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CONFERENCE ON ADVANCEMENTS IN ORGANOID SCIENCES 2023

ABSTRACTS

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Probing and analyzing the activity of brain organoids with advanced high dense microchip technology

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The development of brain organoids opened a window for tapping into the richness and complexity of circuits observed in the brain in vivo. Since the functional properties of high-dimensional neural circuits are key factors disrupted in many highly prevalent neurological disorders, it is fundamental to probe the activity of brain organoids in therapeutical development. However, dissecting the functional organization of brain organoids is still a challenge. Here we present an approach based on high-density multi-electrode array (HD-MEA) to measure and analyze the electrogenic activity observed in brain organoids, and other biological samples. We show the functional characterization of 5-month-old brain organoids generated from isogenic control N6 line. HD-MEA allows for the simultaneous recording of thousands of electrodes in a high signal-to-noise regime. After, we extracted single spikes and isolated the contributions of individual putative neurons to the signal recorded on each electrode. To exemplify the grasp of our system on drug screenings, we measure the activity of the aforementioned organoids immediately before, and after, a treatment with 4-Aminopyridine (4AP) and Cyclothiazide (CTZ). We observed drastic changes in many functional metrics, as well in the network connectivity during drug modulation. Finally, we validate the HD-MEA as a technology to fully exploit the brain organoids capabilities.

Conflict of interest: Beatriz Furones Cuadrado, Chiara Rosa Battaglia and Gabriela Fioreze are employees of 3Brain AG. The rest of authors in this poster declare no conflict of interest for this study.

Generation of a collection of organoids in the Health Biobank of Aragon

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Introduction

3D culture techniques have contributed to the development of organoids, opening the door to a wide field of applications solving part of the limitations of monolayer cultures. They represent the cellular, structural and functional heterogeneity of tissues in vivo, being very useful in the study of therapeutic targets or in drug screening.

Aims

The Biobank of Aragon (BSSA), in coordination with the Spanish Biomodel Platform, has among its objectives the creation of a collection of organoids representative of different human pathologies, to make them available to the scientific community. Firstly, we decided to create a collection of matched tumor and healthy organoids from different tissues.

Methods

To this end, the following specific methods were established: the generation, maintenance and characterization by Hematoxylin/Eosin staining and RT-PCR of colon, kidney and breast organoids from donor fresh tissue and their storage and subsequent thawing.

Results and Conclusions

This work has made possible to obtain and characterize different organoids from healthy or tumor tissues, which has led to the start of a collection of organoids within the BSSA and their transfer to various projects.

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Role of IL-22/IL-17A co-producing CD4 T cells in Intestinal Inflammation and Tissue Regeneration

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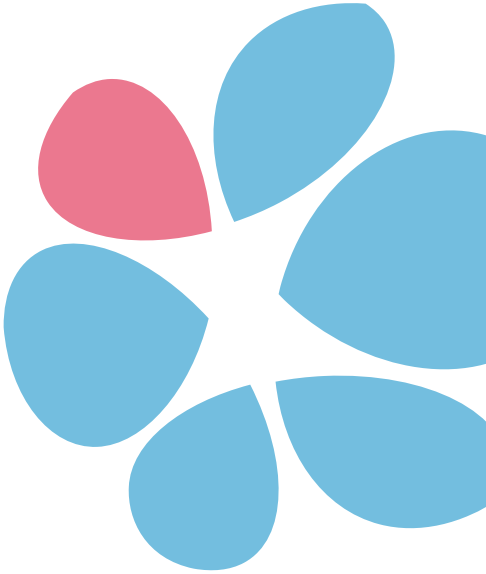
Inflammatory bowel diseases are chronic relapsing inflammatory condition of the gastrointestinal tract. Patients suffering from IBD have an altered gut barrier with impaired mucosal healing properties. The cytokine IL-22 has an important role during tissue regeneration and defense against pathogens. Indeed IL-22 is promoting intestinal proliferation and Paneth cell formation. Accordingly, lack of IL-22 in experimental colitis model results in a more exacerbated phenotype, demonstrating the importance of IL-22 in tissue regeneration. Therefore, IL-22 seems to play a critical in maintaining gut homeostasis and consequentially influencing IBD development. One key source of IL-22 are Th17 cells, a distinct lineage of effector CD4+ T cells characterized by their production of IL-17. In addition, they can also express IL-22 at substantially higher amounts than Th1 or Th2 cells. Of note, IL-22/IL-17A co-producing CD4 Th cells have been shown to contribute to intestinal tumorigenesis. This is in contrast to Th22 cells, which produce IL-22 but not IL-17A and did not impact tumorigenesis. Furthermore, it was shown that some functions of IL-22 are further induced by the presence of IL-17A, for instance, the induction of anti-microbial peptides.

Thus, our study aims to investigate the effect CD4 T cell co-producing IL-17A and IL-22 on tissue regeneration upon inflammatory stimuli. We hypothesized that IL-22 has a different function depending on whether it is produced alone or co-produced with IL-17A, becoming crucial for tissue regeneration. In order to investigate the effect of both cytokines in tissue regeneration, we have generated intestinal organoid from IBD and healthy patients. We have exposed patient-derived intestinal organoids to either IL-22, IL-17A or combination of both cytokines. We have analysed the morphological phenotype of organoids upon stimulation as well as their transcriptome profiles via bulk-RNA sequencing. Microscopic quantifications of the organoids area revealed an increased area in organoids treated with IL-22 compared to the untreated group, confirming what was already shown by others. IL-17A treatment did not promote organoids proliferation compare to untreated group. Surprisingly, organoids treated with both cytokines had a reduced proliferative capacity when they were compared to IL-22 treated organoids, but still more proliferative compare to the untreated group.

This data suggests that IL-17A reduces the proliferative effect of IL-22. Analysis on their transcriptome profiles identified approx. 170 genes that were differentially expressed only in the combination of IL-22 and IL-17A cytokines group compared to the untreated group. Gene ontology analysis identified biological process such as “antigen processing and presentation via MHC II “, “cell chemotaxis”, “positive T cell proliferation” and “immune response” to be upregulated in IL-22/IL-17A stimulated organoids. We validated upregulation of MHC II complex via flow cytometry analysis of HLA-DR,DQ,DP protein expression. Organoids treated with both cytokines showed 4-fold increased expression compared to single-cytokine treatment. Taking together, our data suggest a new role of IL-22 in modulating intestinal epithelial homeostasis and antigen presentation depend on the presence and absence of IL-17A. Thus, we speculate that IL-22/IL-17A co-producing CD4 T cells can orchestrate immune-intestinal epithelial cells crosstalk via regulation of epithelial MHC II molecule. In an inflammatory environment such as in IBD patients, where intestinal barrier is impaired and tissue regeneration is required, understanding the contribution of IL-22/IL-17A co-producing CD4 T cells can shed light on the importance of the source of IL-22 cytokine and their interaction with IL-17A on the intestinal homeostasis and regeneration.



Establishment and characterization of patient-derived organoids from HNSCC for analyzing mechanisms of radioresistance

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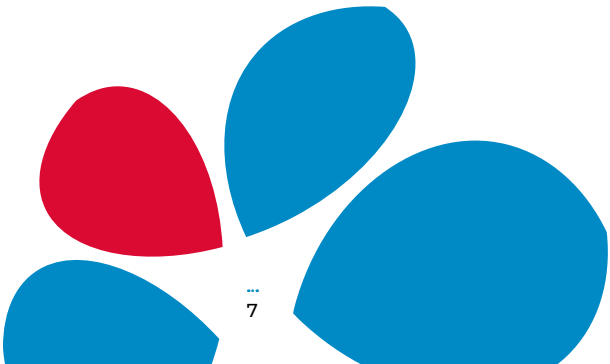
Achieving personalization in treatment of head and neck squamous cell carcinomas (HNSCC) requires adequate models. Biomimetic ex vivo cultures such as organoids increasingly replace simplistic monolayer cell cultures, and are in line with the 3R principle. The study aims at the establishment of patient-derived organoids (PDOs) from HNSCC, and the assessment of their potential value in biomarker discovery for radioresistance and screening for radiosensitizing agents.

The ethics committee of the Charité University Hospital approved this study (EA1/152/10). Tumor tissue from patients with informed consent was collected during diagnostic or curative surgery at the maxillofacial and othorhinolaryngology surgery departments at the Charité. After mechanic and enzymatic dissociation and expansion in monolayer cultures, tumor cells were seeded in Matrigel® to form organoids. Interference of clinical parameters with organoid engraftment was analyzed using SPSS. Formaldehyde-fixed and paraffin-embedded organoid sections were haematoxylin-eosin and immunohistochemically stained for p40, CK5/6 and Ki67. A protocol for ex vivo assessment of radiosensitivity was established using 3D cultures of radioresistant / -sensitive subclones from the HNSCC cell line FaDu. The protocol was then applied to PDO models. Effects of irradiation on organoid volumes, their metabolic activity (Cell Titer Glo®) and clonogenic potential were analyzed.

Overall efficiency of PDO generation from primary tumor specimen (n=170) from HNSCC patients (n=157) was 45% (n= 76). Histopathological characterization confirmed their SCC-phenotype. The majority of models were from male patients (64%) and current or former smokers (71%). Tumors were diagnosed at the oral cavity (n=56), oropharynx (n=14), hypopharynx (n=2), larynx (n=3) or other anatomical sites (n=1). Neither the tumor localization nor the sample type (biopsy vs. surgical specimen) was decisive for successful organoid generation. Samples from recurrent or persistent tumors after radiotherapy showed a significant lower engraftment rate (n=4, 19%) compared to treatment naive specimens (n= 67, 51%) ($p<0.001$). With our ex vivo irradiation protocol, we were able to distinguish radiosensitive from resistant cells in the FaDu model. Preliminary results from PDOs (n=3) showed a dose-dependent decrease in proliferation, cell-viability and clonogenic potential, and a considerable interpatient variability in radiosensitivity.

To our knowledge, this is the largest collection of HNSCC PDO models established so far. Multi-omics characterization (exome, transcriptome and proteome) of the organoids and matching primary tumors is currently ongoing. Our first results suggest that PDOs might be valuable models to investigate individual responses to radiation therapy and the molecular mechanisms underlying radioresistance. In future studies, we will also focus on improving organoid engraftment from radioresistant tumors.



Quality enhancing initiatives in organ model research

Maren Hülsemann, Silke Kniffert, Stefan Hippenstiel, Andreas Hocke, Ulf Tölch, Ulrich Dirnagl

Background and Objectives

Organ models are promising new approaches providing unique opportunities for the study of human diseases and treatment designs. However, proving the reliability and robustness of these novel approaches will be crucial for their broad acceptance and successful application in translational biomedical research. The BIH QUEST Center for Responsible Research is to establish quality standards in organ model research, based on data integrity principles and focusing on scientific value.

Methods

Here, we report on quality measures currently being established at the Einstein Center 3R, a recently founded, Berlin-based consortium of organ model experts.

Additionally, we are leading a large group of model experts conducting a systematic review assessing the contribution of organ model systems to COVID-19 research.

Results

To increase methodological rigor and transparency we are initiating protocol standardization and publication, including the development of minimum reporting standards in organ model research.

The systematic review is focused on the scientific outcome but also elucidates the quality of reporting for 3D cell culture systems in the respective body of literature.

Discussion

Quality measures in an academic research environment have to be pragmatic and science-driven and allow for innovation, ensure data integrity, and an economic use of resources. This approach has to evolve over time and need the continuous contribution and willingness from laboratory-based scientists, as well as a patient but persistent quality management team.

The systematic review will deliver a reliable overview of the content and quality of studies on human organ models in COVID-19 research.

Paneth cells and azathioprine in Crohn's disease: revisiting an old drug

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Crohn's disease (CD) is a chronic inflammatory bowel disease (IBD), characterized by an impairment of ileal Paneth cells' (PCs) function and hence antimicrobial defense mechanisms. While the immunosuppressive drug Azathioprine (AZA) is widely used in CD therapy, the impact of AZA on intestinal epithelial cell (IEC) homeostasis remains elusive.

We aimed to investigate the effect of AZA on PC differentiation utilizing murine small intestinal 2D and 3D cell cultures as well as human patient samples.

AZA-treated CD patients exhibited an improved ileal PC function. Increased differentiation into secretory cells, such as PCs and lowered proliferation were evident in AZA-treated intestinal 2D and 3D-cell cultures. These changes were associated with a metabolic shift from glycolysis to boosted mitochondrial respiration. In vivo, mitochondrial dysfunction was associated with an ileal loss of PC function that was restored by AZA treatment of small intestinal 3D-organoids generated from tissue samples collected from these mice.

AZA has been shown to inhibit proliferation of IEC that is accompanied by boosted mitochondria function and IEC differentiation into PC.
Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Development of a perfusable vascularized in vitro skin model for infection studies with Trypanosoma Brucei

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To investigate infectious diseases such as sleeping sickness in humans, which is caused by the African parasite Trypanosome Brucei there are suitable skin models necessary. The skin is unique with its complex architecture. Due to this complexcity the production of an in vitro skin with hair follicle and skin layers is currently still challenging. Existing simple models lack both complexcity and in addition a perfusable vascularization. Combination of skin organoids with a perfusable vasculaized hydrogel could be used to study interaction of T.Brucei and the skin.

We identified in the hiPSC derived skin organoids several skin-specific cell types such as keratinocytes (CK5), dermal cells (vimentin), adipocytes (nile red) and complex hair-peg formation (indicated by the expression of CK5, CK17). Skin organoids at day 140 were stung by flies infected with Trypanosoma brucei. We saw at the injection site infiltration of Trypanosoma brucei expressing the fluorophore tdTomato into the dermal layer, without infiltration into the epidermal part. After 7 days post infection, the parasites distributed throughout the complete organoid and accumulate in specific areas of the dermal layer without infiltration of epidermal parts. In parallel, the development of a vascularized perfusable hydrogel is realised by using a Scaffold of PcycloPrOx via Melt Electro Writing and a hydrogel composed of GelMA and ColMA in a bioreactor. Vascularization will be realised with iPSC derived vascular organoids. We could identify a vascular network (VE-Cadherin), with endothelial cells (CD31), pericytes (NO-GC, PDGFRß) and smooth muscle cells (SMMHC). This complex skin model is intended to further investigate the mechanism of invasion of the parasite into the human blood stream.

Studies with bat airway organoids of Carollia perspicillata reveal that the respiratory epithelium of bat is not a barrier for interspecies transmission of influenza viruses

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Bats are a natural reservoir for many viruses and therefore are significantly implicated in the interspecies transmission of viral pathogens. To find out whether bat airway cells can be infected by viruses of other mammalian species we developed an organoid culture model derived from the respiratory tract of Carollia perspicillata. The cell composition of organoids resembled that of bat trachea and lungs as determined by immunofluorescence staining. Infection studies revealed that bat airway organoids (AOs) from either the trachea or lung, respectively, are susceptible to infection by two different swine influenza A viruses. The bat AOs were also used to develop an air-liquid-interface (ALI) culture system of filter-grown epithelial cells. Infection of these bat cells by the two swine influenza viruses was as efficient as the infection of porcine ALI cultures. Bat airway cells were found to contain only a low amount of alpha 2,6-linked sialic acids, the preferred receptor determinant for mammalian influenza A viruses. By contrast, alpha 2,3-linked sialic acid, the receptor determinant for avian influenza viruses, is abundantly present on bat cells. Therefore, bat airway cells are expected to be highly susceptible not only to mammalian but also to avian influenza viruses. Our culture models can be extended to other parts of the airways and to other species and thus provide a promising tool to analyze the transmission of viruses both from bats to other species and from other species to bats.

Disclosure statement: The authors declare that they have no competing interests.
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