Mahmoud Abdellatif

Department of Internal Medicine, Division of Cardiology, Medical University of Graz

Project Title:

Targeting pro-aging factors to protect against cardiovascular disease

Background:

Aging is a risk factor for the development of cardiovascular disease, which currently is the leading cause of death worldwide. Without effective interventions capable of influencing the aging process of the cardiovascular system, the medical and socioeconomic burdens of cardiovascular maladies will continue to escalate due to an ever-expanding aging population.

Hypothesis and Objectives:

We have recently identified circulating factors, which exhibit a steady age-dependent rise in humans, while also correlating with traditional risk factors of cardiovascular disease. Intrigued by these observations, we hypothesize that neutralizating these potential pro-aging factors might delay aging and related pathological alterations in the murine cardiovascular system.

Methodology:

The research will employ a comprehensive array of cutting-edge techniques, encompassing, echocardiography, histology, multiomics and molecular biology techniques. Both genetic and immunological interventions will be applied in vivo using relevant mouse models of cardiac or vascular disease, tailored to the candidate's background and interests. The selected PhD candidate will work closely with other fellows who are recruited as part of the same BioTechMed-Graz Flagship project ACE-AGE, coordinated by M. Abdellatif. Additionally, the candidate will have access to the FWF Cluster of Excellence MetAGE activities, including dedicated career development programs for early-career scientists.

References:

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Abdullah Alajati

Department of Urology, Medical University of Graz

Project Titel:

Mechanisms of Therapeutic Resistance and Target Discovery in Uro-Oncology

Background:

Urological cancers such as prostate, renal, and urothelial carcinoma frequently develop resistance to current therapies, limiting long-term clinical benefit. Understanding the molecular mechanisms driving tumor progression and therapy resistance is essential for developing improved treatment strategies and predictive biomarkers.

Hypothesis and Objectives:

We hypothesize that defined molecular alterations drive therapeutic resistance in uro-oncological malignancies and represent actionable targets.

The objectives are:

To investigate molecular mechanisms underlying therapy resistance.

To identify and functionally validate novel therapeutic targets.

To explore biomarker candidates associated with treatment response.

Methodology:

The project will combine translational and experimental approaches, including: Use of transgenic mouse models of uro-oncological cancers. Molecular and cellular analyses (gene and protein expression, signaling pathways, functional assays). Preclinical testing of targeted therapeutic strategies. Histological and immunohistochemical characterization of tumor samples.

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CDCP1 expression is frequently increased in aggressive urothelial carcinoma and promotes urothelial tumor progression. Saponaro M, Flottmann S, Eckstein M, Hommerding O, Klümper N, Corvino D, Hosni S, Schmidt A, Mönig N, Schmidt D, Ellinger J, Toma M, Kristiansen G, Bald T, Alimonti A, Ritter M, Hölzel M, **Alajati A**. Sci Rep. 2023 Jan 2;13(1):73. doi: 10.1038/s41598-022-26579-z.

Adipocyte Precursor-Derived NRG1 Promotes Resistance to FGFR Inhibition in Urothelial Carcinoma. Hosni S, Kilian V, Klümper N, Gabbia D, Sieckmann K, Corvino D, Winkler A, Saponaro M, Wörsdörfer K, Schmidt D, Hahn O, Zanotto I, Bertlich M, Toma M, Bald T, Eckstein M, Hölzel M, Geyer M, Ritter M, Wachten D, De Martin S, **Alajati A**. Cancer Res. 2024 Mar 4;84(5):725-740. doi: 10.1158/0008-5472.CAN-23-1398.

CDCP1 overexpression drives prostate cancer progression and can be targeted in vivo. **Alajati A**, D'Ambrosio M, Troiani M, Mosole S, Pellegrini L, Chen J, Revandkar A, Bolis M, Theurillat JP, Guccini I, Losa M, Calcinotto A, De Bernardis G, Pasquini E, D'Antuono R, Sharp A, Figueiredo I, Nava Rodrigues D, Welti J, Gil V, Yuan W, Vlajnic T, Bubendorf L, Chiorino G, Gnetti L, Torrano V, Carracedo A, Camplese L, Hirabayashi S, Canato E, Pasut G, Montopoli M, Rüschoff JH, Wild P, Moch H, De Bono J, Alimonti A.J Clin Invest. 2020 May 1;130(5):2435-2450. doi: 10.1172/JCI131133.

Patrick David Fischer

Otto Loewi Research Center (Medical Chemistry), Medical University of Graz

Project Title:

Targeting disordered interactions within the BATF/Jun/IRF4 complex

Background:

The BATF/Jun/IRF4 complex drives inflammation in some contexts while promoting T cell exhaustion in others, thereby playing a pivotal role in the regulation of gene expression in immune cells (1,2). Dysregulation of this complex has been implicated in various autoimmune diseases and cancers (3,4). Despite its importance, the structural dynamics of the complex and its interaction with DNA and co-factors remain poorly understood. The project will address key gaps in knowledge by investigating the structural and functional dynamics of the BATF/Jun/IRF4 complex, with the ultimate aim of targeting it with small molecules.

Hypothesis and Objectives:

We hypothesize that targeting the BATF/Jun/IRF4 complex with small molecules can modulate immune cell fate and function by disrupting its dynamic interactions with DNA and co-regulatory proteins. We aim to: (i) Investigate the structure and dynamics of the complex using integrative structural biology (cryo-EM and solution NMR). (ii) Characterize how posttranslational modifications (PTMs) modulate complex formation and function (iii) Lay the groundwork for targeting the complex with small molecules by identifying potential binding sites and testing initial compound interactions in in vitro assays

Methodology:

Protein expression and purification: Complex subunits and interaction partners will be expressed in E. coli whenever possible and in mammalian system when needed, using affinity and gel filtration chromatography for purification. Solution-NMR: using $^{13}C/^{15}N$-labeled protein, we will perform 2- and 3-dimensional NMR experiments to obtain resonance assignments for the dynamic components of the complex. We will also use 15N relaxation measurements (R_1/R_2) to probe conformational dynamics. Cryo-EM: For structural validation at high resolution, we will collaborate with cryo-EM facilities to collect data on the BATF/Jun/IRF4 complex, focusing on the quaternary structure and co-factor interaction. Analysis of PTMs: We will investigate PTMs (e.g. phosphorylation) using mass spectrometry and NMR, assessing their impact on complex formation and function. Small-molecule screening: We will identify initial small-molecule inhibitors by performing ligand-observed NMR and competitive binding assays to evaluate their effect on complex formation and protein interactions.

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Ellen Heitzer

Diagnostic and Research Center for Molecular BioMedicine, Institute of Human Genetics, Medical University of Graz

Project Title:

Multimodal liquid biopsy using plasma and urine ctDNA for detection and monitoring of urogenital cancers

Background:

Urogenital cancers, in particular bladder and prostate cancer, are characterized by heterogeneous clinical courses and therapy responses, and there is a major unmet need for sensitive tools to detect minimal residual disease, monitor treatment response and identify early molecular relapse (1). Liquid biopsy based on circulating tumor DNA (ctDNA) has become a powerful minimally invasive approach, but current clinical applications are largely restricted to plasma-derived ctDNA (2).

In urogenital cancers, urine represents a particularly attractive and still underexploited biofluid, as tumor DNA can be directly shed into the urinary tract and may therefore provide higher sensitivity for local disease than plasma alone, while also containing trans-renal ctDNA derived from the circulation. However, the biological properties of urine ctDNA, its strong fragmentation and its pre-analytical and technical requirements are insufficiently characterized, and standardized combined plasma–urine workflows are lacking (3).

This PhD project is embedded in the Austrian multi-center STRIKE-BC program, which aims to establish a nationwide multimodal precision oncology platform for bladder cancer and to enable biomarker-guided, bladder-preserving treatment strategies. A central component of STRIKE-BC is the longitudinal analysis of circulating and urinary tumor DNA to detect molecular response, minimal residual disease and early recurrence.

The overarching aim of this project is to establish and validate a multimodal liquid biopsy framework based on matched plasma and urine ctDNA for urogenital cancers, with a particular focus on bladder cancer patients undergoing systemic and bladder-preserving treatment. The project will systematically characterize and compare plasma and urine ctDNA features, assess the impact of pre-analytical handling and size-aware workflows on tumor DNA recovery, and determine the added clinical value of urine ctDNA over plasma alone for mutation detection, response assessment and early relapse monitoring. The resulting molecular data will be integrated into the STRIKE-BC data infrastructure to support future data-driven and machine-learning–based prediction models for personalized and bladder-sparing treatment strategies.

Hypothesis and Objectives:

Plasma- and urine-derived ctDNA provide complementary information for monitoring urogenital cancers, and the integration of optimized, size-aware and pre-analytically controlled plasma and urine workflows enables more sensitive detection of molecular response, minimal residual disease

and early relapse than plasma-based liquid biopsy alone, particularly in bladder cancer patients managed within bladder-preserving treatment strategies.

Objectives:

1. To establish and validate a combined plasma–urine ctDNA workflow for urogenital cancers within the clinical framework of the STRIKE-BC program.
2. To systematically characterize and compare mutation and fragmentomic features of ctDNA in matched plasma and urine samples from patients with urogenital cancers, with a focus on bladder cancer.
3. To assess the added clinical and analytical value of urine ctDNA over plasma ctDNA for the detection of molecular response, minimal residual disease and early molecular relapse.
4. To evaluate the performance of urine ctDNA for detecting local disease and early recurrence during active surveillance of bladder-preserved patients.
5. To determine the ability of combined plasma ctDNA and urine-derived tumor DNA to identify molecular complete response and residual disease after neoadjuvant and systemic therapy.
6. To generate high-quality longitudinal plasma and urine ctDNA datasets that can be integrated into the STRIKE-BC multimodal data infrastructure and used for future data-driven and machine-learning–based prediction models supporting personalized and bladder-sparing treatment strategies.

Methodology:

The PhD project will be embedded within the clinical and translational infrastructure of the STRIKE-BC program and its associated prospective observational and interventional studies.

Matched plasma and urine samples will be collected longitudinally from patients with muscle-invasive and metastatic bladder cancer, including patients undergoing bladder-preserving management following systemic therapy. Samples will be obtained at defined clinical time points, including baseline, during treatment, at clinical response assessment, and during follow-up surveillance.

The methodological work will include:

- extraction of cfDNA/ctDNA and utDNA with workflows optimized for highly fragmented and ultrashort DNA species,
- fragment length and fragment end pattern analysis to assess tumor-associated fragmentomic features,
- targeted next-generation sequencing for mutation profiling and detection of minimal residual disease,
- comparative analysis of plasma and urine ctDNA for concordance and complementary detection,
- longitudinal assessment of ctDNA dynamics in relation to clinical response and recurrence,
- integration of molecular readouts with available clinical and imaging data generated within STRIKE-BC.

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Philipp Jost (PI)/ Katarina Vizar Cisarova (Co-PI)

Department of Internal Medicine, Division of Oncology, Medical University of Graz

Project Title:

Multi-omics characterization of immunogenic cell death in non-small cell lung cancer

Background:

Lung cancer is one of the most prevalent cancers in adults and the leading cause of cancer-related deaths worldwide (1). Non-small cell lung cancer (NSCLC), the predominant subtype, accounts for over 85% of cases and includes lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) (2). Therapeutic advances, particularly targeted and immunotherapy-based treatments, have revolutionized the treatment of lung cancer patients with actionable genetic alterations. Inhibitors of *EGFR, ALK, RET, BRAF, ROS1, NTRK, MET*, and *KRAS* are now standard treatments for patients with corresponding molecular drivers (3). Immunotherapy has also become a standard of care (4). However, only about 25% of NSCLC patients benefit from these treatments long-term, as the development of resistance is almost inevitable (5)(6). The primary challenge for long-term survival of NSCLC patients is the high relapse rate, largely driven by immune evasion that prevents immune recognition of NSCLC tumor cells (7). This is, to some extent, counterintuitive, as NSCLC is one of the cancers with the highest mutational load and contains a high number of potentially immunogenic alterations (8). From a clinical perspective, it is crucial to enhance immune recognition and facilitate immune control of NSCLC. One of the ways tumor cells manage to avoid immune destruction is the evasion of programmed cell death, a key hallmark of cancer (9). Immunogenic cell death (ICD), a form of programmed cell death that promotes inflammation, plays a vital role in stimulating the immune system's response against cancer cells. In many cancer types, the key regulators of the necroptotic and pyroptotic pathway are generally downregulated, suggesting that cancer cells survive by escaping ICD (10). Harnessing ICD pathways has therefore emerged as a promising strategy to develop new treatment approaches to shape the tumor immune microenvironment and to enhance existing therapies, particularly immunotherapy (11).

Hypothesis and Objectives:

We hypothesize that specific genetic and epigenetic alterations in key ICD pathway regulators create distinct molecular subtypes in NSCLC characterized by differential immune infiltration patterns and immunotherapy responsiveness. Specifically, we propose that suppression of ICD pathways represents a common mechanism of immune evasion in NSCLC, and that tumors with intact ICD signaling will exhibit increased immune cell infiltration and improved response to checkpoint inhibitor therapy. Our main objectives are to (I) systematically characterize the deregulation of ICD genes and pathways in NSCLC subtypes, (II) identify genetic, epigenetic, and signaling mechanisms behind this deregulation, (III) define pathway-level activities and crosstalks, (IV) investigate the interplay between the tumor

microenvironment (TME) and ICD pathway activities, and (V) experimentally validate the key *in silico* findings.

Approach/methods:

The student will initially apply *in silico* analyses of publicly available and in-house multi-omics datasets, including gene expression, mutational, and methylation data, combined with clinical annotations. A complete multi-omics bioinformatic pipeline will be developed and applied to the datasets. Findings will be validated across cohorts and complemented with spatial and single-cell data analyses. Selected candidate genes will undergo experimental validation using tissue-based approaches and *in vitro* and/or *in vivo* models. This project bridges computational discoveries and functional investigation. Basic knowledge of bioinformatics and/or a strong interest in computational data analysis is required for this project.

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Dagmar Kratky

Gottfried Schatz Center (Molecular Biology and Biochemistry), Medical University of Graz

Project Title:

Identification and characterization of acyltransferases and transacylases involved in glycerophospholipid synthesis

This PhD position is part of the BioTechMed-Graz flagship "Characterization of alternative phospholipid synthesis pathways in mammalian cells (LipoSynth)", a research network between the University of Graz, the Medical University of Graz, and the Graz University of Technology.

Background:

Mammalian cell membranes contain defined amounts of phospholipids required for the maintenance of cell morphology and cell function. According to textbook knowledge, de novo glycerophospholipid synthesis starts with the acylation of glycerol-3-phosphate, leading to the formation of phosphatidic acid, the precursor of all other glycerophospholipids, which are synthesized via the cytidine diphosphate-diacylglycerol [1] or the Kennedy pathway [2]. Subsequently, de novo synthesized glycerophospholipids are reesterified with a fatty acid in a CoA-dependent reaction in the sn-2 position, a process known as Lands cycle [3]. We propose to characterize alternative glycerophospholipid synthesis pathways that have so far not been described in mammalian cells. We found that glycerophosphodiesters (GPDs), the deacylated backbones of glycerophospholipids, can be acylated by acyltransferases. Acylation of glycerophosphoglycerol by CLN8 exemplifies the targeted synthesis of lipids via a GPD-dependent pathway (under review; https://doi.org/10.64898/2025.12.18.693953). Despite associations with human diseases (e.g. Alzheimer and Batten disease [4, 5]), GPD metabolism in mammalian cells has been insufficiently studied.

Hypothesis and Objectives:

We hypothesize that (i) most (if not all) GPDs are reacylated and reused for phospholipid synthesis, depending on the cell type and the prevailing metabolic condition and that (ii) these reactions are catalyzed by currently unknown acyl-CoA-dependent acyltransferases (GPDATs) and transacylases (GPDTAs). The objectives are to identify and characterize acyltansferases/transacylases for all GPDs. Our results will provide essential new insights into mammalian phospholipid metabolism and possibly open new opportunities for therapeutic interventions in lipid-associated metabolic disorders.

Methodology:

To identify GPDATs/GPDTAs, the PhD candidate will screen an enzyme library of ~ 250 enzymes comprising most known lipid hydrolases and acyltransferases/transacylases encoded by the human genome as well as structurally related proteins with unknown function. This library has previously

been successfully used to identify lipid hydrolases as well as transacylases [6, 7] and is currently being expanded with acyl-CoA-dependent acyltransferases. GPDAT activity will be investigated in the presence of ^{13}C-GPDs and acyl-CoA using lysates of cells overexpressing the recombinant enzymes. GPDTA activity will be monitored using a mixture of glycerophospholipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylinositol, and phosphatidylglycerol) as acyl donor and ^{13}C-GPDs as acceptor. To screen for neutral and acidic GPDTs, the student will perform assays at pH 7.4, 6.0, and 4.5. The PhD candidate will use affinity chromatography to confirm acyltransferase/transacylase activity of candidate enzymes with purified proteins in vitro. The characterization of GPDATs and GPDTAs will include optimization of reaction conditions with respect to pH, buffer system, and co-factors, determination of substrate specificity, the investigation of positional preferences for donor lipids and acceptors, and preferences for fatty acid chain length and saturation. Dose- and time-dependent incorporation of GPDs into phospholipids will be investigated in cells lacking and overexpressing GPDATs/GPDTAs. Knockout HAP1 or HEK293 cell lines lacking the candidate proteins will be generated using the CRISPR/Cas9 system. The use of other cell lines than HAP1 or HEK293 cells will be guided by endogenous expression levels of the identified enzymes.

This projectwill reveal whether GPDs are incorporated into cellular lipids without prior degradation. The experiments will also provide information on the time course and saturation of GPD-dependent lipid synthesis pathways. Gain- and loss-of-function experiments will provide insights into the molecular mechanisms mediating GPD acylation.

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Andreas Prokesch

Gottfried Schatz Center for Cell Signaling, Metabolism and Aging, Medical University of Graz

Project Title:

Metabolic regulation through p53/Foxo1 cooperativity in obesity and fasting

Background:

Obesity often associates with comorbidities such as type 2 diabetes, cardiovascular disease, and cancer. While pharmacological means to curtail obesity are beginning to emerge, fasting and caloric restriction remain a proven intervention [1]. The underlying molecular mechanisms are still rather unexplored and appear to go far beyond a simple reduction in energy intake. The transcription factors p53 and Foxo1 are independently known to coordinate genetic programs during feeding/fasting transitions. Our previously published [2-5] and unpublished preliminary data suggest a tight interplay between p53 and Foxo1 in liver, adipose tissue, and skeletal muscle. We found a previously unreported direct interaction of p53 and Foxo1 as well as co-regulatory interdependencies in a tissue-specific manner.

Hypothesis and Objectives:

We hypothesize that a multi-layered nuclear interaction between p53 and FOXO1 regulates feeding/fasting transitions in liver, adipose tissue, and skeletal muscle, and propose that this interaction is required for the salutary effects of cyclic fasting on the mitigation of obesity and its associated co-morbidities. Hence, we plan to investigate the molecular and structural nature of the p53/FOXO1 interaction (direct/indirect, trans/cis), as well as the intricacies of tissue-specific co-regulation of each other and on the level of activation of common target genes and pathways.
This project is part of an Austrian German collaboration as part of the FWF Weave program, and it will focus on p53/FOXO1-mediated metabolic (dis-) regulation of the adipose tissue biology in obesity and weight loss.

Methodology:

We use novel tissue-specific, inducible p53 and/or Foxo1 knock out mouse models with an established protocol of fasting-mediated obesity intervention, in liaison with genome-wide detection of fasting-selective enhancers/promoters (PRO-seq) and transcription factor binding (ChIP-seq) to unravel the specific modes and consequences of p53/FOXO1 interaction in cis. Comprehensive metabolic phenotyping and differential comparison of p53 and/or Foxo1 knock out animals will reveal insight into the impact of the p53/FOXO1-axis on fasting/feeding transitions to tackle obesity. Supported by a proprietary pharmacological agent that disrupts the p53/FOXO1 interaction, in vitro and ex vivo adipocyte models will reveal mechanistic details at the transcriptional and functional level. For that we combine standard molecular biology (luciferase assays, co-IPs) and biochemical methods (e.g.

triglyceride hydrolase assays) with sophisticated state-of-the-art approaches to map intra- and inter-molecular protein-protein interactions.

Understanding the adipose tissue-specific transcriptional networks is imperative to enable the design of fasting regimens and mimetics for preventive, personalized medicine that targets hallmarks of obesity and the metabolic syndrome. Hence, as both p53 and FOXO1 are pharmacological actionable, our results are poised to infuse innovations in the field of obesity research.

Primary researchers involved

Assoc.-Prof. Dr. Andreas Prokesch (lead): Medical University of Graz, Graz, Austria
Prof. Dr. Tobias Madl: Medical University of Graz, Graz, Austria
Prof. Dr. Michael Schupp: Charité Berlin, Berlin, Germany
Prof. Dr. Tim J. Schulz: German Institute of Human Nutrition Potsdam-Rehbruecke, Germany

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Beate Rinner

Diagnostic and Research Institute of Pathology, Medical University of Graz

Project Title:
Precision Oncology: Lipid Emulsions as a Paradigm Shift in Chemotherapy

Background:

Omega-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential nutrients with well-documented anti-proliferative, pro-apoptotic, and anti-metastatic effects. By modulating membrane lipid composition, increasing reactive oxygen species (ROS) generation, and promoting lipid peroxidation and ferroptosis, omega-3 PUFAs can enhance the sensitivity of cancer cells to chemotherapy. Increasing evidence indicates that EPA and DHA not only improve the efficacy of chemotherapeutic agents such as cisplatin but also reduce treatment-related toxicities, including nephrotoxicity, through the regulation of pathways involving Nrf2, GPX4, MAPK, and caspase signaling[1]. Cancer progression is closely linked to profound alterations in lipid metabolism, including increased fatty acid synthesis, uptake, and oxidation. These metabolic adaptations are strongly influenced by the tumor microenvironment, which consists of stromal and immune cells that regulate tumor growth, metastasis, and therapy response. Changes in membrane lipid saturation affect ROS tolerance, endoplasmic reticulum stress, and susceptibility to ferroptosis, while lipid mediators such as prostaglandin E2 and lysophosphatidic acid promote inflammation, angiogenesis, immune evasion, and resistance to therapy[2,3]. However, the biological and clinical relevance of esterified omega-3 fatty acids remains insufficiently understood, highlighting the need for further mechanistic and translational research.

Hypothesis and Objectives:

Metastatic cancer remains difficult to treat due to therapy resistance and severe side effects of conventional chemotherapy. We hypothesize that nano-sized lipid emulsions mimicking physiological lipid carriers can improve the targeted delivery of omega-3 fatty acids to tumors, thereby enhancing chemosensitivity while reducing systemic toxicity. This project will combine patient-derived, tumor and tumor microenvironment models, including autologous primary, metastatic, immune, and iPSC-derived cells, to investigate underlying molecular mechanisms. Complementary in vivo studies will assess biodistribution, metabolism, therapeutic efficacy, and short- and long-term side effects of omega-3-based nanoemulsions in combination with chemotherapy.

Methodology:

The project combines advanced in vitro and in vivo methodologies to investigate omega-3–based lipid emulsions as adjuvants to chemotherapy. High-throughput in vitro screening will first assess synergistic and antagonistic effects of DHA/EPA combined with standard chemotherapeutics across a broad panel of human cancer cell lines using 3D spheroid models. Synergy will be quantified using

established dose–response algorithms, enabling prioritization of responsive tumor entities. Based on these results, patient-derived autologous tumor models will be established. Spatial transcriptomics and molecular characterization will be used to resolve tumor microenvironment heterogeneity. Mechanistic studies will focus on key signaling pathways, lipid metabolism, and regulated cell death mechanisms, particularly ferroptosis, using transcriptomics, proteomics, metabolomics, and functional assays. Cell cycle regulation, ROS production, mitochondrial function, and apoptosis–ferroptosis crosstalk will be analyzed using flow cytometry, live-cell imaging, Western blotting, and inhibitor studies. Metabolic adaptations will be assessed through Seahorse flux analysis and stable isotope–resolved metabolomics. To evaluate therapy-related side effects, cardiotoxicity will be studied using human iPSC-derived cardiomyocytes in mono- and co-culture systems. Selected findings will be validated in vivo using xenograft mouse models to assess biodistribution, immune modulation, tumor progression, and survival outcomes following intravenous lipid emulsion supplementation.

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Project Title:

Modeling of cholesterol binding SK3 K+ channels

Background:

The small conductance KCa2+ (SK) channels are part of the KCa2+ ion channel family. SK channels are voltage-independent that respond to submicromolar calcium levels, which show a complex molecular mechanism. Indeed, the binding of calcium to SK channels is mediated by another protein, calmodulin, which is constitutively bound to the channel. The structure of human SK2 and SK4 have been elucidated and additionally, it is known that the activation of the SK channels also depends on the coupling to other calcium channels (i.e., voltage-gated and voltage-independent variants) and the binding to lipids in the membrane. Cholesterol is one of the lipids which is assmuned to modulate most of the SK channels. As an example, existing evidencies show that the SK3 channel, which is upregulated in breast, colon, and prostate cancer cells,1 co-localizes with Orai1, another calcium channel cholesterol-rich regions.2 However, the co-regulation of these ion channels with their lipid environment, particularly cholesterol, is still intriguing. Given the relevance of SK3 channel, the understanding of the molecular drivers of how SK3 is regulated, will open new therapeutical approaches.

Hypothesis and Objectives:

In this project, we will study the role of cholesterol in SK3 channels regulation within the context of the FWF-funded project 'Role of cholesterol in the regulation of SK3 K+ channels' lead by the Johannes Kepler Universität (JKU) Linz and participated by the Medical University of Graz and the University of Graz.3 In close collaboration with the experimental team in the JKU, the PhD candidate will analyse the effect of mutations disrupting putative cholesterol binding pockets predicted by deep-learning structure algorithms, free energy, and chemical dynamics simulation studies. As outcome, it will allow us to identify crucial sites for cholesterol-mediated modulation and to gain structural information on potential cholesterol binding pockets.

Methodology:

The PhD student will be trained in advanced molecular dynamics simulation techniques as well as in a variety of modeling approaches for biological systems. The PhD candidate will learn different de novo algorithms for the prediction of the structure of SK3 channels and will simulate the dynamics of them using from classical force-field molecular dynamics simulations, metadynamics, accelerated Gaussian molecular dynamics, to coarse-grained approaches, amongst others. Free energy calculation and co-folding algortihms will be used to predict the biniding of cholesterol in SK3 channels from a dynamical perspective and to validate the binding site(s) of cholesterol to SK3 channels.

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