid exposure in the embryo and lava stage of the zebrafish. In this study, the zebrafish embryos were treated with imidacloprid at 0 haf, and kept in the primary concentration till 120 haf. It was found that the body length of the lava treated with 500 ppm were shorter than others. It also found that 500 ppm treatment would induced heart hypertrophy and slower heart rate. Moreover, the movement and freezing behavior were not similar as the other groups. Thus, we suggested that neonicotinoid imidacloprid significantly influences the body length of the zebrafish development and may affect the heart hypertrophy and behavior in the zebrafish lava stage.

Poster #35, Category: Toxicology
Insecticide Paraquat Influences the Development Rate and Growth in the Zebrafish
Li-Wen Chen, National Taichung University of Taiwan

Paraquat is a contact pesticide. It will remain in the water for 80%, and accumulated. Moreover, Affecting the development and growth of aquatic organisms. Therefore, the purpose of this study was to investigate the effects of paraquat on zebrafish embryos and juveniles in the environment. The zebrafish were divided to 6 groups and treated with different concentration of paraquat: 0 ppm, 1.5 ppm, 3 ppm, 5 ppm, 7 ppm, and 10 ppm. It was recorded every 6-12 hours till 120 hours. It was showed that the baby fish LD50 was lower than

adult fish. embryo death in the blastocyst stage will be caused by 7ppm or higher dosage. The hatching rate is about 12.5% ??in/below 5ppm, and the survival rate is 2.5%. At the same time, paraquat may inhibited zebrafish embryos use the yolk nutrient. According to the results, we believe that paraquat inhibits the embryo from obtaining nutrients from the yolk and affects the growth of zebrafish embryos. Therefore, it is speculated that Paraquat will reduce hatching and survival rate during the embryonic period of zebrafish.

Poster #36, Category: Early Development

Zebrafish Klf4 Maintains the Ionocyte Progenitor Population by Regulating Epidermal Stem Cell Proliferation and Lateral Inhibition

Sheng-Ping Hwang, Academia Sinica

In the skin and gill epidermis of fish, ionocytes develop alongside keratinocytes and maintain body fluid ionic homeostasis that is essential for adaptation to environmental fluctuations. Ionocyte progenitors in zebrafish embryos are specified from p63+ epidermal stem cells through DeltaC (Dlc)-Notch-mediated lateral inhibition. However, mechanisms by which the ionocyte progenitor population is modulated remain unclear. Here, we report novel roles for zebrafish Klf4 in the ventral ectoderm during embryonic skin development. We found that Klf4 was expressed in p63+ epidermal stem cells of the ventral ectoderm from 90% epiboly onward. Knockdown or knockout of klf4 expression reduced the proliferation rate of p63+ stem cells, resulting in decreased numbers of p63+ stem cells, dlc-p63+ keratinocyte progenitors and dlc+ p63+ ionocyte progenitor cells. These reductions subsequently led to diminished keratinocyte and ionocyte densities and resulted from upregulation of the well-known cell cycle regulators, p53 and cdkn1a/p21. Moreover, mutation analyses of the KLF motif in the dlc promoter, combined with VP16-klf4 or engrailed-klf4 mRNA overexpression analyses, showed that Klf4 can bind the dlc promoter and modulate lateral inhibition by directly repressing dlc expression. This idea was further supported by observing the lateral inhibition outcomes in klf4-overexpressing or knockdown embryos.