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Thursday February 4st

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Welcome and introduction

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Characterising ontogeny of numerosity discrimination in zebrafish
Eva Sheardown - Queen Mary University of London, UK

15.25 - 15.40
Adrenergic activation modulates the signal from the Reissner fiber to cerebrospinal fluid-contacting neurons during development
Yasmine Cantaut-Belarif - Paris Brain Institute, Sorbonne Université, Paris, France

15.40 - 15.55
Histomolecular characterization of zebrafish models for POLG-related disorders
Raquel Brañas Casas - Department of Biology, University of Padua, Italy

15.55 - 16.10
Arrhythmogenic cardiomyopathy zebrafish models: physical effort implication in pathogenic events and new therapeutic targets identification
Giovanni Risato - Department of Cardiac-Thoracic-Vascular Sciences and Public Health, University of Padua, Italy

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GR and metabolism: analysis of GR function in the regulation of mitochondrial activity
Annachiara Tesoriere - Department of Biology, University of Padua, Italy

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Aging of the zebrafish thyroid gland involves macrophage infiltration inside the thyroid follicle lumen
Sumeet Pal Singh - IRIBHM, Université Libre de Bruxelles, Belgium

16.40 - 16.55
Zebrafish patient avatars in precision cancer therapy
Alice Usai - Department of Biology, University of Pisa, Italy

16.55 - 17.10
Zebrafish models in melanoma research: analysis of coding and non-coding BRAFV600E variants
Raffaella De Paolo - IFC-CNR, Pisa, & Oncogenomics Unit, CRL-ISPRO, Pisa, Italy

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Concluding remarks

Thursday February 11th

15.00 - 15.10
Welcome and introduction

15.10 - 15.40
Potential action of phages as immunomodulators in cystic fibrosis
Anna Pistocchi - Department of Medical Biotechnology and Translational Medicine, University of Milan, Italy

15.40 - 16.10
Deficiency in dTTP metabolism causes a lethal vanishing brain disease
J.M. Vanoevelen - Department of Clinical Genetics, Medical Centre, & GROW - School for Oncology and Developmental Biology, Maastricht University, The Netherlands

16.10 - 16.40
Zebrafish (Danio rerio) as a nutraceutical animal model
Roberta Imperatore - Department of Sciences and Technologies, University of Sannio, Italy

16.40 - 17.10
Zebrafish enrichment: a review of the evidence
Chloe Stevens - Animals in Science Department, RSPCA, Horsham, UK

17.10
Concluding remarks

TITLE

Potential action of phages as immunomodulators in cystic fibrosis

AUTHORS

Marco Cafora[1]; Alessia Brix[2]; Francesca Forti[3]; Nicoletta Loberto[2;] Federica Briani[3]; Massimo Aureli[2]; Anna Pistocchi[2].

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Presenting author: Anna Pistocchi, Associate Professor

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Chronic inflammation caused by bacterial infections is a common feature of patients with Cystic Fibrosis (CF). However, new evidence suggest that constitutive inflammation is present in CF patients even in the absence of bacterial infection. Surprisingly, we previously found that bacteriophages, tha natural enemy of bacteria, might also mitigate the hyper-inflammation of the CF zebrafish model not infected by bacteria. We investigated the mechanism through which phages act as anti-inflammatory agents using the CF zebrafish model and primary human bronchial epithelial cells homozygous for the CFTR mutation F508del. In the zebrafish embryos we characterized the immunomodulatory effects of a phage-cocktail used in our preliminary work. We demonstrated that not only the complete phage cocktail but also each single phage component had anti-inflammatory effects on zebrafish embryos. Moreover, we observed that this effect depends on proteinaceous virion component, but not on phage DNA. We further addressed on the possible mechanisms through which phages modulate the inflammation in zebrafish embryos. We observed lack of immunomodulatory effects in Myd88-deficient embryos, indicating the involvement of the TOLL-like receptor pathway. Moreover, local phage cocktail administration in CF embryos altered neutrophils migration toward an inflammation site, by reducing the chemotactic stimuli. Currently, we are studying the action of the phage cocktail on the human CuFi-1 F508del cell line, characterized by a high basal proinflammatory state. As a read-out of the action of phages as immunomodulators, we will assess the expression of pro-and anti-inflammatory markers by means of qPCR techniques.
The research of new anti-inflammatory agents in a cheap and easy-of-use CF zebrafish model, together with the studies on the effects of phages on CF human cells could speed-up the translational potential of this research and the introduction of bacteriophages into clinics.

TITLE

Deficiency in dTTP metabolism causes a lethal vanishing brain disease

AUTHORS

Vanoevelen J.M.[1,2,*]; Bierau J.[1,*]; Kamsteeg E.J.[3]; Wevers R.A.[4]; Bok L.A.[5]; Abdel-Salam G.M.H.[6]; van der Knaap M.[7]; Bugiani M.[8]; O'Driscoll M.[9], van den Wijngaard A.[1]; Brunner H.G.[1]; Wang L.[11]; Stumpel C.T.R.M.[1,2]

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Human nucleotide metabolism is a complex, tightly controlled pathway regulating numerous cellular processes such as nucleic acid synthesis and repair. Here, we describe DTYMK deficiency as the cause of a severe neurodegenerative disease in two unrelated families.
DTYMK encodes the dTMPK (deoxythymidylate monophosphate kinase) enzyme which catalyzes the penultimate step in the biosynthesis of dTTP. We describe two children showing severe postnatal microcephaly and postnatal growth retardation with extensive atrophy of the cerebral cortex. Exome sequencing identified two variants in DTYMK.
No significant dTMPK enzyme activity could be detected in the patients' fibroblasts, indicating a loss-offunction effect of the variants. Additionally, EdU labelling in fibroblasts confirmed a marked proliferation defect. We generated a dtymk loss-of-function allele in zebrafish. Homozygous dtymk mutant zebrafish are not viable beyond 5dpf and show microcephaly, small eyes, developmental delay, cardiac edema and massive edema of the brain. Biochemical analysis of dTMPK activity in mutant zebrafish larvae confirmed that the allele represents a loss-of-function allele leading to undetectable enzyme activity. Furthermore, impairment of proliferation was detected in the brain of mutant zebrafish larvae. as well as increased apoptosis. Further molecular analysis revealed genome instability due to ribonucleotide incorporation and defects in the DNAdamage response repair mechanism. The striking similarities between the human and zebrafish phenotype strongly suggest a causal link between dTMPK deficiency and the neurodegenerative phenotype, observed in both patients.
In summary, by combining genetic and biochemical approaches in a cellular and a zebrafish model we identified loss-of-function in DTYMK as the cause of a severe neurodegenerative disease. These cases highlight the importance of dTTP synthesis in the survival of neurons.

TITLE

Zebrafish (Danio rerio) as a nutraceutical animal model

AUTHORS

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The term nutraceutical combines the words "nutrition" and "pharmaceutical" to define products which appear to belong to both categories, food and drug. Nutraceuticals are a wide group of compounds comprising substances isolated or extracted from animals or plants. Most commonly used are dietary supplements derived from fruits and vegetables showing nutrition and therapeutic potentials. Indeed, nutraceuticals often show anti-oxidant or anti-inflammatory activity and are used as soothening in many health problems, such as cancer, inflammation, hypertension, cardiovascular diseases, neurodegenerative diseases, obesity, and diabetes, in combination with chemical therapeutics. Widely consumed nutraceuticals include flavonoids, flavonols and polyphenols, plant secondary metabolites commonly used by the plant to fight against infections and diseases. Such plant derived compounds or phytochemicals have been used over centuries for their capacity to promote good health and quality of life. Nutraceuticals commonly have positive physiological effects, but different concentrations and duration can cause adverse effects and toxicity. Being nutraceuticals always more popular in our daily lives, it is necessary to evaluate their possible toxic effects. In the last few decades, ethical considerations have limited the use of higher vertebrates for toxicity tests, inducing the necessity to delineate new, relevant and standardized animal models which allow a complete evaluation of the health benefits and modes of action of nutraceuticals. Among these animal models, the zebrafish has emerged as an excellent in vivo animal model for assessing safety and toxicity of nutraceuticals such as polyphenols. Both adults and embryos of the zebrafish can be used as laboratory models in different research fields, such as biomedicine and aquaculture. Notably, zebrafish are apparently able to capture toxic compounds and allow toxicology assessment due to the transparent nature of embryos, low cost, short cycle, higher fecundity, small size and high conservation of physiology and gene function with humans. Numerous studies are trying to establish a relationship between phytochemicals and their role in health benefits for over two decades. The dosage of phytochemicals and the modality of administration are important factors to determine the effects. The consumption of high doses of nutraceuticals can be toxic, while the oral supplementation of nutraceuticals represents the more physiological and effective approach. In this presentation, we discuss the use of zebrafish as an inflammatory model to define the optimal dose and the administration timing of nutraceuticals. The use of a zebrafish model, allows us to define the safety doses and the timing schedule to prevent, maintain or recover the healthy status. Currently, polyphenols represent a starting point in developing new "natural drugs" to fight chronic inflammatory states and numerous diseases (atherosclerosis, cardiovascular disease, neurodegenerative diseases, obesity). Consequently, we can conclude that the definition of a standard animal model to study the safety and toxic effects of dietary supplements like polyphenols is extremely important in both biomedicine and aquaculture fields.

TITLE

Zebrafish enrichment: a review of the evidence

AUTHORS

Chloe Stevens, PhD - Animals in Science Department, RSPCA, Horsham, UK

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The term 'environmental enrichment' refers to modifications made to the housing or environment of captive animals with the aim of improving animal welfare, and may include increased environmental complexity, the provision of physical items like shelter, food rewards, or sensory stimuli. Enrichment is accepted as an essential requirement for meeting the behavioural needs and improving the welfare of many laboratory animal species, but in general, provision for zebrafish is minimal. Some of those involved in the care and use of zebrafish suggest there is a 'lack of evidence' that enrichment has welfare benefits for this species, or cite a belief that zebrafish do not 'need' enrichment. Concerns are also sometimes raised around the practical challenges of providing enrichments in a laboratory setting, or that they may impact on the science being undertaken. However, there is a growing body of evidence suggesting that various forms of enrichment are preferred by zebrafish over a barren tank, and that enrichment or increased environmental complexity can improve zebrafish welfare by reducing stress and anxiety. This talk will review the evidence in the existing literature relating to the effects that enrichment can have on zebrafish behaviour, physiology and welfare, and considers the challenges to facilities of providing more enrichment for the zebrafish they house.

TITLE

Characterising ontogeny of numerosity discrimination in zebrafish

AUTHORS

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Basic numerical abilities have been demonstrated not only in human infants and non-human primates but also other vertebrates including mammals, birds, amphibians, fish, reptiles, and invertebrates. Numerical competence is widespread within the animal kingdom due to the ecological advantages it provides leading to the hypothesis that numerosity is evolutionarily conserved (Tosto et al, 2014). Despite the wide interest and breadth of studies on the evolution of numerosity, very little is known about the ontogeny of numerosity. It is not clear to what extent number sense is innate, develops with visual acuity or is learned, studying the ontogeny can help answer these questions. We study numerical competence in zebrafish, a model species that allows for biomedical and neurotranslational research due to their high level of both physiological and genetic homology to mammals. To date it has been shown that adult zebrafish can be trained between arrays with a different number of 2d figures (Agrillo et al, 2012) and that males are able to spontaneously discriminate between small and large numerosities, depending on the ratio (Potrich et al, 2015). Here we established an ecological group size preference assay test to investigate the ontogeny and the ability of larval zebrafish to discriminate between different numerical contrasts, as well as whether the ability to discriminate numbers is learned or innate. Fish showed group size preference from 26 days post fertilisation with the ability to discriminate 3 v 1 improve up to 30 days of age. Zebrafish reliably chose the larger group when presented with discrimination ratios from 8:1, through to 3: 2. Newborn humans, chicks, guppies and anuran tadpoles can discriminate numbers smaller than 4 from birth. Our findings show number discrimination improves with age in zebrafish and the ratio limit of discrimination matches those found in human infants, chicks, guppies and angelfish with an upper limit of 2:3.

TITLE

Adrenergic activation modulates the signal from the Reissner fiber to cerebrospinal fluid-contacting neurons during development

AUTHORS

Cantaut-Belarif Yasmine; Orts-Del'Immagine Adeline; Penru Margot; Pézeron Guillaume; Wyart Claire; Bardet Pierre-Luc.

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The cerebrospinal fluid (CSF) contains an extracellular thread conserved in vertebrates, the Reissner fiber, which controls body axis morphogenesis in the zebrafish embryo. Yet, the signaling cascade originating from this fiber to ensure body axis straightening is not understood. Here, we explore the functional link between the Reissner fiber and undifferentiated spinal neurons contacting the CSF (CSF-cNs). First, by performing a transcriptome analysis of scospondin mutants devoid of the fiber, we show that the Reissner fiber is required in vivo for the expression of urp2, a neuropeptide expressed in CSF-cNs. Next, using in vivo imaging in the embryonic zebrafish, we show that the Reissner fiber is also required for early calcium transients in these spinal neurons. Finally, using a gain-of-function approach, we study how local adrenergic activation can substitute for the Reissner fiber-signaling pathway to CSF-cNs and rescue body axis morphogenesis. Our results show that the Reissner fiber acts on CSF-cNs and thereby contributes to establish body axis morphogenesis. They suggest it does so by controlling the availability of a chemical signal in the CSF. The dissection of the signaling steps identified here will allow understand better how this fiber bathing in the CSF facilitates a long-range signaling to tune the geometry of the trunk in a developing vertebrate, but also the formation of the spine during the juvenile stage.

Keywords: morphogenesis, cerebrospinal fluid, Reissner fiber

TITLE

Histomolecular characterization of zebrafish models for POLG-related disorders

AUTHORS

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The only polymerase found in mitochondria is γ-polymerase (POLG), which is essential for replication and repair of mitochondrial DNA (mtDNA). In humans, POLG is composed of a catalytic subunit encoded by the POLG nuclear gene and a dimeric accessory subunit encoded by the POLG2 nuclear gene. Most of the mutations in mtDNA are due to errors of POLG-mediated replication mechanism, thus rendering the POLG nuclear gene the main locus for human mitochondrial disease with a mutation frequency higher than 2%. POLG-related disorders are a group of mitochondrial diseases caused by mutations of polymerase gamma associated with a spectrum of clinical presentations, ranging from infantile-onset epilepsies, liver failure, polyneuropathy, ataxia, dilated/hypertrophic cardiomyopathy, male infertility and premature menopause, to late-onset ophthalmoplegia and muscle weakness.

The aim of our study is to characterize Polg mutant lines in zebrafish as models for POLG-related mitochondrial diseases, since the homologous zebrafish enzyme Polg includes the same functional domains present in humans, and the identity found between the two polymerases is 69%, with a similarity of 79%.

In this project, stable zebrafish Polg mutants have been studied: polgsa$^{9574/sa9574,}$ obtained by ENU technology, bearing a hypomorphic point mutation with the loss of the last 6 aminoacids; polgia3$^{02/ia302}$, generated by CRISPR/Cas9 strategy, with a 16-nucleotide deletion in the exon 2 that brings to a premature stop codon; and polg2$^{ia303/ia303,}$ produced by CRISPR/Cas9 method, carrying a 10-nucleotide deletion.

Morphological and functional characterizations detected a set of phenotypes remarkably associated to POLG disorders. Polg mutant lines display different survival rates: polg$^{ia302/ia302}$ and polg2$^{ia303/ia303}$ animals fail to survive up to 4 weeks post fertilization, while polg$^{sa9574/sa9574}$ can reach adulthood, even though homozygous mutants show decreased survival in relation to wild type. All three mutants display a reduced body size at larval stages, and this reduced growth is maintained in polg$^{sa9574/sa9574}$ mutants until adult phase. A mitochondrial-targeted transgene has been used to assess the mitochondrial organization, allowing the fluorescent visualization of these organelles in vivo: an alteration of mitochondrial distribution along the fibers and a decrease of mitochondrial mass has been detected in polg$^{sa9574/sa9574}$; polg2$^{ia303/ia303}$ is currently being monitored. Furthermore, mtDNA content has been quantified by qPCR, showing a significant reduction in polg$^{sa9574/sa9574}$ and polg$^{ia303/a303}$ individuals. Finally, the hypoxia pathway seems to be upregulated in all three mutants, suggesting that this retrograde signalling pattern could represent a common molecular signature of the Polg dysfunction.

All in all, these models are promising systems to analyze Polg dysfunction during early developmental phases and adulthood. Furthermore, all POLG models produced so far show phenotypes that resemble human conditions, indicating that pharmaceutical treatments could be worth to be investigated in these systems to discover efficient drugs that rescue Polg-related defects.

Keywords: zebrafish, POLG, POLG2, mitochondria, mtDNA.

TITLE

Arrhythmogenic cardiomyopathy zebrafish models: physical effort implication in pathogenic events and new therapeutic targets identification

AUTHORS

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Arrhythmogenic cardiomyopathy (AC) is an inherited primary disorder of the heart muscle characterized pathologically by progressive fibro-fatty replacement of the ventricular myocardium and clinically by life-threatening ventricular arrhythmias, palpitations, syncope and increased high risk of sudden cardiac death in young people and athletes. Despite the discovery of causative genes, early molecular events leading to tissue damage and arrhythmias remain elusive. About 50-60% of AC cases carry one or more mutations in genes encoding for proteins of the intercellular junctional complexes known as desmosomes, which ensure the mechanical interaction of myocytes and maintain the structural integrity of the myocardium. The AC form linked to mutations in Desmoplakin (DSP) gene is the most challenging AC type, being more difficult to identify using classical ECG and echocardiographic tools, due to a high prevalence of left dominant forms. The aim of our study is the characterization of stable knock-out (KO) zebrafish AC models to identify in vivo early pathogenic events leading to the onset and progression of the disease due to dsp (zebrafish dspa and dspb genes) dysfunction at rest and under physical effort. The final goal is the assessment of our zebrafish AC models as suitable tools to evaluate the role of the physical exercise in AC and test the efficacy of pathway-directed drugs. Our zebrafish models include a dspa mutant line obtained by ENU mutagenesis, a dspb mutant line generated by CRISPR/Cas9 strategy and dspa/dspb double mutants. All AC models have been characterized at embryonic, larval and adult stages. At developmental phase, our models display a down-regulated expression of both Dsp-encoding mRNA and the protein itself, with the absence of the protein-mutated forms. The morphological analysis of the cardiac region shows alterations in 30% of mutated larvae, very similar to humans, such as changes in the heart morphology, edema or blood effusion. Moreover, heart rate reduction is observed at 2-3 and 7 days post fertilization in the mutant lines, in particular in the double heterozygous lines that show the worst condition. The mortality analysis also points out that the heterozygous double line shows the lowest percentage of survival, around 20%. After an intensive physical exercise protocol, in both the mutated and WT control fish, the number of animal death per day has been counted: the control pool survives longer than the trained one, suggesting that the physical exercise increases mortality and may accelerate the progression of the disease, leading to a worse condition in larvae. Double heterozygous adults display irregular heart morphology and dilated ventricle. Furthermore, histological analysis has been performed, showing contractile structures reduced in number and age-related alterations in the distribution and organization. In conclusion, all our models recapitulate AC features pointing at zebrafish as a suitable system for in vivo screening of molecular-targeted drugs at rest and under stress-induced conditions.

Keywords: arrhythmogenic cardiomyopathy, Desmoplakin, zebrafish, physical effort, therapeutic targets

Oral presentations junior speakers

TITLE

GR AND METABOLISM: ANALYSIS OF GR FUNCTION IN THE REGULATION OF MITOCHONDRIAL ACTIVITY

AUTHORS

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TEXT

The glucocorticoids receptor is a protein involved in a wide variety of biological processes, such as inflammation, stress response, hypoxia and metabolism. In details, after interaction with its ligand, GR translocates in the nucleus, where acts as transcription factor regulating glucocorticoids cell responses. The GR nuclear activity is also modulated by the interaction with other proteins like STAT3, a fundamental transcription factor involved in cell survival, proliferation and migration. It was demonstrated that GR-STAT3 interaction regulates the transcriptional activity of the two transcription factors resulting in both synergistic and antagonistic effects.

More recently the presence of GR in the mitochondria was detected but, while the nuclear GR activities have been widely studied, the GR mitochondrial function is still not completely characterized. At the nuclear level, it is already demonstrated that GR stimulates the expression of some proteins involved in mitochondrial homeostasis and the presence of GR in mitochondria confirms that it regulates multiple processes in the organelle, even if part of these regulatory mechanisms is not clear. So, our aim consists in the identification of nuclear and mitochondrial GR involvement in the regulation of mitochondrial activities.

To better investigate the possible GR roles in the mitochondrial metabolism and homeostasis, we are using zebrafish as a model organism and, in particular the gr knock-out zebrafish line generated with CRISPR-Cas9 technology. This mutant line is characterized by a 5-nucleotide insertion and shows severe defects in behaviour and stress response.

RNA sequencing analysis performed on gr+/+ and gr-/- 6-dpf larvae demonstrated that some genes involved in mitochondrial correct functionality are affected by gr mutation. In particular, nuclear genes that regulate ion transport in mitochondria (slc25a25, slc25a43, ucp2, ucp3) appear to be GR target genes. The downregulation of these genes in gr mutant larvae compared to WT siblings confirms that nuclear GR regulates mitochondrial homeostasis.

On the other hand, the presence of GR in mitochondria suggests also a more direct control of the organelle activity. To better investigate the mitochondrial role of GR, we are currently screening the potential targets of this protein in mitochondrial DNA and we are investigating about other GR potential interactors.

Particularly, we detected a downregulated expression of some mitochondrial genes (mt_nd1, mt_nd2, mt_nd4, co1, co2, co3) in gr knock-out larvae comparing to WT. These data suggest that GR regulates mitochondrial genes expression through unknown mechanisms.

Interestingly, STAT3 can also migrate to mitochondria where -basing on our recent data - induces the expression of mitochondrial genes. Contrary to the wild type condition, this increase was not detected in gr KO larvae, suggesting that GR and STAT3 can functionally interact also in mitochondrion. The possible GR-STAT3 connection seems to be confirmed by the fact that the chemical inhibition of JAK/STAT3 pathway with AG490 reduced significantly mitochondrial gene expression in WT larvae, but no further reduction was detected in gr mutant after AG490 treatment.

Finally, we are planning to perform mitochondrial Gr overexpression experiments to better understand the role of this receptor in mitochondria and to describe its possible connections with STAT3.

TITLE

Aging of the zebrafish thyroid gland involves macrophage infiltration inside the thyroid follicle lumen

AUTHORS

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Thyroid gland is responsible for generation, storage and secretion of thyroid hormones and is a master regulator of growth and metabolism. Thyroid hormone is produced by thyroid follicular cells (TFCs), the functional unit of the gland, and stored as a colloid in the follicular lumen. Using single-cell RNA-Seq. of the adult thyroid gland from zebrafish, our previous work has documented the presence of macrophages in the organ. However, the lack of spatial information in single-cell profiling restricts our understanding of the interaction between macrophages and thyroid follicular cells (TFCs). Using transgenic lines labeling macrophages and TFCs with complementary fluorescence, we document physical association between the two cell types in adult organ. Using in vivo live imaging at larval stages, a technical advantage provided by the zebrafish model, we have characterized the dynamic nature of the interaction, which includes phagocytosis of thyrocytes by macrophages. In adult zebrafish, we have documented presence of macrophages in the interfollicular regions, an observation that matches well with the organization of macrophages in mammalian thyroid gland.
Intriguingly, with age, we observe an infiltration of macrophages inside the lumen of the follicle. The follicles with macrophages accumulated inside the follicle might represent damaged, dysfunctional follicles. Infiltration could be triggered by TFCs death. To understand the cellular and molecular mechanisms underlying macrophage infiltration, we are utilizing genetic ablation and premature aging models, with the hypothesis that disruption of cellular polarity leads to thyroid dysfunction, death and macrophage attraction. Together, our work elucidates how damage to thyroid follicle, an epithelial structure, leads to immune-attraction; a mechanism that could be conserved during aging, failure and cancer in other epithelial structures.
Keywords: Aging, Macrophage, Dysfunction, Thyroid, Cell Death

TITLE

Zebrafish patient avatars in precision cancer therapy

AUTHORS

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TEXT

Cancer is a major public health problem worldwide. Despite great advances in precision medicine, there is still a lack of methods to personalize the cancer therapy. Animal avatar has emerged as a powerful method to predict individual patient response. They consist in cancer cell Patient-Derived Xenograft (PDX) in animal model and they are used to run co-clinical trial, as an alternative to the traditional cancer cell culture method.
In a previous work, we developed an experimental approach to create zebrafish avatar consisting in the xenotransplantation of pieces of patient tumor tissue after surgery and histopathology screening. The xenografts were performed into the perivitelline space of zebrafish embryos 2 days post fertilization, allowing preserving the tumor microenvironment. Experimental data confirm that the cells of the tissue implanted could engraft in the host, survive, spread and migrate, maintaining the primary tumor phenotypic traits based on histology [1].
In this work the zebrafish avatar was validated in a co-clinical trial (XenoZ, NCT03668418) in which zebrafish embryos received fresh tissue fragments of patients with hepato-biliary-pancreatic cancer or gastro-intestinal cancer. In the XenoZ study, patients were treated with standard chemotherapeutic regimens as determined by the oncologist, while the avatars were treated with different traditional chemotherapeutic regimens to test chemosensitivity.
We adopted a linear mixed effect model to analyze the effects of the treatments on tumors xenografted and to find a PDX stratification with respect to the treatments.
The model showed that for some PDX, the treatments could reduce the size of the tumor xenograft, hindering the tumor cell growth compared to the absence of chemotherapy.
In the case of pancreatic cancer, the model allows to discriminate between PDX that benefits receiving chemotherapy (responders) and those who does not (non-responders); and interestingly, comparing the PDX chemosensitivity with the matching patients, we could anticipate relapse/no relapse within one year after surgery in six out of seven pancreatic cancer patients enrolled.
On the contrary in colorectal PDX was evident a PDX specific response to different treatment group, let us to clusterize the ones that could be benefit from a specific treatment.
In conclusion our experimental data have shown good agreement with observations registered in the common clinical practice, and even though with few cases we have demonstrated that zebrafish avatars have great value in predicting the outcome of therapy for individual patients. Nowadays is clear that patients respond differently to treatments, for this reason in the future animal avatar could improved the personalized medicine, helping the oncologists to make the

best prescription for each patient.

[1] Usai, A., et al., A Model of a Zebrafish Avatar for Co-Clinical Trials. Cancers (Basel), 2020. 12(3).

Keywords: patient-derived xenograft, chemosensitivity, equivalent dose, translational research

TITLE

Zebrafish models in melanoma research: analysis of coding and non-coding BRAFV600E variants

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Malignant melanoma is one of the most aggressive types of cancer.While early-stage melanoma can be cured by surgical excision, late-stage melanoma remains a highly lethal disease. Current therapeutic strategies, which include single agents or combined therapies, are in fact hampered by low response rates and by the development of diverse resistance mechanisms.
The most common mutation associated with melanoma is the V600E substitution of BRAF oncogene, which makes it constitutively active as protein kinase. Many BRAFV600E inhibitors (BRAFi) have been recently developed and are very effective at first, but they can be used for just a short period of time (4-6 months), due to quick development of acquired resistance.
With the final aim to identify new molecular factors involved in BRAFV600E-driven malignant transformation and in response to BRAFi, we are developing and characterizing new melanoma models in zebrafish.
Using Tol2 transposon system, we generated new melanoma-prone transgenic lines in which melanomas are driven by BRAFV600E in its reference or X1 isoforms (BRAFV600E-ref and BRAFV600E-X1), the latter recently identified and characterized in our laboratory.These lines, which express either BRAFV600E coding sequence only or BRAFV600E coding sequence plus 3'UTR, show altered pigmentation patterns and nevi, from which tumors originate.They will be used to analyze similarities and differences between the two isoforms, in terms of tumorigenicity, coding-dependent and coding-independent activities, as well as drug sensitivity in adult fish.
In the melanoma-prone BRAFV600E/p53null genetic background, melanoma tumors that form in adult zebrafish show a re-activation of the neural crest signature that is present in progenitors during early stages of embryonic development. In particular, they uniformly re-acquire the expression of the neural crest marker Crestin. Therefore, we intend to use Tg(mitfa:BRAFV600E);p53-/- embryos and take a decrease in Crestin levels within 72hpf as read-out of the activity of anti-melanoma drugs. Accordingly, we preliminarily confirmed higher expression of Crestin in embryos of the BRAFV600E transgenic line compared to the wt line. Moreover, we observed a reduced expression of this marker by treating BRAFV600E embryos with anticancer drugs, including BRAFi. Currently, we are working on the creation of a new zebrafish transgenic line that expresses luciferase reporter gene under the control of Crestin promoter. Crossed with the melanoma prone lines mentioned above, this Crestin line can be used as a platform for quantitative high-throughput screening of new BRAFi-centered pharmacological combinations in zebrafish embryos.

Keywords: melanoma, skin pigmentation, BRAFV600E-ref, BRAFV600E-X1, Crestin