THE CYPRUS INSTITUTE OF
NEUROLOGY & GENETICS

PhD day

2024

PhD Day: 03.04.2024

Welcome to our annual PhD Day event!

PhD Day is an event dedicated to our Doctoral students, to showcase their work and to gain valuable presenting experience and feedback on their work so far.

This year, we are honoured to have Prof. Nektarios Tavernarakis as our Keynote Speaker, presenting his work on "The Mystery of Ageing and Neurodegeneration: What does modern scientific research teach us?" Prof. Tavernarakis will present his lecture during the morning session, after which our PhD students will present their work to date.

Poster presentations will take place in the area outside the Amphitheatre throughout the day, refer to the schedule in this booklet for a full breakdown of the event.

Please show your support to our guest speaker and to our students by joining us for PhD Day 2024!

NEKTARIOS TAVERNARAKIS

SHORT BIOGRAPHY

Nektarios Tavernarakis is Professor of Molecular Systems Biology at the Medical School of the University of Crete, in Heraklion, Greece. He is also the Chairman of the Board of Directors at the Foundation for Research and Technology-Hellas (FORTH), and Research Director at the Institute of Molecular Biology and Biotechnology (IMBB) of FORTH, where he is heading the Neurogenetics and Ageing laboratory. He is the Founder and first Director of the Graduate Program on BioInformatics at the University of Crete. He is Chairman of the European Institute of Innovation and Technology (EIT) Governing Board, and has served as Vice President of the Scientific Council of the European Research Council (ERC), and Director of IMBB. He is a member of the American Association for the Advancement of Science (AAAS), the European Molecular Biology Organization (EMBO), the German National Academy of Sciences (Leopoldina), Academia Europaea, and the Academy of Athens. He earned his Ph.D. degree at the University of Crete, and trained as a postdoctoral researcher at Rutgers University in New Jersey, USA. His work focuses on the molecular mechanisms of necrotic cell death and neurodegeneration, the interplay between cellular metabolism and ageing, the mechanisms of sensory transduction and integration by the nervous system, and the development of novel genetic tools for biomedical research. He has published numerous scientific papers in top-tier, cross-discipline, international scientific journals, in addition to invited book chapters, and other publications, including editorials, commentaries, and science-popularizing articles. He has received several notable scientific prizes, including two ERC Advanced Investigator Grants, and an innovation-supporting ERC Proof of Concept Grant. He is also the recipient of the EMBO Young Investigator award, the Alexander von Humboldt Foundation, Friedrich Wilhelm Bessel research award, the Helmholtz International Fellow Award, the Galien Scientific Research Award, the BioMedical Research Award of the Academy of Athens, the Bodossaki Foundation Scientific Prize for Medicine and Biology, and the Empeirikeion Foundation Academic Excellence Prize.

PhD Day Schedule - 03.04.2024

9:30 a.m.	Welcome from the Dean **Prof. Kyproula Christodoulou** Welcome from the Provost **Prof. Leonidas Phylactou**
9:45–10:45	Keynote Speaker **Prof. Nektarios Tavernarakis**
10:45–11:00	**C O F F E E B R E A K**
11:00–11:20	SESSION 1: Sereen Abbara Investigation of acid ceramidase depletion in exosome formation/excretion, autophagy, and spliceosomal machinery formation processes
11:20–11:40	SESSION 2: Mikaella Kontopoulou Investigating the global burden of hemoglobinopathies: a data-driven approach
11:40–12:00	SESSION 3: Demos Kynigopoulos Treatment of a murine model of familial Alzheimer's disease with insulin and cholesterol-lowering medications
12:00–13:00	**POSTER PRESENTATIONS**
13:00–14:00	**L U N C H B R E A K**
14:00–14:20	SESSION 4: Anna Pafiti Interactions between complement proteins and clotting factors in multiple sclerosis and their role in pathegenesis
14:20–14:40	SESSION 5: Antri Florentia Romanou Development of comprehensive preimplantation genetic testing for monogenic diseases, the road toward an all-embracing universal application
14:40–15:00	SESSION 6: Giorgos Solomonidis Intrathecal delivery of AAV-HSP70-mediated expression in ALS mice
15:00–15:20	**CLOSING REMARKS**
15:20–16:00	**POSTER SESSION**

SELECTED ABSTRACTS

SA1 **Investigation of acid ceramidase depletion in exosome formation/excretion, autophagy, and spliceosomal machinery formation processes.**
Sereen Abbara

SA2 **Investigating the global burden of hemoglobinopathies: a data-driven approach**
Mikaella Kontopoulou

SA3 **Treatment of a murine model of familial Alzheimer's disease with insulin and cholesterol-lowering medications**
Demos Kynigopoulos

SA4 **Interactions between complement proteins and clotting factors in multiple sclerosis and their role in pathogenesis**
Anna Pafiti

SA5 **Development of comprehensive preimplantation genetic testing for monogenic diseases, the road toward an all-embracing universal application**
Antri Florentia Romanou

SA6 **Intrathecal delivery of AAV-HSP70-mediated expression in ALS mice**
Giorgos Solomonidis

SA1 INVESTIGATION OF ACID CERAMIDASE DEPLETION IN EXOSOME FORMATION/EXCRETION, AUTOPHAGY, AND SPLICEOSOMAL MACHINERY FORMATION PROCESSES

SEREEN ABBARA
Biochemical Genetics Department
Research Advisor/s: Dr. Anna Malekkou

Abstract: Acid ceramidase (AC) is a lysosomal key regulatory enzyme of ceramide metabolism. AC deficiency due to mutations in the ASAH1 gene leads to Farber disease, a fatal lysosomal storage disorder, and to Spinal Muscular Atrophy (SMA) with/without progressive myoclonic epilepsy. SMA is a neuromuscular disorder characterized by progressive proximal muscle weakness and atrophy, primarily caused by the deletion or mutation of the Survival Motor Neuron 1 (SMN1) gene. In our previous work, a stable neuroblastoma SH-SY5Y ASAH1 knockdown (shASAH1) cell line was established and used to evaluate the functional significance of AC depletion. Phenotypic alterations commonly observed in neurodegenerative diseases were detected in shASAH1 cells. In an attempt to identify alterations in the proteome profile resulting from AC depletion, a proteomic analysis was performed. The identified altered proteins in shASAH1 cells were classified according to their biological role and their involvement in particular pathways. In this work, to gain further insights into the role of AC in specific processes, we evaluated the expression levels of multiple components of selected pathways using real-time PCR, western blot analysis and immunofluorescence. AC depletion resulted in significantly elevated mRNA and protein levels of several subunits of the ESCRT-III complex, which drives the formation of intraluminal vesicles inside multivesicular bodies and proteins that actively facilitate the attachment of vesicles to the plasma membrane, destined for exosome excretion. Furthermore, AC depletion resulted in increased expression of p-mTOR and disruption of autophagic flux. Lastly, the mRNA and protein levels of various factors of spliceosome machinery were found upregulated in shASAH1 cells, indicating dysregulation of RNA splicing. These findings suggest an important role for AC in fundamental processes related to normal neuronal function.

SA2 INVESTIGATING THE GLOBAL BURDEN OF HEMOGLOBINOPATHIES: A DATA-DRIVEN APPROACH

MIKAELLA KONTOPOULOU
Molecular Genetics Thalassaemia Department
Research Advisor/s: Dr Petros Kountouris / Dr Coralea Stephanou

Abstract

Hemoglobinopathies are a group of inherited diseases arising from genetic mutations within globin gene clusters, thereby influencing the synthesis and structure of hemoglobin. They encompass sickle-cell disease, thalassemia syndromes, and other rare blood diseases. Historically, hemoglobinopathies have been subjected to positive natural selection in regions experiencing high malaria transmission rates due to conferring protection against this infectious disease. However, human migration and interbreeding have rendered hemoglobinopathies a global health burden.

The primary objective of this study was to assess the global spatial distribution of the health burden caused by hemoglobinopathies. To accomplish this goal, demographic and epidemiological data from registries, digital repositories and published literature were assimilated into a comprehensive, quality appraised and systematically biocurated database that will be incorporated into the publicly available ITHANET community portal. The database encompasses national and regional statistics on the prevalence and incidence rates of hemoglobinopathies, as well as the genetic diversity observed among affected individuals and carriers. Moreover, the database offers insights into the availability and nature of interventions for the management and prevention of hemoglobinopathies.

To demonstrate the practical utility of this database, a Bayesian geostatistical modeling framework was employed. This approach facilitated the generation of continuous, high-resolution maps depicting the predicted frequency of β-thalassemia variation in India. Additionally, it provided estimates of both β-thalassemia patients and affected live births across India.

By leveraging the database and estimation maps, healthcare practitioners and policymakers can gain access to an invaluable tool. This tool can facilitate the evaluation of current prevention and management strategies, enable the monitoring of trends over time, identify regions with a high burden of hemoglobinopathies, and inform data-driven policy decisions on the implementation of targeted interventions in these regions. In summary, the database and estimation maps offer a comprehensive and evidence-based approach to tackle and mitigate the global hemoglobinopathy burden, ultimately aiming to reduce its impact on affected populations.

SA3 TREATMENT OF A MURINE MODEL OF FAMILIAL ALZHEIMER'S DISEASE WITH INSULIN AND CHOLESTEROL-LOWERING MEDICATIONS

DEMOS KYNIGOPOULOS
Neuropathology Department
Research Advisor/s: Dr Elena Panagiotou - Worth

Alzheimer's disease (AD) is a chronic neurodegenerative disorder of the central nervous system (CNS) and the most common cause of dementia. Studies suggest a link between Metabolic Syndrome (MetS) and AD; essentially linking unhealthy nutritional behaviors and associated disorders such as obesity, hypertension, type 2 diabetes mellitus to the onset and progression of dementia including syndromes; such as AD.

To test that hypothesis, we administered two drugs, Diamicron® (Gliclazide) and Praluent® (Alirocumab) involved in the insulin and cholesterol metabolism respectively to mice models of AD (5xFAD).

Post-treatment groups (5xFAD and controls) underwent the non-reward Y-maze behavioral test. Results suggest significant cognitive reduction in the untreated 5xFAD mice when compared to the untreated control group. 5xFAD mice treated with Diamicron or Praluent exhibited increased performance when compared to their untreated 5xFAD counterparts.

Electrophysiological experiments were carried out to assess both synaptic response and Long-Term Potentiation (LTP) in the CA1 area of the hippocampus in brain slices (ex vivo); since the hippocampal function is heavily affected during the progression of AD. The drugs were found to increase synaptic response in control mice but did not affect their LTP values. Whilst, in the 5xFAD mice, both drugs did not appear to alter synaptic response but were instead significantly enhanced LTP.

Brain tissue, visceral fat, and blood serum from all mice used in the study were collected and used in Enzyme-Linked Immunosorbent Assay (ELISA) to measure directly the expression levels of beta-amyloid, a main hallmark of AD, as well as several other markers that commonly represent neurons, astrocytes and macrophages. Cholesterol and triglyceride levels were also measured.

So far, Praluent® seems to be the most promising drug as it was found to slow down hippocampal atrophy while significantly increasing LTP. However, further analysis is required to establish if the drugs are effective against AD.

SA4 INTERACTIONS BETWEEN COMPLEMENT PROTEINS AND CLOTTING FACTORS IN MULTIPLE SCLEROSIS AND THEIR ROLE IN PATHOGENESIS

ANNA PAFITI
Neuroimmunology Department
Research Advisor/s: Dr Nancy Lambrianides

Introduction: Thrombosis is a cardinal feature of many complement-mediated disorders, resolved through complement inhibitors. This connection highlights the bidirectional link between two proteolytic cascades, namely coagulation and complement. Multiple sclerosis (MS) is a chronic immune-mediated inflammatory disease affecting the central nervous system (CNS). During inflammatory disease conditions, coagulation and complement systems shift from their protective roles to a potentially harmful state through various mechanisms. Early-onset MS patients face an elevated risk of thrombosis, indicating the significance of the coagulation system in MS. Furthermore, consistent complement protein depositions have been observed in all demyelinating lesions within the brain of MS patients, signifying an overactivation of this system.

Methods: Using commercially available ELISA and Luminex kits, clotting factors, Factor XI, XII, XII, IX, CRP, thrombin, protein C and S as well as complement proteins C1q, C2, C3, C3a, C3b/C3i, C4, C4b, C5, C5a, SC5b9, C9, mannose-binding, factors D, B, H, and I were quantified in the serum and plasma samples of 46 MS patients and 30 healthy controls (HC).

Results: We evaluated the relationship between each complement protein and clotting factor using Pearson's correlation, showing 70 positive correlations and 21 negative correlations in MS patients. In HCs, 38 positive and 41 negative correlations were found. Notably, the majority of positive correlations in MS were seen between complement proteins to clotting factors, CRP, Factor IX, Protein S and Protein C. Such associations were not prominent in HCs.

Conclusion: Our findings emphasize the evident connection between complement and coagulation cascades. However, as yet no firm conclusions can be drawn regarding their relevance in MS pathogenesis. The existing literature has not thoroughly explored the interactions between all complement proteins and clotting factors. To address this, we have established a protocol for the purification of clotting factors from serum, enabling us to investigate these interactions further in vitro.

SA5 DEVELOPMENT OF COMPREHENSIVE PREIMPLANTATION GENETIC TESTING FOR MONOGENIC DISEASES, THE ROAD TOWARD AN ALL-EMBRACING UNIVERSAL APPLICATION

ANTRI FLORENTIA ROMANOU
Molecular Genetics Thalassaemia Department
Research Advisor/s: Dr Papasavva Thessalia

Background
Preimplantation Genetic Testing (PGT) is a test performed to analyze the DNA from oocytes or embryos for HLA-typing or for determining genetic abnormalities. This study focuses on developing an all-encompassing Preimplantation Genetic Testing for monogenic disorders (PGT-M).

Methods
An empirical analysis of our 19-year PGT-M and Prenatal Diagnosis (PND) data was conducted, revealing a significant increase in the number of cases per year, particularly for couples seeking PGT-M to prevent β-thalassemia. Additionally, three Bespoke PGT-M panels targeting variations in the SPTB, PIEZO1, and BRCA2 genes were designed and developed. The optimization and validation of the PGT methodologies require cell-samples resembling the genomic DNA (gDNA) obtained from an actual embryo biopsy. Manual cell collection, which has been employed since 2004, and used in PGT optimization processes, has proven effective but is time-consuming, painful, and susceptible to contamination. To address this, we focused on optimizing and validating an automated cell collection method using the BD FACSAria III Cell Sorter. Our primary objective is to implement a whole genome amplification (WGA) protocol capable of amplifying genomic DNA (gDNA) from cell-samples. For this purpose, we selected the MDA isothermal amplification approach. The ongoing study presents our examination of the REPLI-g DNA Single Cell Kit (Qiagen Sciences, USA) as a potential tool for WGA prior to PGT-M. We evaluate various metrics of interest, including amplification specificity, uniformity of genome coverage, and the performance of downstream methods in identifying single nucleotide variants (SNVs).

Results
Although still in the early stages, the preliminary results demonstrate the kit's potential for use in PGT-M procedures. The development of tailor-made assays, highlighted the technical challenges, specialized training requirements, and substantial waiting time associated with designing a PGT-M assay.

Conclusion
Our findings underscore the need to upgrade our PGT-M procedures to achieve a comprehensive and universal assay. At the same time, it suggests and evaluates tools that contribute to the upgrade of the established PGT-M protocol.

SA6 INTRATHECAL DELIVERY OF AAV-HSP70-MEDIATED EXPRESSION IN ALS MICE

GIORGOS SOLOMONIDIS
Molecular Virology Department
Research Advisor/s: Dr Jan Richter

Amyotrophic lateral sclerosis (ALS) is a rapidly progressive neurodegenerative disease that primarily affects motor neurons in the central nervous system, the spinal cord and brain. Current literature has highlighted the role of TAR DNA binding protein 43 kDa (TDP-43) in developing various neurological and neuromuscular diseases, including ALS. In the cellular milieu, TDP-43 dysfunction leads to impaired gene expression, RNA instability and aggregation in neurons, with the latter being present in more than 95% of ALS patients. Heat shock proteins (HSPs) or chaperones are molecular machinery fundamentally involved in protein quality control, and many studies have shown the HSP70 importance in modulating TDP-43 behaviour leading to cytoprotective effects also in other neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.

The proposed project seeks to investigate the suitability of the HSP70-isoform HSPA1A, as a pharmacological intervention for in vivo gene therapy in an ALS mouse model exhibiting TDP-43 proteinopathy, specifically the rNLS8 mouse with inducible overexpression of a mutated human TDP-43 protein in neurons.

For this purpose, we utilise recombinant adeno-associated virus 9-mediated expression of HSPA1A under hSyn1, a neuron-specific promoter delivered in a single dose [S(1] directly to the cerebrospinal fluid intrathecally in adult mice. The mutated hTDP-43 temporal overexpression is achieved with the Tet-off system three weeks post-delivery and maintained for six weeks until mice are sacrificed for immunohistochemical and western blot analysis and gene expression changes, while during the life phase, behavioural tests are performed to assess motor system function.

This study explores a novel therapeutic approach to improving the ALS phenotype associated with TDP-43 pathology. Additionally, it aims to contribute to the cellular and molecular underpinnings of TDP-43 proteinopathies.

POSTER PRESENTATIONS

PA1 **Integrating imaging and omics data to unravel signatures of heterogeneity of Mild Cognitive Impairment in Alzheimer's disease**
Sotiroula Afxenti

PA2 **Multi-omics factor analysis to detect subgroups of Parkinson's disease patients with differences in clinical severity and progression**
Efi Athieniti

PA3 **Whole exome sequencing of Breast Cancer cases and controls in the Cypriot population**
Dimitra Christaki

PA4 **Computational Investigation on the Progression of Monoclonal Gammopathies to Multiple Myeloma**
Grigoris Georgiou

PA5 **Exploring the therapeutic potential of current and experimental epigenetic drug compounds in human malignant melanoma**
Sergei Gorbunov

PA6 **Investigation of the polygenic burden of epilepsy in Cyprus**
Aristotelis Karamousoulakis

PA7 **Frequency of intronic FGF14 GAA repeat expansion in Cypriot patients with late-onset cerebellar ataxia**
Ioannis Livanos

PA8 **Investigation of a novel muscle communication pathway**
Christodoulos Messios

PA9 **Identification of extracellular vesicles as biomarkers for abnormal puberty**
Maria Morrou

PA10 **Computational investigation of cell-to-cell communication networks and signalling mechanisms in Multiple Myeloma, inferred by single-cell transcriptomics**
Eleni Nicolaidou

POSTER PRESENTATIONS

PA11 **Identification of novel γ-globin repressors through a custom CRISPR knockout screen and validation of these repressors for the treatment of β-hemoglobinopathies**
Sevgi Özkaramehmet

PA12 **Therapeutic Double Base Editing for Fetal Hemoglobin induction in β-Hemoglobinopathies**
Nikoletta Papaioannou

PA13 **Mapping breakpoints and identifying cryptic chromosomal rearrangements using next-generation cytogenomic tools**
Soteria Polyviou

PA14 **Stbd1 enhances AMPK signalling and improves insulin resistance**
Andria Theodoulou

PA15 **Role of Connexin 47 in the inflammatory and demyelinating response in the cuprizone animal model of multiple sclerosis**
Styliani Theophanous

PA16 **Phytochemicals as Novel Epigenetic Regulators in skin cancer Therapeutics**
Venetia Tragkola

PA1 INTEGRATING IMAGING AND OMICS DATA TO UNRAVEL SIGNATURES OF HETEROGENEITY OF MILD COGNITIVE IMPAIRMENT IN ALZHEIMER'S DISEASE

SOTIROULA AFXENTI

Bioinformatics Department and Neuroimmunology Department
Research Advisor/s: Prof. George Spyrou, Dr. Marios Pantzaris, and Dr. Nancy Lambrianides

Mild Cognitive Impairment (MCI) is a critical stage between the expected ageing-related memory decline and Alzheimer's disease (AD), and is characterized by heterogeneous etiology. Although this heterogeneity is ubiquitous, it is poorly understood and remains a great challenge for the research community. Subtyping of MCI subjects is crucial for implementing precision medicine approaches and facilitating early intervention strategies. Subtyping using multi-modal data may provide a more precise subtyping result. However, only a handful of studies have considered the subtyping of MCI based on integrative, simultaneous multi-modal data.

In this study, we capitalized on multi-modal data, including imaging features and proteomics data from 119 MCI subjects from the Alzheimer's Disease Neuroimaging Initiative (ADNI) database. More specifically, we integrated structural MRI measurements (cortical thickness, cortical and subcortical volumes and surface area measurements), protein expression data, biological markers (CSF amyloid-b, CSF total-tau, CSF phosphorylated - ptau), and clinical assessments (Rey Auditory Verbal Learning Test, Trail Making Test, etc.) for the baseline visit. We used the Similarity Network Fusion (SNF) algorithm to fuse the different modalities and Spectral Clustering based on the fused network to cluster the subjects into two clusters. Clinical differences between the two clusters, including the status of progression to AD, are discussed. Specifically, we focused on identifying individuals who progress to AD within specific timeframes: 6-12 months as early MCI, 18-24 months as middle MCI, and beyond 30 months as late MCI, as well as those who remain stable.

Cluster analysis delineated distinct pathologies, as shown by the molecular and imaging differences among clusters. Additionally, Canonical Correlation Analysis and Partial Least Squares analysis uncovered unique correlations between modalities for each cluster. Finally, within the two identified clusters we compared the progressors with the stable subgroup, to identify molecular and imaging features associated with different progression trajectories of the disease.

Overall, our approach offers insights into personalized management strategies for MCI subjects, with implications for early AD detection and targeted intervention.

PA2 MULTI-OMICS FACTOR ANALYSIS TO DETECT SUBGROUPS OF PARKINSON'S DISEASE PATIENTS WITH DIFFERENCES IN CLINICAL SEVERITY AND PROGRESSION

EFI ATHIENITI

Bioinformatics Department
Research Advisor/s: Prof. George M. Spyrou

Parkinson's Disease (PD) manifests with a spectrum of motor and non-motor symptoms, presenting challenges in assessing disease progression and treatment efficacy. Traditional clinical scores like MDS-UPDRS and MoCa lack the precision required for molecular-level disease monitoring. Adding molecular endpoints is important to accurately assess the success of clinical trials. To address this gap, we utilise multi-omics data from the Parkinson's Progression Marker Initiative (PPMI) dataset to identify molecular markers indicative of PD progression stages.
Integrating omics data from blood mRNA, miRNA, plasma, and CSF proteomics, collected longitudinally over three years after disease diagnosis, we applied Multi-Omics Factor Analysis (MOFA) to identify molecular factors associated with clinical scores. These factors were then utilized in k-means clustering to separate patients into subgroups reflecting different disease stages.
Our analysis revealed distinct molecular signatures associated with disease severity across MDS-UPDRS-II, MDS-UPDRS-III, and MoCa scores. These signatures uncovered potential monitoring biomarkers covarying in CSF, blood, and plasma. Subgroups exhibiting higher disease severity displayed, alterations in peripheral immune cell proportions and activation, including natural killer cells, CD4+ naive cells, and B-cells, suggesting that monitoring these dysregulations could be useful in assessing interventions. Furthermore, differences in CSF proteomics profiles, including previously suggested diagnostic markers, were observed in these subgroups.
Our findings underscore the utility of multi-omics approaches in elucidating PD progression stages and identifying potential biomarkers for disease monitoring and therapeutic efficacy assessment. By bridging the gap between clinical assessment and molecular insights, this study contributes to the advancement of personalized medicine strategies for PD management.

PA3 WHOLE EXOME SEQUENCING OF BREAST CANCER CASES AND CONTROLS IN THE CYPRIOT POPULATION

DIMITRA CHRISTAKI

Biostatistics Unit
Research Advisor/s: Dr Kyriaki Michailidou

Breast cancer (BC), is the most commonly diagnosed cancer. The risk of developing breast cancer increases for an individual that carries a pathogenic variant in the high or moderate established breast cancer risk genes, such as *BRCA1, BRCA2, PALB2, CHEK2* and *ATM*. In Cyprus, there has not been an extensive evaluation of whole-exome sequencing (WES) data in breast cancer cases and controls. In order to evaluate the prevalence of known high and moderate risk genes and the potential identification of novel breast cancer risk genes in the Cypriot population, we used WES data from the *MASTOS* study. The dataset included 736 women, of which 339 were diagnosed with breast cancer and 397 were healthy controls. Logistic regression analysis was performed to evaluate the association of protein truncating variants (PTVs) in respect to breast cancer risk and estimate the effect of association using odds ratios (OR). Statistically significant associations were explored for genes with p value less than 0.005 and OR > 1. Eight genes were associated with nominal significance, *PTER, TAOK3, ADCY4, ZNF223, HADHA, CCDC110, SHRF2* and *TAS2R40*. Moreover, none of the known established genes *BRCA1, BRCA2, CHEK2* and *ATM* indicated statistical significance although their ORs were in the right direction. We did not identify any individuals carrying *PALB2* PTVs and therefore we could not assess its significance in our population. Although we present a relatively large case-control cohort for breast cancer in our population, it is clear that replication is needed in larger cohort before we can conclude on the association of these genes and breast cancer risk.

PA4 COMPUTATIONAL INVESTIGATION ON THE PROGRESSION OF MONOCLONAL GAMMOPATHIES TO MULTIPLE MYELOMA

GRIGORIS GEORGIOU

Bioinformatics Department
Research Advisor/s: Prof. George Spyrou, Dr Anastasios Oulas, Dr George Minadakis, Dr Margarita Zachariou, Dr Marios Tomazou

Multiple Myeloma (MM) is a hematologic malignancy that is characterised by the accumulation of antibody-producing malignant Plasma Cells (PCs) in the Bone Marrow. Those PCs are capable of producing huge amounts of monoclonal protein, the so-called M-protein, that can be measured through blood or urine. Precursor diseases, Monoclonal Gammopathy of Undetermined Significance (MGUS) and Smoldering Myeloma (SMM) are asymptomatic forms of MM. The biology behind the progression of those two stages to active MM is still poorly understood. Different combinations of genetic abnormalities like IgH translocations and gene mutations seem to promote the progression from the asymptomatic to the active form of the disease.
The diagnosis of the disease involves a combination of laboratory tests, imaging and bone marrow biopsies that differ for each disease stage. To identify a patient with MM a combination of myeloma-defining events (MDE) has to be present. To prevent late or false diagnosis, biomarkers can be crucial for identifying the different disease states. In this direction, we proceeded with the analysis of two transcriptomics datasets, where genes with monotonic behaviour regarding their expression across the three stages of the disease were highlighted. Those so-called monotonically expressed genes (MEGs) were then used in a methodology for biomarker discovery. Some of the investigated gene combinations can separate the disease stages from healthy controls, but further analysis with more datasets has to be made to find the best biomarker or combination of biomarkers that could separate both the disease stages from the healthy controls or between the disease stages.

PA5 EXPLORING THE THERAPEUTIC POTENTIAL OF CURRENT AND EXPERIMENTAL EPIGENETIC DRUG COMPOUNDS IN HUMAN MALIGNANT MELANOMA

SERGEI GORBUNOV

Cancer Genetics, Therapeutics & Ultrastructural Pathology Department
Research Advisor/s: Prof. Michail Panagiotidis, Dr. Sotiris Kyriakou, Dr. Ioannis Anestopoulos

Aberrant alterations of epigenetic marks are well-known contributors to the development of cancer. Several epigenetic drugs have been discovered as chemotherapeutic agents in the last few decades, including DNA methyltransferase (DNMT), histone deacetylase (HDAC), and histone methyltransferase (HMT) inhibitors. Particularly, tazemetostat (a HMTi) revealed significant effectiveness in the treatment of some solid tumors (metastatic and locally advanced epithelioid sarcomas) and was approved by the FDA in 2020. The action of tazemetostat is based on selective inhibition of the histone-lysine N-methyltransferase; Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit (EZH2). In vitro and in vivo studies showed that pharmacological inhibition of EZH2 can also suppress tumor growth and metastasis in melanoma. As part of our research, we aimed to investigate some existing as well as newly synthesized EZH2 inhibitors as potential anti-melanoma drugs. We have tested a known potent EZH2 inhibitor; PF-06726304. Also, based on in silico studies, we designed and synthesized two novel compounds with predicted EZH2-inhibitory activity, with the possibility of crossing the blood-brain barrier. The latter is important because it can be utilized against metastatic melanoma, since this type of cancer can metastasize in the brain. These molecules have the same basic structural motif as PF-06726304. The compounds were obtained by the standard procedures of organic synthesis and the chemical structures were confirmed by nuclear magnetic resonance (NMR) and mass spectrometry (MS). The cytotoxic profile of these three compounds has been assessed via Alamar Blue Assay on primary (A375) human malignant melanoma cells and their potency has been compared with Tazemetostat at its EC50 concentration.

PA6 INVESTIGATION OF THE POLYGENIC BURDEN OF EPILEPSY IN CYPRUS

ARISTOTELIS KARAMOUSOULAKIS

Department of Neurophysiology
Biostatistics Unit
Research Advisor/s: Dr. Andreas Koupparis, Dr Ioanna Kousiappa

Purpose: Several common variants have been identified to date that contribute to epilepsy risk through a cumulative effect. This study aims to examine the polygenic burden of epilepsy in the Cypriot Epi25 cohort by examining Single Nucleotide Polymorphisms (SNPs) available from Polygenic Risk Scores (PRS) as based upon the International League Against Epilepsy Consortium on Complex Epilepsies (ILAE) Gene Wide Association Studies (GWASs).

Method: The 2018 and 2022 ILAE GWAS studies identified 20 and 30 SNPs, respectively, associated with epilepsy (46 unique SNPs). A final list of 43 SNPs was available in our post-imputed Cypriot Epi25 data (n = 174 cases, 29 controls). The PRS was categorized based on the median score and Cohen's d was computed to quantify effect size. Receiver Operating Characteristic (ROC) curve analysis evaluated the power of the PRS model and its ability to distinguish between conditions. A t-test was performed to determine the statistical significance of differences observed between cases and controls.

Results: We calculated the PRS using published effect estimates from the 2018 and 2022 ILAE GWAS studies. PRS was able to discriminate between people with epilepsy and controls (Area Under the ROC; AUC=0.73), giving a combined Odds Ratio (OR) of 1,78 and a mean value and standard deviation of 1,73±1,62 for epilepsy^{+} cases and 0,15±1,83 for the control group in a respective range of -2,33 to 6,68 and -4,50 to 3,14. The t-test (T-Statistic: 4.75, P-Value: 3.86x10-06) supports the significant difference between groups.

Conclusion: The PRS model demonstrated a moderate capacity to accurately discriminate between cases and controls with an AUC indicative of a predictive model that performs significantly better than by chance. This suggests that the PRS is an effective tool for risk classification even in small populations, thus highlighting its potential within personalized medicine approaches in the management and prevention of epilepsy.

PA7 FREQUENCY OF INTRONIC FGF14 GAA REPEAT EXPANSION IN CYPRIOT PATIENTS WITH LATE-ONSET CEREBELLAR ATAXIA

IOANNIS LIVANOS

Neurogenetics Department
Research Advisor/s: Prof. Kyproula Christodoulou, Dr. Christina Votsi

Dominantly inherited intronic GAA repeat expansion in the fibroblast growth factor 14 (FGF14) gene has been recently associated with spinocerebellar ataxia type 27B (SCA27B) and is rapidly gaining global recognition as one of the most prevalent forms of ataxia. Currently, the pathogenicity threshold of (GAA)≥300 is disease-causing and fully penetrant, while expansions of (GAA)250-299 are likely pathogenic with reduced penetrance. In this study, we investigated the presence of FGF14 GAA pathogenic repeat expansions in a cohort of Cypriot patients with genetically unsolved late-onset cerebellar ataxia (LOCA). We also determined these repeat allele ranges and their distribution in the patient's cohort of 155 individuals, and 227 non-neurological disease controls. The repeat locus was interrogated using long-range PCR (LR-PCR) followed by fragment analysis using capillary electrophoresis, agarose gel electrophoresis and automated electrophoresis. Bidirectional repeat-primed PCR (RP-PCR) and Sanger sequencing were also performed to confirm that the pathogenic expansions were not interrupted by other motifs. The comparative analysis of methods revealed that accurate size estimation of large alleles presents a significant challenge. Consequently, appropriate adjustments were performed by employing controls that were analysed by long-read sequencing. Of the 155 tested patients, 11 (7%) carried a pure (GAA)≥250 repeat expansion, consistent with a SCA27B diagnosis. The mean age at the first examination of FGF14 GAA-positive patients was 67 ± 15 years. One patient carrying a (GAA)287 repeat expansion was diagnosed with ataxia at the age of 28. Additionally, a repeat expansion was identified in 2 (0.9%) controls. This study highlights the importance of FGF14 GAA repeat analysis in individuals presented with LOCA. Our findings indicate that SCA27B represents the predominant aetiology of autosomal dominant cerebellar ataxia (ADCA) within the Cypriot population, as this is the first dominant repeat expansion ataxia type detected in this population. Furthermore, our data strongly advocate including FGF14 GAA repeat expansion testing as a first-tier genetic diagnostic approach for patients presenting with LOCA.

PA8 INVESTIGATION OF A NOVEL MUSCLE COMMUNICATION PATHWAY

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Background: MicroRNAs (miRNAs) are small non-coding RNA molecules that have a regulatory role in multiple cellular processes. They negatively regulate gene expression through miRNA-mRNA interactions that lead to inhibition of translation. MiRNAs are involved in muscle cell communication, especially during development. Previously reported data suggested that muscles communicate locally by transferring small RNAs. The aim of this study is to investigate for the first time whether muscles communicate with other distant tissues through small RNAs.

Methods: To achieve our aim we designed oligonucleotides and administered them intramuscularly in mice. Antisense oligonucleotides (antagomiRs) targeting a muscle-specific miRNA (miR-133b), a universally expressed miRNA (miR-16) and an antagomiR with no target (scrambled) were used to assess biodistribution and efficacy. Intramuscular and intravenous administration methods were also compared. Moreover, fluorescently labelled miR-133b mimic was designed in order to assess its biodistribution.

Results: Intramuscular injections in the tibialis anterior (TA) muscle of mice showed remarkable systemic delivery, particularly with the antagomiR-133b. Interestingly, the antagomiR-133b showed enhanced biodistribution in various skeletal muscles and heart, compared to antagomiR-16 and scrambled. Moreover, the antagomiR-133b showed enhanced efficacy in distant skeletal muscles and heart compared to the antagomiR-16. Intramuscular administration of antagomiR-133b showed enhanced inhibition of target miRNAs in skeletal muscles compared to intravenous. The miR-133b mimic showed similar biodistribution to distant skeletal muscles.

Conclusion: Our data show that intramuscularly administered antagomiR and mimic oligonucleotides are transported to other muscles, implying distant tissue communication through molecular cargo. This pathway could help us understand more about how muscles develop and function and might prove useful in the delivery of therapeutics for muscle diseases.

PA9 IDENTIFICATION OF EXTRACELLULAR VESICLES AS BIOMARKERS FOR ABNORMAL PUBERTY

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Background: Puberty is defined as the transition from childhood to sexual maturation. Puberty onset is marked by the hypothalamic pulsatile release of GnRH, which stimulates the release of gonadotropins in the pituitary and leads to the production of sex hormones. The pubertal timing is highly regulated by a complex network of hormonal signals with excitatory or inhibitory properties. Influenced by genetic, epigenetic and environmental factors, pubertal timing varies while it can appear as pathological when exceeds the normally expected period. Precocious puberty appears in <8 years in girls and in <9 years in boys often with unknown aetiology, thus it is of paramount importance to reveal the underlying molecular mechanisms. The discovery of extracellular vesicles (EVs) as cell and tissue communication molecules, through proteins and small noncoding regulatory RNAs (sncRNAs), holds promise in unravelling the mechanisms of puberty. This project aims the identification and characterization of EVs in patients with central precocious puberty (CPP).

Methods: Collection of serum samples from CPP female patients and healthy controls. EV isolation using polymer–based precipitation (Exo-Quick) and Size Exclusion Chromatography. Characterization of EV type, size and abundance by Electron microscopy and Flow cytometry. The surface composition and protein content of EVs can be analysed by using Western Blotting and Mass Spectrometry, along with profiling the RNA content using small RNA sequencing.

Results: EV isolation method was confirmed through Electron microscopy. Serum EVs were found positive for EV markers; proteins CD63, Flot1 and Tsg101 and negative for non-endosomal markers; Nucleoporin and Cytochrome C, using western blot. Preliminary results of Flow Cytometry analysis confirmed the existence of EV markers. As a next step, flow cytometric experiments will be completed, along with the small RNA and protein profiling of EVs.

Conclusion: To our knowledge, this is the first ever attempt of EV characterization on CPP patients. The results will assist towards the discovery of different molecules and enhance our understanding of the molecular mechanisms involved in CPP and puberty. Moreover, the identification of circulating biomarkers will offer a prognostic/diagnostic potential on several other pubertal disorders.

PA10 COMPUTATIONAL INVESTIGATION OF CELL-TO-CELL COMMUNICATION NETWORKS AND SIGNALLING MECHANISMS IN MULTIPLE MYELOMA, INFERRED BY SINGLE-CELL TRANSCRIPTOMICS

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Single-cell RNA sequencing (scRNAseq) is a cutting-edge technique, crucial for understanding cellular composition, heterogeneity and gene expression at the single-cell level. This powerful method provides insights into cellular heterogeneity, developmental processes, disease mechanisms, and therapeutic approaches. Importantly, scRNAseq enables the study of intercellular communication, uncovering signalling patterns in fields such as cancer and immunology. Bioinformatics have now a crucial role in scRNAseq analyses, by providing computational methods and tools for the processing and interpretation of biologically meaningful results.

Multiple Myeloma (MM) is a hematologic malignancy characterized by cancerous myeloma cells, that produce abnormal immunoglobulins. MM has three stages: Monoclonal Gammopathy of Uncertain Significance (MGUS), Smoldering MM (SMM) and MM. Intercellular communication, mediated by ligand-receptor pairs, and cell-to-cell communication (CCC) network reconstruction is of utmost importance to understand MM initiation and progression. CCC networks visualize the communication patterns between cells and how they change across the stages of MM. The most rewired nodes and edges in the networks could provide insights for the biology of the disease suggesting potential targets for therapeutic approaches, by methods such as drug repurposing.

Three publicly available scRNAseq datasets with samples from the three stages of MM were analysed and CCC networks were reconstructed. Global communication differs across the stages in each dataset, with trachea neutrophils, liver dendritic cells, bone marrow plasma cells and lymph node neutrophils being the most rewired cell types. Interactions of these cell types may imply an interesting role in MM progression. In the future, significant ligand-receptor pairs and intercellular interactions can be investigated further for therapeutic targeting.

By these scRNAseq analyses, crucial insights of the biology of MM at the single-cell level and how intercellular communication changes in response to the disease have emerged. Future directions for targeting abnormal CCCs across the stages of MM and focusing on personalized medicine will be of high importance.

PA11 IDENTIFICATION OF NOVEL γ-GLOBIN REPRESSORS THROUGH A CUSTOM CRISPR KNOCKOUT SCREEN AND VALIDATION OF THESE REPRESSORS FOR THE TREATMENT OF β-HEMOGLOBINOPATHIES

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Introduction

Haemoglobinopathies, a group of conditions affecting haemoglobin, result from mutations in the HBB gene, leading to prevalent monogenic disorders such as β-thalassaemia and sickle cell disease. Reactivating the γ-globin gene for fetal haemoglobin (HbF) production emerges as a promising therapeutic strategy. However, current gene therapy approaches face limitations in terms of risks, costs, and accessibility. Additionally, pharmacologically targeting key regulators BCL11A and LRF (ZBTB7A) proves challenging due to their involvement in the regulation of non-erythroid genes. Identifying new factors amenable to pharmacological control is crucial for effective treatment of β-haemoglobinopathies.

Materials & Methods

Building on a prior custom CRISPR/Cas9 knockout screen targeting 293 genes, this study focuses on validating candidate genes associated with the screening phenotype, specifically HbF upregulation. Two candidate genes were selected for in-depth investigation. CRISPR/Cas-mediated knockouts were conducted through lentiviral transduction and nucleofection with ribonucleoproteins.

hCD34+ cells were isolated from peripheral blood using magnetic-activated cell sorting. Nucleofections involved two single and one double nucleofection per candidate gene, aiming to enhance disruption efficiency. Nucleofected cells were expanded, and erythroid differentiation spanned 11 days, with cell collection on the final day. HPLC analysis was used for assessing the levels of globins in the samples. Furhter, the HPLC analysis was quantified in order to detect the ratios of β/α and (Gγ+Aγ)/α.

Results

The CRISPR/Cas-mediated knockdown of Gene A demonstrated a more pronounced upregulation of γ-globin compared to Gene B. Remarkably, employing a double nucleofection strategy for both Gene A and Gene B resulted in a more significant increase in γ-globin levels compared to the individual knockdowns. The observed increase in γ-globin levels with the double nucleofections highlights the potential for independent contributions of Gene A and Gene B to γ-globin regulation. Further dissection of their individual mechanisms is necessary for a comprehensive understanding of their roles in γ-globin modulation.

Discussion & Conclusions

This project lays the groundwork for potentially validating new HbF regulators identified through a CRISPR-knockout screen. Ongoing investigations into the mechanisms of Gene A and Gene B, particularly their impact on HbF upregulation in hCD34+ cells, hold promise for identifying novel therapeutic targets. The outcomes may contribute to innovative strategies in treating β-haemoglobinopathies, offering significant advancements in genetic therapies for these inherited monogenic disorders.

PA12 THERAPEUTIC DOUBLE BASE EDITING FOR FETAL HEMOGLOBIN INDUCTION IN β-HEMOGLOBINOPATHIES

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Introduction/Aim: Beta-haemoglobinopathies are the most common monogenic diseases. Of these, β-thalassemia results from decreased (β+) or absence (β0) of β-globin chain production, causing hemolysis and ineffective erythropoiesis. Elevated γ-globin levels, the fetal hemoglobin (HbF, α2γ2), confer major clinical benefits in β-thalassemic patients. Recent research demonstrates that targeting HbF modifiers such as BCL11A and HBG genes, enhances γ-globin expression. Genome editing tools show therapeutic promise such as base editors (BEs) which are safer and likely more efficient than traditional DSB-dependent CRISPR/Cas technology. However, safety concerns regarding chromosomal aberrations still remain a risk. The project aims to adopt BEs for simultaneously editing both BCL11A erythroid enhancer and HBG promoter for correspondingly increased clinical potential and investigate the potential of genomic alterations using CAST-Seq analysis.

Methods: In this study, the HUDEP-2 cell-line and patient-derived CD34+ cells were used. Cells were nucleofected with three different guide RNAs (gRNAs) for single or double target editing. Editing efficiency and functional studies at the DNA, RNA, and protein level were conducted. Lastly, CAST-Seq analysis was performed, for the assessment of chromosomal aberrations.

Results: A successful double base editing protocol was established resulting in high editing efficiency after targeting both BCL11A and HBG loci. Our study reveals that multiplex base editing of both BCL11A enhancer and HBG promoter (2xBE) in patient-derived CD34+ cells induces robust γ-globin and HbF induction reaching to 56.86% HbF increase, indicating a potential therapeutic benefit of 2xBE approach. Our study shows that single and, most importantly, double base editing offers a safe editing option, resulting in a low incidence of genomic alterations in these therapeutically relevant target loci.

Conclusions: In this pioneering study we demonstrate the effectiveness of multiplex base editing targeting both, BCL11A and HBG loci. This approach induces potent fetal hemoglobin with negligible chromosomal aberrations, highlighting the therapeutic potential and safety benefits.

PA13 MAPPING BREAKPOINTS AND IDENTIFYING CRYPTIC CHROMOSOMAL REARRANGEMENTS USING NEXT-GENERATION CYTOGENOMIC TOOLS

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Chromosomal rearrangements, including structural variants (SVs), constitute one of the main causes of human genetic disorders. Their detection still relies on conventional methods, such as chromosomal analysis, Fluorescence In Situ Hybridization and microarrays. Besides their limitations, a combinatorial approach is often required to reach a genetic diagnosis for the patient, resulting in prolonged turnaround time and increased costs. Optical Genome Mapping (OGM) is a high-resolution cytogenomic tool offering a universal approach as it is capable of detecting nearly all types of chromosomal aberrations, and could potentially replace classical cytogenetic workflows. OGM employs the conversion of linearized ultra-high molecular weight labelled DNA into genomic maps enabling the visualization of SVs through the comparison to a reference genome. The aim of this study is to utilize OGM for the investigation of 120 patients with intellectual disability (ID) and/or other congenital developmental disorders (DDs). Specifically, the study cohort will consist of cases that remain undiagnosed after negative prior testing, SV carriers with unexplained phenotypes, or cases that will be blindly tested with OGM along with conventional SV detection methods. Our goal is to delineate the genomic landscape in these cases, precisely map SV breakpoints, and possibly reveal cryptic chromosomal abnormalities relevant to the observed phenotypes. The use of OGM will enable the discovery of new data that will provide a better insight into the genetic aetiology of ID/DDs and the underlying disease mechanisms in a more accurate and affordable manner. We anticipate that OGM will increase the diagnostic rate for patients with ID/DDs by identifying SVs that were previously missed by conventional methods and/or refining rearrangement complexity. Deciphering and associating patient phenotypes with the underlying genetic factors will generate further evidence supporting the potential of OGM as a comprehensive approach for the investigation of patients with ID/DDs.

PA14 Stbd1 ENHANCES AMPK SIGNALLING AND IMPROVES INSULIN RESISTANCE

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Energy balance and metabolic homeostasis are fundamental processes largely regulated by the anabolic hormone insulin. Impaired response of tissues to insulin is described as insulin resistance (IR), the major risk factor for type 2 diabetes. An association between perturbations in endoplasmic reticulum mitochondria contact sites (ERMCs) integrity and IR has recently gained increased attention. Nevertheless, because of contradicting findings, the precise role of ERMCs in IR remains elusive. Starch binding domain-containing protein 1 (Stbd1) is a glycogen-binding protein which undergoes co-translational N-myristoylation that largely determines its subcellular targeting. While N-myristoylated Stbd1(WT) is mostly localized at the ER membrane, a non-N-myristoylated form Stbd1(G2A) is preferentially targeted to ERMCs and enhances ER-mitochondria association. We have recently reported that Stbd1-/- mice display IR associated with enhanced hepatic ERMCs suggesting a role for Stbd1 in the regulation of glucose homeostasis. In the present study, we sought to address the effects of Stbd1 overexpression and ERMCs on insulin signalling and further dissect the importance of N-myristoylation in an in vitro hepatocyte model. To this end, AML12 stable cell lines overexpressing either Stbd1(WT) or Stbd1(G2A) were generated. AML12 cells overexpressing the unrelated GFP protein served as controls. Overexpression of Stbd1 was found to improve insulin signalling and ameliorate IR. Interestingly, this effect was not influenced by changes in ERMCs. We provide evidence that the improved response to insulin is associated with activation of AMP-activated protein kinase (AMPK), a highly conserved regulator of cellular metabolism. Our findings further demonstrate that AMPK activation occurs independently of observed differences between Stbd1(WT) and Stbd1(G2A) cells in parameters known to influence AMPK signalling. These include cellular glycogen levels, mitochondrial morphology and Ca^{2+} balance and activity of respiratory chain complexes.
Taken together, our findings strongly suggest a direct impact of Stbd1 overexpression on AMPK signalling resulting in improved response to insulin and identify Stbd1 as a potential target for the management of IR.

PA15 ROLE OF CONNEXIN 47 IN THE INFLAMMATORY AND DEMYELINATING RESPONSE IN THE CUPRIZONE ANIMAL MODEL OF MULTIPLE SCLEROSIS

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Multiple sclerosis is the commonest autoimmune demyelinating disease affecting young adults. It is a primarily inflammatory disorder of the central nervous system, during which infiltrating activated lymphocytes cross the blood-brain barrier (BBB) leading to dysfunction and apoptosis of oligodendrocytes, demyelination and axonal degeneration. Glial gap junction (GJ) coupling is vital for oligodendrocyte function. More specifically, connexin 47 (Cx47), which is present in most oligodendrocyte-astrocyte GJs, was previously shown to be crucial for the myelination process both in post-mortem brain tissue of MS patients, as well as in the animal mouse model experimental autoimmune encephalomyelitis (EAE). This study focuses on the mechanisms of de- and remyelination in Cx47-/- mice using the toxin-induced cuprizone animal model, in which infiltrating immune cells are less prominent than in EAE. Surprisingly, immunofluorescence shows that demyelination in the medial corpus callosum (mCC) of the Cx47-/- mice is absent after 5 weeks of cuprizone administration, contrary to the wildtype (WT). Demyelination is also absent at different administration durations, i.e. earlier, at 3 weeks, and later, at 7 weeks. Additionally, cuprizone-administered Cx47-/- mice show not statistically significant astrogliosis nor microgliosis, contrary to the WT. However, oligodendrocyte precursor cell (OPC) recruitment is increased at 5 weeks, indicating the response to proliferation and migration signalling. Interestingly, other brain areas of the Cx47-/- mice, including the cortex and thalamus, show comparable demyelination as the WT, further indicating that mCC may have a different immune regulation in the absence of Cx47. Moreover, multiplex bead-based immunoassay shows increased secretion of pro- and anti-inflammatory cytokines like CCL2, CCL5, IL-1 alpha and GM-CSF, and chemokines CXCL10 and CXCL1, in the Cx47-/- cuprizone-administered mice compared to the WT at 3 weeks post administration. These findings clearly indicate that there is an involvement of Cx47 in the inflammatory and demyelinating process that needs to be further investigated.

PA16 PHYTOCHEMICALS AS NOVEL EPIGENETIC REGULATORS IN SKIN CANCER THERAPEUTICS

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Malignant melanoma is one of the most aggressive types of cancer with an increasing global incidence, and as such, the development of innovative therapeutic options is of utmost importance. Recently, the scientific interest in cancer therapeutics has orientated towards the development of epigenetic regulating agents. Currently, there are three generations of epigenetic drugs in clinical practice against various malignancies. Among them, Zebularine (a DNA methyltransferase inhibitor), Tazemetostat (Histone methyltransferase inhibitor) and Entinostat (a histone deacetylase inhibitor) demonstrate improved therapeutic outcomes, especially against haematological cancers. On the other hand, watercress is a rich source of various phytochemicals, including Phenethyl Isothiocyanate (PEITC) whose anticancer properties have been widely studied in the past. In this present study, we evaluated the anticancer potency of characterised PEITC-enriched watercress fraction (PhEF) in an in vitro model of human malignant melanoma, and also the therapeutic effect that the three epigenetic drugs could exert in combination with PhEF. The cytotoxic profile of the PhEF was evaluated via Alamar blue, suggesting time- and dose-dependent cytotoxicity in malignant melanoma cell lines. In contrast, non-melanoma cells exhibited increased resistance in treatment. The reduced viability levels were associated with the induction of mitochondrial apoptosis as proved via the associated gene (qRT-PCR) and protein expression (western immunoblotting) levels. Out of 32 apoptosis-related genes tested, 8 were finally selected as the most significant (dysregulated) for further investigation. Furthermore, combinatorial treatments were performed, using various concentrations of each of the epigenetic drugs, with a fixed concentration of PhEF. Once the optimal experimental conditions of combinatorial treatments were determined, the gene expression of the 8 apoptosis-related genes and the protein expression of the key protein targets of epigenetic drugs were re-assessed. These results suggest the involvement of combinatorial treatments in the enhanced regulation of both the epigenome and apoptosis, hence highlighting the role of a PhEF as an adjuvant in the development of novel combinatorial protocols for the clinical management of malignant melanoma.

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