UNLOCKING THE EPIGENOME

NEW HORIZONS IN MOLECULAR MEDICINE AND RARE DISEASES

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Book of Abstracts

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Foreword

It is a great privilege to welcome you to the Book of Abstracts for the international symposium, "Unlocking the Epigenome: New Horizons in Molecular Medicine and Rare Diseases." As we enter the era of precision medicine, understanding the epigenetic landscape is becoming essential for unraveling the complexities of rare genetic disorders. Our primary aim is to bring together leading scientists, clinicians, and biotechnology innovators from around the world. The abstracts compiled in this volume reflect the core scientific themes driving our field forward. These contributions span fundamental Epigenetic Regulation Mechanisms—including DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs—to the direct role of epigenetics in rare diseases, with a strong emphasis on diagnostics and emerging therapeutic targets. We also explore new horizons in molecular medicine through genome editing, multi-omics integration, and precision medicine, alongside diagnostic frontiers utilizing liquid biopsies, molecular biomarkers, and AI-driven approaches. Crucially, the program addresses the vital human dimension through patient perspectives, quality of life, ethics, and patient advocacy, while showcasing the latest therapeutic strategies and drug development in clinical trials. We hope that these abstracts spark deep clinical and scientific curiosity, leading to long-lasting international connections. Thank you for your invaluable contributions to this scientific program.



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Key Speakers



Prof. Tapas Kumar Kundu

Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), India

TALK TITLE: Epigenetic Regulation of Neurological Disorders: Role of Lysine Acetyltransferases p300/CBP

Prof. Tapas Kumar Kundu is an internationally recognized expert in molecular biology, epigenetics, and chemical biology. He is the former Director of the CSIR-Central Drug Research Institute (CDRI) in Lucknow. Currently, he leads the Transcription and Disease Laboratory at the Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR) in Bengaluru, India.
His pioneering research focuses on gene regulation, functional chromatin dynamics, and their roles in cellular physiology and disease, including cancer, HIV/AIDS, and neurological disorders. Prof. Kundu's major contributions include discoveries related to the chromatin protein PC4, the histone chaperone NPM1, and the epigenetic regulator p300. His group has also developed novel small-molecule modulators of epigenetic enzymes and innovative carbon-nanosphere-based drug delivery systems. These discoveries have opened promising avenues for next-generation diagnostics and therapies, particularly in cancer, neurodegenerative diseases, and brain-targeted drug delivery.



Prof. Patrick Varga-Weisz

University of Essex, UK

TALK TITLE: Chromatin dynamics in host-microbiota crosstalk and intestinal biology

Prof. Patrick Varga-Weisz is an internationally recognized expert in epigenetics and chromatin biology, whose pioneering research has significantly advanced our understanding of how genome organization regulates gene expression, stem cell function, ageing, and host–microbiome interactions.
Prof. Varga-Weisz is a Senior Lecturer in the School of Life Sciences at the University of Essex, United Kingdom. Previously, he led a research group at the Babraham Institute in Cambridge, where he made seminal contributions to the field of chromatin remodeling and epigenetic inheritance. His current research focuses on the molecular mechanisms by which chromatin dynamics and the gut microbiota influence gene regulation, tissue homeostasis, and disease.



Prof. Rumen Stefanov

Medical University of Plovdiv, Bulgaria

TALK TITLE: From molecular diagnosis to integrated care: bridging the implementation gap in rare diseases

Prof. Dr. Rumen Stefanov is an internationally recognized expert in public health, epidemiology, and social medicine. His transformative research and advocacy have contributed significantly to the advancement of healthcare policies, orphan drug development, and epidemiological registries for rare diseases, both in Bulgaria and on a global scale.
Prof. Stefanov is the Head of the Department of Social Medicine and Public Health at the Medical University of Plovdiv and the founder of the Bulgarian Institute for Rare Diseases. He serves as the President of the International Conference on Rare Diseases and Orphan Drugs (ICORD). His work focuses on healthcare systems evaluation, rare disease policy formulation, access to innovative treatments, and the coordination of European research networks aimed at improving the quality of life for patients with rare conditions.

Key Speakers



Prof. George Garinis

University of Crete and IMBB-FORTH, Greece

TALK TITLE: DNA damage and innate immune responses in health and disease

Prof. George A. Garinis is an internationally recognized expert in molecular genetics, genome stability, and the biology of ageing, whose pioneering research has provided important insights into the mechanisms linking DNA damage, genome maintenance, and human disease.
Prof. Garinis is Professor at the University of Crete and a Principal Investigator at the Institute of Molecular Biology and Biotechnology (IMBB–FORTH), Greece, where he leads a research group focused on gene regulation and epigenetics. His research explores how defects in DNA repair pathways and transcriptional regulation contribute to genome instability, premature ageing, developmental disorders, and age-related diseases.



Prof. Christophe Arnoult

Institute for Advanced Biosciences (IAB) Grenoble, France

TALK TITLE: Oligogenism as a cause of human infertility

Prof. Christophe Arnoult is an internationally recognized expert in reproductive biology, male infertility, and genetic diseases affecting human reproduction. His pioneering research has contributed significantly to the understanding of sperm development, fertilization mechanisms, and the molecular causes of inherited reproductive disorders.
Prof. Arnoult is Research Director at the French National Centre for Scientific Research (CNRS) and leads research activities at the Institute for Advanced Biosciences (IAB) at the University of Grenoble, France. His work focuses on the genetic and molecular mechanisms underlying human fertility, with particular emphasis on sperm function, ion channels involved in sperm motility, and the identification of genetic causes of male infertility.



Prof. Albena Jordanova

VIB Center for Molecular Neurology, University of Antwerp, Belgium/ Medical University of Sofia, Bulgaria

TALK TITLE: Modeling rare diseases: the example of hereditary peripheral neuropathies

Prof. Dr. Albena Yordanova is one of the world's leading scientists in molecular neurogenetics and the recipient of the prestigious 2025 Belgian Generet Award, worth €1 million, for her groundbreaking research on rare diseases. She is Professor of Molecular Neuroscience at the University of Antwerp and Principal Investigator at the Flemish Institute of Biotechnology (VIB), Belgium, where she leads the Molecular Neurogenomics Group. In Bulgaria, she is a member of the National Genetics Laboratory and a co-founder of the Center for Molecular Medicine at the Medical University of Sofia. Her research focuses on the discovery and characterization of genes responsible for inherited neurological disorders, as well as genetic studies in isolated and inbred populations.

Key Speakers



Prof. Julien Thévenon

Grenoble Alpes University Hospital, France

TALK TITLE: Genomic research in rare disorders

Dr. Julien Thévenon MD, is an internationally recognized expert in clinical genetics and rare developmental disorders, whose research has significantly contributed to the discovery of genetic causes underlying neurodevelopmental diseases and congenital anomalies.
Dr. Thévenon is an Inserm researcher at the Institute for Advanced Biosciences (IAB; Inserm/CNRS/Université Grenoble Alpes), a clinical geneticist at Grenoble Alpes University Hospital, France, and coordinator of a Centre of excellence for rare diseases with developmental abnormalities and malformation syndromes, within the AnDDI-Rare (French rare diseases network). He leads research activities in translational genomics, focusing on the identification and interpretation of disease-causing genetic variants and the integration of genomic data into clinical practice.



Dr. Seyit Kale

Izmir Biomedicine and Genome Center, Türkiye

TALK TITLE: Chromatin dynamics in health and disease

Dr. Seyit Kale is an internationally recognized computational biophysicist and epigenetics researcher whose work combines molecular modeling, high-performance computing, and artificial intelligence to uncover the molecular mechanisms underlying chromatin organization, rare diseases, and gene regulation.
Dr. Kale is Principal Investigator and head of the Omics and Computational Biophysics Group at the Izmir Biomedicine and Genome Center (IBG), Türkiye. His research focuses on the molecular mechanisms that regulate chromatin structure and epigenetic inheritance, as well as the development of computational approaches for the design of therapeutic small molecules, peptides, and antibodies.



Prof. Ivanka Dimova

Medical University, Sofia, Bulgaria

TALK TITLE: Imprinting disorders - or how the genes escape from the Mendel laws

Prof. Dr. Ivanka Dimova, MD, PhD is an internationally recognized expert in medical genetics, genomics, and oncogenetics. Her transformative research and diagnostic innovation have contributed significantly to the advancement of genomic medicine, precision oncology, and molecular diagnostics for complex and rare conditions, both in Bulgaria and on a global scale.
Prof. Dimova is a leading professor at the Department of Medical Genetics and the Head of the Laboratory of Genomic Diagnostics at the Center for Molecular Medicine, Medical University of Sofia. She also serves as the Geneticist-in-Chief at the Genetic Laboratory of the "Dr. Shterev" Medical Complex. Her work focuses on high-throughput genetic analyses, hereditary cancer syndromes, reproductive genetics, and the coordination of international scientific networks aimed at pioneering breakthrough DNA microarray technologies and NGS-based diagnostics to improve clinical decision-making and patient care.

Key Speakers



Dr. Rumen Stamatov

Institute of Molecular Biology, BAS, Bulgaria

TALK TITLE: An automatic tracker for chromosome trajectories in mitotic and interphase chromosomes

Dr. Rumen Stamatov is one of the most brilliant young minds at the "Acad. Roumen Tsanev" Institute of Molecular Biology within the Bulgarian Academy of Sciences (BAS). He earned widespread scientific acclaim as the lead author of a pioneering research paper published on the cover of the prestigious journal Nature Cell Biology in April 2025. Dr. Stamatov holds an exceptional international educational background, having completed his undergraduate studies at the University of Pennsylvania and his master's degree at Duke University in the United States. As a vital researcher in the Laboratory of Genomic Stability, his work bridges cutting-edge artificial intelligence with live-cell imaging. His research focuses on how chromosomes move and change over time during cell division, utilizing custom-built neural networks to track individual chromosomes in 3D with unprecedented speed and precision.



Dr. Nikolay Vasev

CEO of Epix AI, Bulgaria

TALK TITLE: From biomarkers to dynamic biological age: An AI-driven framework for personalized health monitoring

Dr. Nikolay Vassev is an experienced healthcare and technology leader with a strong focus on the intersection of artificial intelligence, preventive medicine, and digital health innovation. His work is dedicated to leveraging emerging technologies to improve healthcare decision-making, enable personalized approaches, and address major challenges in disease prevention and management. Dr. Vassev is Chief Operating Officer of Epix AI, a company developing AI-driven solutions at the intersection of healthcare, biotechnology, and data science. His professional background combines expertise in European healthcare policy, health systems, pharmaceutical innovation, and strategic development of technology-based healthcare solutions.



Dr. Jérôme Govin

Inserm and University of Grenoble, France

TALK TITLE: Drugging epigenetic pathways to treat fungal infections

Dr. Jérôme Govin is an internationally recognized expert in molecular biology, epigenetics, and chromatin structure. His pioneering research has contributed significantly to the understanding of epigenetic regulation, cellular differentiation processes (such as gametogenesis), and the molecular mechanisms behind fungal infections and aggressive cancers. Dr. Govin is Research Director at the French National Institute of Health and Medical Research (Inserm) and leads the "Translational Epigenetics" research team at the Institute for Advanced Biosciences (IAB) at the University of Grenoble, France. His work focuses on the genetic and molecular mechanisms governing cell fate, with particular emphasis on chromatin remodeling, the regulation of fungal pathogen development, and the identification of epigenetic anomalies linked to hematological malignancies.

Key Speakers



Gogo Krastev

Country Lead, Genesis Pharma, Bulgaria

TALK TITLE: From scientific discovery to patient access: The role of the pharmaceutical industry in rare disease innovation

Mr. Gogo Krastev is an experienced leader in the biopharmaceutical industry with a strong focus on innovative medicines, rare diseases, and improving patient access to advanced therapies. Through his work in the pharmaceutical sector, he helps bridge scientific innovation and healthcare systems and supports the introduction of novel treatments for patients with unmet medical needs.
Mr. Krastev is Country Lead at GENESIS Pharma Bulgaria, where he is responsible for driving the company's strategic activities and expanding access to innovative biopharmaceutical solutions in the Bulgarian healthcare environment. GENESIS Pharma specializes in the commercialization of innovative therapies, with a particular focus on rare and chronic diseases across multiple therapeutic areas, including neurology, genetic diseases, oncology, hematology, and other specialized fields.



Prof. Boris Minev

CSO, Cure Science Institute; UCSD Moores Cancer Center, USA

TALK TITLE: Optimized cell-based delivery & potentiation of oncolytic virotherapies

Prof. Boris Minev is a leading expert in tumor immunology, cancer vaccines, and cell-based immunotherapy. He serves as the Chief Scientific Officer (CSO) at the CureScience Institute and is an Adjunct Professor at the UCSD Moores Cancer Center in San Diego. With an extensive background bridging academia and the biotech industry, Prof. Minev's research focuses on tumor antigen discovery and the integration of immunotherapy with precision medicine to treat advanced malignancies. He has held key scientific leadership roles in industry-leading biotech corporations and serves on the advisory boards of several international translational medicine societies, contributing significantly to next-generation cancer therapeutics.



Prof. Aladar Szalay

CEO, Founder, Chairman, StemImmune Inc.; UCSD Moores Cancer Center USA & University of Würzburg, Germany

TALK TITLE: Optimized cell-based delivery & potentiation of oncolytic virotherapies

Prof. Aladar Szalay is an internationally renowned scientist and entrepreneur with over three decades of pioneer research in immuno-oncology, molecular imaging, and oncolytic virotherapy. He is the Founder, CEO, and Chairman of StemImmune Inc. and holds dual professorships at the UCSD Moores Cancer Center (USA) and the University of Würzburg (Germany). Prof. Szalay is globally recognized for developing tumor-targeted oncolytic vaccinia virus strains for cancer therapy and pioneering the use of bioluminescent imaging for early cancer detection. Throughout his distinguished career, he has successfully transitioned laboratory discoveries into advanced clinical trials, founding multiple biotechnology companies and advancing the field of translational medicine.

Unlocking the Epigenome:
New Horizons in Molecular Medicine and Rare Diseases

20–21 October 2026 | Sofia, Bulgaria

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Abstract

ABSTRACT #1 **ORAL**

Epigenetic Regulation of Neurological Disorders: Role of Lysine Acetyltransferases p300/CBP

Tapas K. Kundu

Transcription and Disease Laboratory, Molecular Biology and Genetics Unit, Jawaharlal Nehru Centre for Advanced Scientific Research, Jakkur P.O, Bangalore 56064, INDIA

Epigenetics is gene function beyond the DNA sequence, operated by DNA modifications, DNA-associated protein modifications, and noncoding RNA. It is reversible and metabolically regulated, thus directly related to habit and lifestyle. Reversible histone acetylation is one of the most extensively investigated epigenetic modifications, shown to be involved in diverse physiological and pathological phenomena. However, its role in memory and neurological disorders is not fully understood. Several studies have demonstrated that the catalytic activity of the master Lysine acetyltransferases CBP/p300 could be critical for long-term memory formation. We have shown that specific activation of p300/CBP KAT activity significantly prolongs the long-term memory in mice. We have discovered a small-molecule activator of CBP/p300 (TTK21) that, after conjugation to glucose-derived carbon nanospheres (CSP), crosses the blood-brain barrier and reaches different parts of the brain without apparent toxicity. It induces adult neurogenesis and long-term memory. By administering this activator to the tauopathy mouse model of Alzheimer's Disease (AD), we significantly reversed memory loss in both young and older mice. In the course, we identified that the cholesterol biosynthesis pathway is severely deregulated in older AD mice, which could be correlated with available human patient data. Activating p300/CBP KAT activity could normalize cholesterol biosynthesis, reversing cognitive function. Recently, we have found that in Syngap1+/-, a mouse model of intellectual disability (ID) and autism spectrum disorder (ASD), the p300 KAT activity is dramatically reduced in the CA1 region of the hippocampus. Our results demonstrate that the oral administration of CSP-TTK21 in adult Syngap1+/- mice rescued physiological and cognitive/emotional functions, presumably by restoring p300/CBP-mediated histone acetylation and adult neurogenesis. The molecular pathways underlying symptom amelioration are being elucidated. Recently, we have also established the critical role of p300/CBP catalytic activity in depression and related neurogenesis in a chronic stress model system.

Abstract

ABSTRACT #2 **ORAL**

Chromatin dynamics in host-microbiota crosstalk and intestinal biology

Patrick Varga-Weisz and collaborators

School of Life Sciences, University of Essex, UK and Institute of Biology, University of Campinas, São Paulo State, Brazil

Our work highlights the importance of fundamental chromatin packaging mechanisms in intestinal biology and microbiota-host interaction and, surprisingly, that the sex of the organism plays a critical role in shaping this.

The intestinal epithelium is a highly regenerative single-cell-thick tissue layer critical for nutrient absorption and for hosting the gut microbiota. The gut microbiota generate large amounts of metabolites, such as short-chain fatty acids from fermentation of dietary fibers. These metabolites are taken up by our cells and impact physiology systemically. Importantly, microbiota-derived metabolites affect epigenetic processes, such as histone modifications and, thus, are part of an inter-kingdom dialogue. Our current research is centered on chromatin dynamics and epigenetic regulation, and how these processes shape intestinal biology, particularly in contexts of gut-microbiome interaction, ageing, gene expression, and disease. Our work connects chromatin biology with questions that are relevant to gut inflammation and epithelial function. We use mouse models and other approaches (e.g., gut organoid culture, single cell transcriptomics, chromatin immuno-precipitation sequencing) to illuminate relevant pathways (e.g., how the gut microbiota affect gene expression in the colon epithelium, ref 1).

We have shown that the gut microbiota impacts non-acetyl histone acylations, such as crotonylation (e.g., ref. 2, 3). These modifications function in part by generating binding platforms for 'reader' proteins. I will present our research highlighting the important role of such a crotonylation reader, Yeats4, in intestinal biology, presenting evidence that Yeats4 links histone crotonylation to epithelial gene regulation in the colon.

In addition to histone modifications, histone variants, such as histone H3.3 play an important role in epigenetic processes. The deposition of these variants, in turn, is regulated by specific histone chaperones, such as the protein Daxx. I will present our research showcasing the function of the H3.3-Daxx pair in constraining inflammation of the colon epithelium.

References:

(1) Microbiota shape the colon epithelium controlling inter-crypt absorptive goblet cells via butyrate-GPR109A signalling. Dias Nirello et al. Gut Microbes. 2025;17(1):2573045.

(2) Improved Mass Spectrometry-based methods reveal abundant propionylation and tissue specific histone propionylation profiles. Vai et al. Mol Cell Proteomics. 2024:100799.

(3) Microbiota derived short chain fatty acids promote histone crotonylation in the colon through histone deacetylases. Fellows et al. Nature Communications 2018, DOI: 10.1038/s41467-017-02651-5.

Abstract

ABSTRACT #3 **ORAL**

From genotype to gonadal fate: A functional validation of WT1 variants

Mehdi Totonchi

Institute of Molecular Biology, "Roumen Tsanev", Bulgarian Academy of Sciences

KEYWORDS WT1; genotype–phenotype correlation; functional validation; differences of sex development; gene regulation

Background: Connecting genetic variation to clinical phenotype requires understanding how variants alter developmental regulatory networks. SRY-negative 46,XX testicular and ovotesticular differences of sex development (DSD) provide a model for investigating how disruption of these networks redirects gonadal fate. This presentation uses our published WT1 study to illustrate an integrated approach linking clinical phenotyping, variant discovery, and downstream functional validation.

Methods: Exome sequencing was performed in 78 individuals with unexplained 46,XX testicular or ovotesticular DSD. Clinical and histological findings were integrated with variant analysis. Functional investigations included transcriptional reporter assays, endogenous gene-expression analysis in human granulosa cells, protein-interaction assays, and a genome-edited mouse model.

Results: Seven individuals (8.97%) carried five distinct heterozygous variants affecting the fourth zinc-finger domain of WT1. Phenotypes ranged from testicular to ovotesticular DSD; the recurrent p.Arg495Gln variant was also associated with 46,XY gonadal dysgenesis in an affected sibling, highlighting the importance of chromosomal context. Functional studies centered on p.Arg495Gly demonstrated altered transcriptional activity, including increased testis-pathway activity when coexpressed with wild-type WT1. Enhanced interaction with β-catenin supported a proposed mechanism involving interference with pro-ovarian signaling. Homozygous mutant XX mouse gonads showed partial masculinization, with SOX9-positive cells and reduced FOXL2 expression; heterozygous mice did not reproduce this fetal gonadal phenotype.

Conclusion: These findings illustrate how protein-domain specificity, chromosomal context, and dosage-dependent effects shape genotype–phenotype relationships. Functional validation is essential for moving beyond variant detection toward biological interpretation. Building on this example, the presentation will outline a broader framework integrating detailed phenotyping with complementary functional models and discuss how future transcriptomic and chromatin studies could investigate regulatory mechanisms underlying phenotypic variability in rare developmental disorders.

Abstract

ABSTRACT #4 **ORAL**

Analysis of the function of H2A.Z by using mini-auxin-inducible degron 2 (mAID2) technology

Dimitar Iliev, Tanya Gyuleva, Banko Bankov, Momchil Popov, Aleksander Aleksandrov, Stefan Dimitrov

Institute of Molecular Biology, "Roumen Tsanev", Bulgarian Academy of Sciences

The histone variant H2A.Z is an epigenetic factor implicated in regulation of the structure and the function of the genetic material in eukaryotic cells. Alterations in H2A.Z chromatin are associated with perturbed segregation of chromosomes and, thereby, pathologies such as spontaneous abortions, infertility, birth defects as well as development of cancer. H2A.Z promotes proliferation by selectively regulating cell cycle components, suggesting an H2A.Z-mediated mechanism for cancer progression. Detailed studies on cell cycle regulators are difficult without implementation of systems for rapid control of protein expression. In the current project, we will take advantage of the well-established auxin-inducible degron 2 (mAID2) technology. This system provides a sharp degradation control of a target protein and it will be used to deplete H2A.Z at specific cell cycle stages in U2OS cancer cells. This project is expected to advance our understanding of the detailed molecular mechanisms that give rise to the above-mentioned disorders.

Abstract

ABSTRACT #5 **ORAL**

Existence of analytic expression for stationary total current of a single cycle process as a starting point of analysis for various complex biological systems

Alexander G . Dimitrov

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KEYWORDS cycle, driving force, affinity, analysis

Background. Some time ago to solve his specific problem the author created a general method that allowed to represent analytically the current flowing through a stationary cyclic process. However, cycles often quasi stationary, are present all over the entire nature and current through every one of them could be represented in the same way.

Methods. Theoretical and mathematical.

Results. An expression for stationary total current that equilibrates the existing forward and backward cycle currents is presented. The total current naturally factors into driving force related part and affinities related part. The former determines the general behavior of the current as it depends on the size of the driving force that in turn, could be written directly and straightforwardly from the stoichiometry of the process and the rules of thermodynamics. The latter has a potential to attenuate the size of the current. Thus different isoforms in a cycle with a same stoichiometry and so driving force could differ in their affinity to some substance(s) thus altering their behavior.

Conclusion. To form a cycle one needs only 3 states. Much more common is the situation when there are many possible interacting cycles. Writing approximation to any one of them would be straightforward. Analysis of interactions between cycles, could then be studied by means of theoretical biology and thus provide additional point of view on practically any field in biology. In epigenetics one of the main questions under research could be formulated as: “When individual processes are turned on or off?” In such situation the ability to write analytically individual currents would be of great value and their details could explain lots of things. All of this would depend on the concentrations of the involved substances as they would form both the possible driving force and affinities.

Acknowledgment. Supported by BNSF КП-06-Н93/2 from 08.12.2025

Abstract

ABSTRACT #6 **ORAL**

Effects of Changes in Chromatin Structure on Energy Source Utilization, Nutrient Sensing, and Ageing

Penyo Ivanov[1], Dessislava Staneva[1], Alastair Fleming[2], Milena Georgieva[1], George Miloshev[1]

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Chromatin structure and its dynamic organization are among the most complex epigenetic regulatory mechanisms, and therefore, their contribution to epigenetic inheritance remains far from completely understood. It is well established that alterations in chromatin structure can reshape the epigenetic landscape of the genome and, consequently, influence cellular responses to both intrinsic and environmental factors, including nutrient sensing and energy source utilization. Ageing and age-associated pathological conditions are accompanied by profound changes in energy metabolism and in cellular responses to, and utilization of, nutrients. In this study, we investigated how alterations in chromatin structure affect cellular responses to different energy sources and calorie restriction, with the broader aim of exploring the relationship between chromatin organization and cellular adaptation to fluctuations in the environment.

We used the budding yeast Saccharomyces cerevisiae, a well-established model organism with highly tractable genetics, to investigate the effects of chromatin alterations on cellular metabolism and ageing. We analyzed strains carrying deletions of genes encoding two chromatin-associated proteins: the linker histone Hho1p and the high-mobility-group (HMG) protein Hmo1p. Replicative ageing, cell survival, metabolite accumulation, mitochondrial activity, and nutrient sensing were assessed under different nutritional and metabolic conditions.

Alterations in chromatin structure were associated with changes in several key cellular processes. Notably, deletion of HHO1 or HMO1 resulted in opposite effects on cellular growth, indicating distinct roles for these chromatin proteins. Mitochondrial membrane potential and cellular responses to different energy sources were also altered. Although these changes were not severe, they were associated with differences in cellular morphology, mitochondrial state, and nutrient-sensing responses. Importantly, the phenotypic effects were strongly dependent on cellular age, suggesting that the consequences of altered chromatin structure become increasingly apparent during ageing.

Our results highlight a potentially important connection between chromatin organization, metabolic regulation, and ageing and may contribute to a better understanding of the molecular mechanisms linking chromatin alterations to age-associated cellular dysfunction.

This work is supported from BNSF by Grant number KP-06-H81/3.

Abstract

ABSTRACT #7 **ORAL**

From molecular diagnosis to integrated care: bridging the implementation gap in rare diseases

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Institute for Rare Diseases, Bulgaria

Advances in genomics, epigenetics and molecular diagnostics are expanding the possibilities for diagnosing rare diseases and developing personalised interventions. Diagnostic innovation alone, however, does not ensure timely referral, appropriate treatment or continuity of care. This presentation examines the health system conditions required to translate molecular progress into benefit for patients.

A narrative analysis of European rare disease policy frameworks and of Bulgarian regulatory and organisational experience was conducted. On this basis, an integrated patient pathway is proposed, linking clinical suspicion, molecular diagnosis where indicated, expert assessment, individual care planning and long-term follow-up. Its key enabling mechanisms are centres of expertise, European Reference Networks, standardised disease identification using the Orphanet nomenclature and ORPHA codes, interoperable health information systems, and clearly defined and coordinated responsibilities across primary and specialised care. The Bulgarian experience illustrates how formally established policy instruments may remain disconnected from routine clinical practice. The main gaps concern referral pathways, data exchange between information systems, continuity of care, and the alignment of expert recommendations with healthcare financing and patient follow-up. The proposed model distinguishes existing arrangements from the regulatory and organisational changes needed for implementation.

Realising the potential of molecular medicine in rare diseases therefore depends as much on how care is organised as on diagnostic technology. An integrated patient pathway offers a framework for linking scientific progress with clinical expertise and long-term follow-up. Its feasibility and its effect on access to care and patient outcomes require further evaluation.

Abstract

ABSTRACT #8 **ORAL**

DNA damage and innate immune responses in health and disease

George Garinis

University of Crete and IMBB-FORTH, Greece

We will provide evidence that DNA damage extends beyond genome instability to become a potent regulator of innate immunity. Persistent DNA lesions, defective repair and transcriptional stress generate aberrant or misplaced self-nucleic acids that activate innate immune sensors, including cGAS–STING and related DNA- and RNA-sensing pathways. While these responses are initially protective, their persistence can drive chronic sterile inflammation, cellular senescence and tissue dysfunction. Using examples from DNA-repair disorders, ageing and inflammatory disease, the talk will explore how failure to resolve genome-damage-induced immune signalling may provide a mechanistic link between accumulated molecular damage and systemic pathology, and may reveal new therapeutic opportunities for restoring tissue homeostasis.

Abstract

ABSTRACT #9 **ORAL**

Oligogenism as a cause of human infertility

Christophe Arnoult

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Male gametogenesis, or spermatogenesis, is a highly complex physiological process which involves several thousands of genes of which a large part expresses almost exclusively in the testis. Because of this multiplicity of genes, spermatogenesis is strongly affected by genetic alterations, leading to male infertility.

Given the substantial contribution of genetic defects to male infertility, numerous rare genetic disorders affecting male reproduction have been described. A significant proportion of male infertility results from impaired spermatogenesis, leading to the production of spermatozoa with a reduced or absent ability to support natural fertilization and, in some cases, normal embryonic development. Among the different male infertility phenotype, our lab is working on a phenotype knows as MMAF for multiple morphological abnormalities of the sperm flagella (MMAF), a severe form of asthenoteratozoospermia (OMIM 72 entry).

The MMAF syndrome is characterized by a heterogenous phenotype with several types of flagellar defects, including short, coiled, bent, irregular or missing flagella. Flagellar defects have a severe negative effect on sperm motility, with sperm showing reduced or absence of motility. MMAF is a recessive disease and he number of genes linked to the MMAF phenotype has been rising drastically: now more 72 genes have been associated with MMAF in humans and/ or in mice and our team has substantially contributed by the identification of 22 genes among the 72. We have investigated the possibility that infertility could be due to oligogenism and that accumulation of heterozygote mutations in genes involved in MMAF could induce a spermatogenic failure. We show that qualitative abnormalities in sperm can arise from the accumulation of heterozygous variants in distinct genes, thereby establishing oligogenicity as a genuine mode of inheritance in male infertility. Drawing on our demonstration of oligogenicity for morphological sperm defects, we initiated investigations to determine whether concentration sperm defects can likewise result from combinations of genes within the ncRNA pathway (PIWI/piRNA) and involved in the same phenotype of total absence of spermatozoa in the ejaculate (called azoospermia). We focused on the PIWI/piRNA pathway, a conserved, germline-specific small RNA–mediated mechanism essential for safeguarding genome integrity. Biallelic variants in its components is a recurrent cause of spermatogenic failure with azoospermia in humans and animal models.

Abstract

ABSTRACT #10 **ORAL**

Modeling rare diseases: the example of hereditary peripheral neuropathies

Albena Jordanova

VIB Center for Molecular Neurology, University of Antwerp, Belgium, Molecular Medicine Center, Department of Biochemistry, Medical University-Sofia, Bulgaria

To address the etiological complexity and data scarcity inherent to small patient populations, rare disease research increasingly relies on advanced disease modeling. Robust disease models provide the foundational framework required to decipher cellular and molecular pathomechanisms and engineer targeted therapeutic interventions. This utility is exemplified by hereditary peripheral neuropathies, which affect approximately 1 in 2,500 individuals and are characterized by dying-back axonal degeneration and demyelination of motor and sensory peripheral neurons. While mammalian models are widely used due to their evolutionary proximity and physiological alignment with humans, non-mammalian vertebrate, invertebrate, and simple eukaryotic models also play critical roles in uncovering the mechanisms of peripheral nerve degeneration. Because no single model captures the full spectrum of human pathology, an integrative framework combining multiple organisms offers the highest probability of success. Consequently, establishing unified disease modeling protocols across diverse species is essential to ensure experimental consistency and maximize data comparability.

Abstract

ABSTRACT #11 **ORAL**

Genomic research in rare disorders

Julien Thévenon

Grenoble Alpes University Hospital, France

Genomic research in rare disorders increasingly relies on the tight integration of clinical diagnostics, variant interpretation, and technological innovation. At Grenoble Alpes University Hospital, our Department of Genetics, Genomics, and Reproduction has developed a translational approach that combines high-throughput sequencing, customized bioinformatics pipelines, and artificial intelligence tools to accelerate the identification of pathogenic variants.

Our workflow is built on systematic analysis of large-scale VCF files, exploitation of variant-impact scores such as AlphaGenome/AVI, and allelic data visualization (VAF plots, heatmaps) to prioritize diagnostic candidates. These Python-based pipelines ensure reproducible, scalable interpretation suited to both routine diagnostics and research projects on intellectual disability and ultra-rare conditions.

Beyond technical analysis, we coordinate an inter-university hospital alliance aimed at pooling genomic resources and deploying clinically validated AI tools (SeqOne, Diagho) for patients across the Alpine region. This strategy aligns with the French National Rare Disease Plan 4 and the France Genomic Medicine 2025 initiative, emphasizing equitable access, diagnostic quality, and multi-omics data integration.

This presentation will illustrate how the synergy between medical expertise, data engineering, and territorial cooperation is transforming rare disease care—shortening diagnostic odysseys and enabling targeted interventions.

Abstract

ABSTRACT #12 **ORAL**

Chromatinopathies: from altered genome structure to disease etiology

Stefan Dimitrov

Institute of Molecular Biology, "Roumen Tsanev", Bulgarian Academy of Sciences

My talk will be focused on the molecular etiology of a set of rare diseases due to alterations in the 3D structure of the human genome. A rare disease is defined as one that affects less than 1 in 2000 people in Europe. It is estimated that 25-30 000 000 of people are suffering of rare diseases in Europe and in Bulgaria, the number of rare diseases suffering people is believed to exceed 350-400,000. Rare diseases are a worldwide healthcare challenge with over 7000-8000 different disorders affecting at average more than 6% of the population in western societies. These disorders are disabling, expensive to manage and one third of children with a rare disease die before their fifth birthday. The patients need personalized care and medicine and understanding the etiology of the disease is mandatory.

Yet, the etiology of the majority of the rare diseases is either poorly understood or unknown. Furthermore, the scientific reports on rare diseases are generally describing the disease at "bird eye flight" view and the use of the modern biology techniques to shed light on the disease origins is very restricted and unsatisfactory. To be able to help efficiently the rare disease patients, it is absolutely necessary to overcome this gap. With this in mind, we are developing and applying a novel, unique and highly original workflow, which, in addition to the standard approaches including diagnosis with NGS, is using a large cohort of the cutting edge molecular and cellular biology, biochemistry and omics methods combined with structural approaches and generation of animal models with the help of CRISPR/CAS technology and mouse genetics.

By using this approach, we have shed light on the molecular etiology of Rett syndrome, a very severe neurodevelopmental rare disease, due to mutation in the methyl-DNA binding protein MeCP2. These data, as well as the data on the molecular mechanism of Rahman syndrome, a recently described neurogenerative diseases, associated with the expression of the truncated form of the linker histone H1.4, will be in the focus of my talk.

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Abstract

ABSTRACT #13 **ORAL**

Chromatin dynamics in health and disease

Seyit Kale

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The integrity of our genomic material is critical for cell survival and faithful inheritance. The recently characterized Rahman syndrome (RS) is a rare genetic disorder associated with frameshift mutations in the gene encoding the linker-histone H1.4 protein, a key structural component of chromatin. A broad and heterogeneous phenotypic spectrum has been reported in RS patients, including intellectual disability, skeletal and cardiac anomalies, and Down syndrome-like facial gestalt. All known disease-causing mutations are located within a narrow locus encoding the carboxy-terminal region (CTB) of H1.4. Using atomistic molecular dynamics simulations validated by a broad set of structural experiments, we reported that the altered electrochemical composition of the RS H1.4 CTB disrupts genome packaging in one patient. However, it is unclear to what extent the same mechanism applies to other RS patients with a diagnosis. Extending our prior result to now about a dozen patients, we demonstrate that the loss of H1.4's DNA-packaging ability is not only the origin of the disease but also a direct predictor of the patient's clinical profile. In the second part of my talk, I will show how mitosis-related biophysical changes in the cell directly affect chromatin in an epigenetically dependent manner.

Abstract

ABSTRACT #14 **ORAL**

Imprinting disorders - or how the genes escape from the Mendel laws

Ivanka Dimova

Medical University, Sofia, Bulgaria

Genomic imprinting is an epigenetic mechanism that determines the monoallelic expression of specific genes according to their parental origin. This process is primarily regulated by allele-specific DNA methylation at imprinting control regions, established during gametogenesis and maintained after fertilization. Imprinting disorders encompass a broad spectrum of clinical manifestations affecting growth, neurodevelopment, and metabolic regulation. Their underlying molecular mechanisms include uniparental disomy (UPD), copy number alterations, pathogenic sequence variants, and epimutations.

This presentation explores the mechanisms and clinical relevance of genomic imprinting through a diagnostically challenging case of a child with severe developmental delay and isovaleric acidemia. The identification of a homozygous variant in the IVD gene, with only the father carrying the variant, prompted investigation of an unusual inheritance mechanism. Analysis of polymorphic markers and chromosome-wide homozygosity supported paternal uniparental isodisomy of chromosome 15. This mechanism explains both the autosomal recessive metabolic disorder and Angelman syndrome, illustrating how a single genomic event can result in two distinct diagnoses.

Results from MLPA testing of 119 prenatal and postnatal samples are also presented. Overall, 16 abnormal findings were identified: 2 among 16 prenatal samples (12.5%) and 14 among 103 postnatal samples (13.6%). Postnatal findings included methylation abnormalities associated with Prader–Willi, Silver–Russell, Beckwith–Wiedemann, and Angelman syndromes, as well as copy number alterations.

These findings highlight the importance of integrating clinical assessment, sequencing, parental-origin analysis, and methylation testing in the diagnostic evaluation of suspected imprinting disorders. Defining the underlying molecular mechanism is essential for establishing an accurate diagnosis, assessing recurrence risk, and providing appropriate genetic counseling. Prenatal diagnosis requires consideration of tissue-specific methylation patterns and the use of methods validated for the relevant locus and sample type.

Abstract

ABSTRACT #15 **ORAL**

Epigenetic Alterations in Breast Cancer: From Molecular Dysregulation to Diagnostic, Prognostic and Predictive Biomarkers

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KEYWORDS breast cancer; epigenetics; DNA methylation; non-coding RNA; liquid biopsy

Background: Breast cancer is a molecularly heterogeneous disease in which genetic alterations interact with dynamic epigenetic mechanisms to drive tumor initiation, progression, and therapeutic response. DNA methylation, histone modifications, chromatin remodeling, and non-coding RNAs contribute to aberrant gene regulation and may offer molecular insight beyond conventional genetic and histopathological characterization.

Methods: We performed a focused literature-based synthesis of the major epigenetic mechanisms in breast cancer and their potential as diagnostic, prognostic, and predictive biomarkers, with emphasis on DNA methylation, microRNAs, long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs), and their detection in tissue and liquid biopsy specimens, including cell-free DNA and circulating RNAs.

Results: Epigenetic alterations are implicated in breast cancer initiation, molecular subtyping, progression, recurrence, and treatment resistance. Aberrant DNA methylation patterns support early detection and molecular classification. Circulating microRNAs show meta-analytic evidence for both diagnostic and prognostic utility: miR-155 has demonstrated diagnostic accuracy for breast cancer detection, while miR-21 and the lncRNA MALAT1 are consistently associated with poor prognosis and survival outcomes across pooled analyses. Circulating circRNAs are emerging as promising diagnostic and prognostic candidates detectable in plasma. Liquid biopsy approaches enable minimally invasive, longitudinal monitoring of these tumor-associated molecular changes and may support assessment of treatment response, including lncRNA-mediated mechanisms of drug resistance. However, biological and analytical heterogeneity, the absence of standardized cut-off values, and limited independent clinical validation remain major barriers to routine clinical implementation.

Conclusion: The epigenome constitutes a dynamic molecular layer linking genomic alterations to breast cancer phenotype and clinical behavior. Epigenetic biomarkers hold promise for advancing precision breast cancer medicine, but their clinical translation will require standardized analytical platforms, robust multi-cohort validation, and demonstrated clinical utility.

Abstract

ABSTRACT #16 **ORAL**

The antagonistic regulation of gene transcription by the chromatin remodeler and co-activator Swi-Snf and the co-repressor Tup1-Cyc8 in yeast

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KEYWORDS chromatin, Swi-Snf and Tup1-Cyc8 interplay, active inactive gene promoters

The yeast Swi-Snf co-activator was the first chromatin remodelling complex discovered which can disrupt promoter nucleosomes to enable transcription. The Tup1-Cyc8 complex was the first global co-repressor identified which can position nucleosomes across promoters to block transcription. However, the global extent of their antagonistic regulation of gene transcription in yeast is unknown.

We have identified the genes repressed by Tup1-Cyc8 and activated by Swi-Snf in glucose-grown cells which include inactive and actively transcribed genes. We show that Swi-Snf and Tup1-Cyc8 are present at both active and inactive gene promoters, where SNF2 is required for full Tup1-Cyc8 occupancy. The influence of Swi-Snf and Tup1-Cyc8 upon nucleosome positioning is specific to each co-regulated gene and extends over the available upstream intergenic region.

We propose Tup1-Cyc8 and Swi-Snf are constitutively present at co-regulated gene promoters where their interplay influences the entire intergenic upstream chromatin environment of target genes and transcription.

Abstract

ABSTRACT #17 **ORAL**

An automatic tracker for chromosome trajectories in mitotic and interphase chromosomes

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The three-dimensional organization of chromatin inside the cell is essential for gene regulation. Several case studies have pinpointed faulty spatial locations of chromatin as the driver of rare diseases - for example, the Rett syndrome [1] and the Hutchinson-Gilford progeria syndrome [2] – emphasizing the importance of studying chromatin 3D dynamics. Previously, we developed a method for segmentation and tracking of mitotic chromosomes in single, live cells, using microscopy and deep learning [3]. However, the procedure is specific to mitosis and requires time-consuming manual corrections, which limit its throughput. Here we introduce its successor – Fully Automatic Tracking of Chromosomes in Mitosis Pipeline (FAT CHIMP). It decimates the time required to run the analysis and greatly reduces the required manual corrections. Importantly, FAT CHIMP was implemented with a graphical user interphase, eliminating the need for python coding completely. By combining FAT CHIMP with centromere tracking in a dual-tagged cell line, we demonstrate for the first time a workflow for identifying and tracking chromosomes over the whole cell cycle. Therefore, we envision FAT CHIMP as a go-to approach in studies aiming to interrogate potential irregularities in chromatin organization in rare pathologies in a high throughput manner.

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Abstract

ABSTRACT #18 **ORAL**

The Role of a Differentiated Approach in the Screening and Diagnosis of Breast Cancer in Women with Genetic Mutations and Rare Diseases

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KEYWORDS breast cancer, genetic mutations, rare diseases, microRNA, liquid biopsy, differentiated screening, cost-effectiveness, artificial intelligence

Introduction: Standard age-based breast cancer screening is often insufficient for women with hereditary predisposition or rare genetic syndromes. Carriers of pathogenic variants in BRCA1/2, PALB2, TP53, CHEK2, ATM, and other genes require a risk-tailored approach that accounts for individual heterogeneity in cancer risk. Modern molecular biomarkers — including circulating microRNAs (miRNAs), liquid biopsy, and AI-based image analysis — expand the possibilities for earlier and more precise diagnosis.

Objective: To justify the need for a differentiated, genetically informed model of breast cancer screening and diagnosis in high-risk women and patients with rare diseases, and to assess its potential health-economic impact relative to current practice in Bulgaria.

Methods: We propose a conceptual model incorporating initial genetic triage via multigene panels for patients with a family history, early age of onset, multiple primary tumors, or clinical features suggestive of a hereditary syndrome. The resulting differentiated pathways integrate annual MRI, molecular biomarkers, circulating miRNAs, liquid biopsy, and AI-assisted imaging analysis. Circulating miRNAs are promising non-invasive biomarkers for early detection, risk stratification, and monitoring of disease progression, although their routine clinical use remains under validation.

Results: In Bulgaria, only an estimated 12% of breast cancer cases are detected at an early stage (source to be confirmed — see reviewer note), and as of 2026 no organized national population-based breast cancer screening program exists. A differentiated approach would improve identification of high-risk patients for whom standard mammography is insufficient, and would establish a clearer diagnostic pathway for women with rare conditions. Expected outcomes include earlier-stage diagnosis, less invasive treatment, reduced direct and indirect healthcare costs, and improved patient quality of life.

Conclusion: Transitioning to personalized screening based on genetic risk, innovative biomarkers, and patient-centered care is strategically essential. Integrating multigene panels, MRI, liquid biopsy, and miRNA profiling has the potential to increase diagnostic sensitivity, improve cost-effectiveness, and expand access to personalized cancer care for vulnerable populations.

Abstract

ABSTRACT #19 **ORAL**

From biomarkers to dynamic biological age: an AI-driven framework for personalized health monitoring

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KEYWORDS biological aging; biomarkers; epigenetics; artificial intelligence; precision health

Background: Biological ageing is a complex, multidimensional process that neither chronological age nor individual biomarkers fully capture. DNA methylation-based ageing measures provide valuable molecular information, but integrating them with continuously collected physiological and lifestyle data could enhance their potential for precision health. EPIX.AI is developing an AI-driven framework to transform heterogeneous biomarker data into a dynamic representation of an individual's biological state.

Methods: The EPIX.AI platform integrates DNA methylation biomarkers, genetic information, blood-based parameters and longitudinal physiological data, including heart rate, sleep and activity measurements obtained from wearable devices. Machine-learning approaches establish relationships between physiological patterns and validated biological-age measures. We developed a PhenoAge-calibrated model to translate longitudinal physiological signals into a continuously updated indicator of biological aging state, while a separate longitudinal component captures changes in this state over time.

Results: The resulting framework distinguishes between current biological state and its longitudinal dynamics. The EPIX Daily Aging Index provides a continuously updated, PhenoAge-calibrated representation of physiological aging state, whereas EPIX Aging Velocity characterizes the direction and magnitude of changes in this state. This architecture enables integration of molecular and real-world longitudinal biomarkers and creates a framework for monitoring individual trajectories and responses to health interventions.

Conclusion: EPIX.AI demonstrates how AI can move biological-age assessment from a static biomarker measurement toward a dynamic, multimodal biomarker framework. Integrating epigenetic, genetic, clinical, and wearable-derived data may provide a foundation for individualized risk assessment, precision prevention, and quantitative monitoring of responses to interventions.

Abstract

ABSTRACT #20 **ORAL**

Beyond the Genetic Code: DNA Screening, Nutrigenetics and Epigenetics in the Dynamic Response to Diet and Disease Prevention

Olga Antonova

Medical University Sofia, Bulgaria

Abstract

ABSTRACT #21 **ORAL**

Drugging epigenetic pathways to treat fungal infections

Jérôme Govin

Inserm and University of Grenoble, France

Fungal infections can be life-threatening, particularly in immunocompromised patients. Treatment options remain limited, and the emergence of drug resistance highlights the need for new therapeutic strategies. Our research explores whether targeting the molecular machinery that controls fungal gene expression could provide a new approach to antifungal treatment.

We focus on Bdf1, a protein that recognizes acetylation on histones and helps regulate gene activity. Combining genetics, structural biology and chemical screening, we showed that its recognition domains, known as bromodomains, are essential for the survival of two major fungal pathogens, Candida albicans and Candida glabrata. We identified compounds that preferentially inhibit fungal bromodomains over their human counterparts. We also demonstrated that a BET bromodomain inhibitor acts against several Candida species, including drug-resistant clinical isolates, and shows efficacy in an invertebrate infection model.

This talk will illustrate how insights into chromatin regulation can guide antifungal discovery, while highlighting the challenges of developing compounds that effectively target fungi and spare human cells.

Abstract

ABSTRACT #22_1 **ORAL**

From Scientific Discovery to Patient Access: The Role of the Pharmaceutical Industry in Rare Disease Innovation

Gogo Krastev

Country Lead, Genesis Pharma, Bulgaria

KEYWORDS rare diseases; RNA interference; RNAi therapeutics; pharmaceutical innovation; orphan drugs; market access; health policy; hereditary transthyretin amyloidosis; precision medicine.

Rare diseases represent one of the greatest challenges and opportunities in modern medicine. Although more than 300 million people worldwide are affected by over 7,000 rare diseases, effective therapies remain available for only a small proportion of these conditions. The journey from scientific discovery to patient access is typically long, costly, and characterized by a high rate of failure, highlighting the critical role of the pharmaceutical industry in translating basic science into clinical benefit.

The development of RNA interference (RNAi) therapeutics provides a compelling illustration of this process. The first observations of gene silencing phenomena emerged in plants in 1990, when Napoli and Jorgensen described unexpected suppression of pigment production in petunias. This fundamental biological observation was transformed into a therapeutic platform after Fire and Mello demonstrated sequence-specific gene silencing in Caenorhabditis elegans in 1998, work that was subsequently recognized with the Nobel Prize in Physiology or Medicine in 2006. Industry investment, particularly through the establishment of Alnylam Pharmaceuticals in 2002, enabled the translation of RNAi biology into a drug development platform capable of addressing previously untreatable rare diseases.

However, scientific promise alone does not guarantee success. Numerous RNAi programs were discontinued because of delivery challenges, insufficient efficacy, or safety concerns before reaching patients. Similar patterns have been observed across the rare disease field. Gene therapies for several metabolic disorders, antisense oligonucleotide programs, and enzyme replacement approaches have failed during clinical development despite strong preclinical rationale. Industry-sponsored research and development must therefore navigate a complex landscape of scientific uncertainty, regulatory requirements, manufacturing challenges, and financial risk.

Abstract

ABSTRACT #22_2 **ORAL**

From Scientific Discovery to Patient Access: The Role of the Pharmaceutical Industry in Rare Disease Innovation

Gogo Krastev

Country Lead, Genesis Pharma, Bulgaria

KEYWORDS rare diseases; RNA interference; RNAi therapeutics; pharmaceutical innovation; orphan drugs; market access; health policy; hereditary transthyretin amyloidosis; precision medicine.

Conversely, successful examples demonstrate the transformative potential of sustained investment. Therapies such as nusinersen for spinal muscular atrophy, patisiran and vutrisiran for hereditary transthyretin amyloidosis, and several gene therapies for inherited retinal and hematologic disorders have shown how innovative platforms can alter the natural history of severe rare diseases. These achievements required decades of collaboration among academic researchers, biotechnology companies, pharmaceutical manufacturers, regulators, patient organizations, and healthcare systems.

Yet regulatory approval represents only one milestone. Market access remains a major barrier for rare disease therapies. In the United States, high treatment costs, payer restrictions, and prior authorization requirements may delay access even after Food and Drug Administration approval. In Europe, disparities in health technology assessment processes, reimbursement negotiations, and national budget constraints contribute to significant inequalities in treatment availability between countries. Patients in lower-income healthcare systems often experience prolonged delays before gaining access to innovative therapies.

These challenges are particularly evident in Bulgaria, where limited healthcare resources, lengthy reimbursement procedures, restricted diagnostic pathways, and delayed adoption of orphan medicines continue to affect patients with rare diseases. The gap between regulatory approval and actual patient access remains substantial, emphasizing the need for stronger collaboration among policymakers, healthcare institutions, patient organizations, and industry stakeholders.

The history of rare disease innovation demonstrates that breakthrough discoveries are only the starting point. Pharmaceutical companies play a pivotal role not only in drug discovery and clinical development but also in overcoming the regulatory, economic, and logistical barriers that determine whether scientific innovation ultimately reaches patients. Understanding and addressing these challenges will be essential for translating future advances in molecular medicine and epigenetic science into meaningful health outcomes for individuals living with rare diseases.

Abstract

ABSTRACT #23 **ORAL**

Neurotensin(8-13) Analogs Targeting NTS1 and NTS2 Receptors

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KEYWORDS neurotensin (NT); neurotensin receptors type 1 and 2; molecular modeling; NT(8-13) analogs

Background: The simultaneous activation of both neurotensin type 1 and 2 receptors (NTS1R and NTS2R) through the neuronal peptide neurotensin (NT), activating the dopamine (DA) release and DA signaling within the dopaminergic system in the brain, suggest that NTS1R/NTS2R dual-specific NT analogs may represent an attractive tool in the treatment of Parkinson's disease (PD) and/or other related conditions.1,2
Methods: Herein, we report in silico exploration of NTS1R and NTS2R driven by in vitro pharmacological evaluation of the linear hexapeptide NT analogs 3 (sequence Lys8-Cav9-Pro10-Tyr11-Ile12-Leu13) and 4 (Arg8-Cav9-Pro10-Tyr11-Ile12-Leu13), both active towards the human NTS1R and NTS2R.
Results: Compared to the parent peptide NT(8-13) (2), compounds 3 and 4 showed improved in vitro human plasma stability and BBB permeability. Moreover, in silico ADMET evaluation indicated that both NT-analogs have strong pharmacological properties combined with good safety profiles, highlighting their potential for further structural improvements. Furthermore, we applied an AI-based approach to generate the homology models of hNTS1R and hNTS2R, followed by MD simulations of their ligand-free state and molecular docking in order to estimate the most probable protein–ligand complexes of peptides 3 and 4. Binding interaction/affinity analysis of the best-ranked docking modes, obtained with selected time-frames from the respective MD trajectories, suggest that the receptor activation occurs via a ligand-receptor binding into the initial "entry" conformation of hNTS1R and hNTS2R. This assumption is supported by additional HYDE analysis confirming the binding affinities of peptides 3 and 4 towards hNTS1R and hNTS2R obtained by radioligand binding experiments.3
Conclusion: The reported study may serve as a ready-to-use in silico approach for further development of therapeutic options against PD and potentially other neurological disorders.

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Abstract

ABSTRACT #24 **ORAL**

Optimized cell-based delivery & potentiation of oncolytic virotherapies

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Oncolytic virotherapy represents a promising approach to cancer treatment through selective viral replication, tumor destruction, and activation of antitumor immunity. Dr. Aladar Szalay will introduce the scientific foundations of genetically engineered oncolytic vaccinia viruses, highlighting their tumor-selective replication, molecular imaging capabilities, and preclinical antitumor activity. Emphasis will be placed on innovative cell-based delivery strategies that protect viruses from immune neutralization, enhance systemic tumor targeting, and improve therapeutic efficacy.

Building on these advances, Dr. Boris Minev will discuss the clinical relevance and translational development of oncolytic vaccinia platforms, including experience with cell-based virotherapy approaches in patients with advanced solid tumors. Clinical findings involving systemic and regional administration, viral replication, tumor localization, and combination treatment strategies will be presented. The discussion will further explore emerging approaches integrating oncolytic vaccinia viruses with engineered immune cells, including CAR-T cells, to overcome limitations of conventional immunotherapies in solid tumors.

Together, these presentations will illustrate how advances in viral engineering, cellular delivery, and clinical development may enable more effective, targeted, and potentially synergistic cancer immunotherapies.

Abstract

ABSTRACT #1 **POSTER**

Engineered Dendrimers as Advanced Antimicrobial Nanotools

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KEYWORDS Dendrimers, Antimicrobial Resistance, Nanomedicine, Biofilm Disruption

Background: The global rise of antimicrobial resistance poses a serious threat to modern healthcare, necessitating the development of innovative therapeutic strategies to combat resistant pathogens. Engineered dendrimers have emerged as multifunctional nanoplatforms capable of addressing these challenges. Characterized by a highly branched structure, precise monodispersity, and densely packed surface functional groups, these nanomacromolecules offer unique opportunities for specialized biomedical applications. This study analyzes dendrimers as potent antimicrobial agents by investigating their interactions with model lipid membrane systems that mimic the composition of bacterial membranes.

Methods: Advanced biophysical techniques, including Langmuir monolayers, Thin Liquid Films, Giant Unilamellar Vesicles, Large Unilamellar Vesicles, and electrostatic profiling, are applied to elucidate the antibacterial mechanisms of novel and established dendrimers.

Results: Through the modern and highly informative biophysical methods we established that studied dendrimers exert their antibacterial effect primarily through direct physical adhesion and interaction with model membranes. Due to the presence of cationic surface groups, they bind electrostatically to negatively charged lipid headgroups in the mimicking membranes. This disrupts the lipid bilayer, alters membrane potential, increases permeability, causes leakage of intracellular contents, and leads to cell lysis without triggering rapid bacterial resistance.

Conclusion: Structural modifications of dendrimers, including cationic surface groups enhance their ability to penetrate resilient bacterial biofilms and eradicate intracellular infections. Overall, engineered dendrimers represent a transformative paradigm at the intersection of nanomedicine and infectious disease control. Future efforts focused on optimizing biocompatibility and scalable synthesis will be pivotal for translating these antimicrobial nanotools into clinical practice.

Funding: This study is financed by the Scientific Research Fund of the Ministry of Education and Science - project КП-06-Н51/15, 19.11.2021.

Abstract

ABSTRACT #2 **POSTER**

The Role of a Differentiated Approach in Screening and Diagnosis of Pancreatic Cancer in Individuals with Genetic Mutations, High-Risk Syndromes, and Pancreatitis

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KEYWORDS Pancreatic cancer, genetic screening, high-risk syndromes, risk-stratified screening, microRNA, ctDNA, medico-economic effect, EUS, MRI, pancreatitis

Introduction: Pancreatic cancer (PC) remains one of the most lethal malignancies, with over 75% of cases diagnosed at an advanced stage. Approximately 5–10% of cases are linked to hereditary predisposition from germline mutations or rare cancer syndromes. Standard clinical pathways fail to detect PC early, resulting in poor outcomes.

Objective: To propose a risk-stratified, genetically informed screening model for high-risk individuals and evaluate its medico-economic impact in the Bulgarian healthcare context.

Methods: A conceptual model for early detection is presented, based on initial genetic screening using multigene panels in individuals with family history or known predisposing syndromes (e.g., Lynch, Peutz-Jeghers, BRCA1/2, CDKN2A). Differentiated surveillance includes annual MRI/MRCP and endoscopic ultrasound (EUS). The approach integrates innovative tools such as ctDNA, microRNA biomarkers, and AI-supported image analysis. This model is compared to current clinical practice in Bulgaria, where no organized screening program exists. High-risk groups with acute and chronic pancreatitis are also included, as these conditions confer additional genetic predisposition.

Results: Bulgaria lacks a national PC screening strategy, leading to delayed diagnosis and high treatment costs—often exceeding 100,000 BGN per patient in advanced stages. In contrast, early detection through resection is significantly less costly (30,000–50,000 BGN) and associated with improved survival. The annual cost of surveillance per high-risk individual (1,500–2,500 BGN) is minimal compared to the late-stage economic burden.

Conclusion: Transitioning to a risk-adapted, precision-based screening model for pancreatic cancer is clinically and economically justified. Integrating genetic risk assessment, advanced imaging, and molecular biomarkers offers a sustainable strategy for improving early detection, outcomes, and healthcare efficiency—especially in countries like Bulgaria with limited resources and high unmet needs.

Abstract

ABSTRACT #3 **POSTER**

Galectin-3 – A possible regulator of complement system activity?

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KEYWORDS Galectin-3, C1q, C3

Background: Galectin-3 is a multifunctional protein, localised in the cytoplasm, in the nucleus, on the cell surface as well as in the extracellular spaces. Galectin-3 acts as a regulatory protein in various biological proccesses, including the immune response. Currently, the immune function of the extracellular Galectin-3 is reffered to the activated lymphocytes. However, in the serum there are molecules, which are implicated in the early phases of the immune response like the proteins of the Complement system. Some of these proteins might be subjected to a functional regulation by the extracellular Galectin-3.

Methods: To investigate a possible interaction of Gal-3 with C1q and C3 we performed ELISA analysis, Dot blot assay, fluorescent spectroscopy and antibody phage display.

Results: Gal-3 and C1q interacted with a pronounced affinity based on electrostatic attraction, aided by a plethora of hydrogen bonds. Fluorescence spectroscopy gave quantitative assessment to the recognition with Kd value of 0,04 μM. It was a protein-protein interaction between the globular "head" regions of C1q and the backside of the lectin domain of Gal-3. Gal-3 formed complexes with C3 in both immobilised and soluble state and the nature of the interaction is again protein-protein. Dot blot analysis and ELISA assay showed that the bindidng site for Gal-3 was within the C3c fragment. We selected high affinity monoclonal anti-Gal-3 scFv antibodies from the phage library „Griffin.1". Ten clones were able to inhibit the binding of Gal-3 to C1q and C3.

Conclusion: C1q and C3 form complexes with Gal-3 through portein-protein interaction. These results could shed light onto understanding the uderlying molecular mechanisms of the interactions concerning the pathogenesis of various diseases. The selected monoclonal scFv ati-Gal-3 could be applied as competitors.

Acknowledgements: The research was financially supported by Grant KP-06-N41-9 of the Bulgarian NSF.

Abstract

ABSTRACT #4 **POSTER**

Dynamics of the replication fork stalling and restart in BRCA2/PALB1 deficient cells

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The BRCA2 protein plays a central role in maintaining genome stability during DNA replication, particularly under conditions of replication stress. Replication forks can stall when they encounter DNA damage, difficult-to-replicate regions, nucleotide depletion, or other sources of stress. BRCA2 is recruited to stalled replication forks through its interaction with PALB2. At these sites, BRCA2 and PALB2 promote the loading and stabilization of RAD51 onto single-stranded DNA. The resulting RAD51 nucleoprotein filament protects nascent DNA from degradation and facilitates repair through homologous recombination. Current evidence suggests that BRCA2 and PALB2, via RAD51, shield newly synthesized DNA strands and prevent excessive nucleolytic resection. To address this, we analysed the dynamics of replication fork stalling and restart by quantifying the kinetics of recruitment and removal of RPA1 and PCNA. in PALB2/BRCA2 deficient cells.

Abstract

ABSTRACT #5 **POSTER**

Design and development of novel low-molecular-weight carboxamides as dual modulators of MAO-B and α-Synuclein

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Neurodegenerative disorders such as Parkinson's disease (PD) are characterized by progressive dopaminergic neuronal loss and the pathological aggregation of α-synuclein (α-syn), accompanied by dysregulation of monoaminergic neurotransmission. Monoamine oxidase B (MAO-B) represents a validated enzymatic target involved in dopamine metabolism and oxidative stress, while α-syn constitutes a central disease-modifying target associated with protein misfolding, aggregation, and neurotoxicity. Simultaneous modulation of both targets represents a rational multitarget strategy for the development of small-molecule therapeutics, as for example, indazole-5-carboxamide derivatives.

Inhibition of MAO-B reduces dopamine degradation and limits the formation of reactive oxygen species1, thereby attenuating oxidative stress–mediated neuronal damage, while modulation of α-Syn–related processes interfere with protein aggregation, toxicity, and pathological spreading. Targeting both mechanisms within a single low-molecular-weight framework is expected to address complementary pathological pathways implicated in neurodegeneration and may contribute to slowing disease progression through combined symptomatic and disease-modifying effects.

This work is focused on the design of novel low-molecular-weight compounds acting as dual MAO-B/α-syn inhibitors, employing an integrated strategy based on molecular docking, molecular dynamics simulations, virtual screening, synthesis, and in vitro physicochemical and biological evaluation. The combined application of these approaches provides a systematic framework for the identification and optimization of small molecules with potential relevance for neurodegenerative disease research.

Acknowledgements: This work is financially supported by the Bulgarian National Science Fund project KP-06-N93/10 (КП-06-Н93/10).

Abstract

ABSTRACT #6 **POSTER**

miR-19b-3p as an oncogenic regulator in the epigenetic reprogramming of metastatic melanoma

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KEYWORDS miR-19b-3p; metastatic melanoma; epigenetics; microRNA; NanoString technology

Introduction: Epigenetic mechanisms are key regulators of gene expression and cellular plasticity without alterations in the DNA sequence. In metastatic melanoma, pronounced epigenetic remodeling drives activation of oncogenic programs and suppression of tumor-suppressor pathways. miRNAs represent an essential component of this regulatory network, functioning as post-transcriptional modulators of gene expression.

Materials and Methods: FFPE tissue samples from metastatic melanoma (n=15) and normal skin (n=6) were analyzed. miRNA and mRNA expression profiling was performed using NanoString nCounter platforms. BRAF V600E mutational status was determined by qPCR. Differential expression analysis was conducted after normalization using statistical thresholds (p=0.01),predefined expression cut-offs end fold-change analysis.

Results: miR-19b-3p was significantly overexpressed in metastatic melanoma compared to normal skin (fold-change > 4.4, p=0.01), independent of BRAF status. The predicted targets NOTCH1 (FC = -0.49, p=0.01), F2RL1 (FC = -0.43, p=0.01), CNTFR (FC = -0.28, p=0.01), CXCL12 (FC = -0.26, p=0.01), and PIK3R1 (FC = -0.48, p=0.01) showed significant downregulation, suggesting coordinated post-transcriptional regulation and concerted transcriptional repression of functionally related genes.

Conclusion: The data demonstrate that miR-19b-3p is consistently overexpressed in metastatic melanoma and associated with coordinated downregulation of key target genes, supporting its role as a relevant post-transcriptional regulator involved in maintaining an epigenetically altered transcriptional landscape characteristic of progressive disease. This expression pattern identifies miR-19b-3p as a potential biomarker of aggressive clinical behavior and a putative therapeutic target.

Abstract

ABSTRACT #7 **POSTER**

From small molecules investigated as Factor XIIIa Inhibitors to biological target diversity

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Activated Factor XIIIa (FXIIIa) is a key enzyme in the final stage of the blood coagulation cascade. While clotting is essential, excessive clot formation can cause serious conditions like heart attack or stroke. Inhibition of FXIIIa represents an interesting anticoagulant strategy, as reduced fibrin crosslinking is known to produce mechanically weaker clots that are more susceptible to fibrinolysis and may lower the risk of side-effect bleeding.1 Despite reported FXIIIa inhibitors, to date, there is no medically approved drug specifically targeting this enzyme.

The study identifies and characterizes small-molecules with potential activity against multiple biological targets, initially evaluated as FXIIIa inhibitors. Structure-based molecular docking was combined with ligand-based target prediction approaches to evaluate reference and query drug sets against selected biological targets.

The investigated compounds are comprised of two sets: (i) a reference set of molecules with documented physicochemical and biological relevance, including the approved drugs donepezil, safinamide, and the investigational drug NTZ-1091 (a monoamine oxidase B inhibitor) (ii) a drug test set derived from novel scaffolds as well as previously synthesized and characterized by our group.2,3 Based on target prediction results obtained from target-prediction sources, additional docking experiments were performed against biologically relevant enzymes, including human monoamine oxidase A and B (MAO-A/B) as a starting point of our investigations.

The identification of MAO as a potential secondary target (off-target) is supported further by literature evidence demonstrating that structurally optimized small molecules can achieve high potency and selectivity towards MAO-B. In particular, the subnanomolar indazole-5-carboxamide inhibitors with slight modifications enhance MAO-B affinity, selectivity, and drug-like properties.2,3 Higher similarity scores indicate greater chemical similarity between compounds and, based on the similarity principle, structurally related analogs are more likely to exhibit activity toward the same biological target. These findings therefore provide a promising basis for subsequent compound optimization and experimental validation.

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Abstract

ABSTRACT #8 **POSTER**

Germline was identified a known founder BRCA1 variant and highlights HRR/DDR genetic heterogeneity in breast cancer

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KEYWORDS Breast cancer; Germline WES; HRR/DDR; Clinico-genomic integration

Background. Germline variants in genes involved in homologous recombination repair (HRR) and the DNA damage response (DDR) contribute to hereditary breast cancer susceptibility. Germline BRCA1/2 mutations are clinically relevant markers for PARP inhibitor treatment, whereas other HRR/DDR gene mutations have more conditional therapeutic value. In this study, we aimed to identify priority germline HRR/DDR mutations and associations with clinical and tumor features.

Methods. We performed WES analysis of germline DNA from 47 breast cancer patients who received genetic testing for hereditary HRR/DDR genes in the context of potential olaparib treatment. Mutation prioritization criteria included ClinVar classification, prediction of functional effect, allele frequency in the population, and biological relevance. Clinical and tumor features include age at diagnosis, family history, TNBC, and ER, PR, HER2, and Ki-67 expression.

Results. Fifteen patients (31.9%) possessed at least one ClinVar-reported P/LP mutation. The HRR gene mutation spectrum included BRCA1/2, PALB2, CHEK2, and FANCM genes. Other germline P/LP mutations involved MUTYH and LZTR1 genes and ABRAXAS1 unclassified variant. Eight patients (17.0%) carried P/LP germline mutations in BRCA1/2. Recurrent BRCA1 c.5266dupC mutation was detected in 3 patients (6.4%). For patients with available biomarker data, ER and PR biomarkers showed positive correlations, whereas high hormone receptor expression correlated with lower Ki-67 values. Median age at diagnosis of BRCA1/2 P/LP carriers was lower than that of non-carriers (39 years and 53.5 years, respectively; p=0.026), although this difference lost its statistical significance after Benjamini-Hochberg correction (q=0.233).

Conclusion. Clinically relevant P/LP germline mutations in BRCA1/2 and other HRR/DDR genes were identified by WES in breast cancer patients under hereditary HRR/DDR gene testing in the context of potential PARPi therapy. In view of the small size of the clinically relevant cohort and exploratory analyses, genotype-phenotype associations need further validation in large independent cohorts.

Grants: D01-302/17.12.2021,D01-165/28.07.2022,BG-RRP-2.004-0004-C01.

Abstract

ABSTRACT #9 **POSTER**

Cannabidiol induces cell line-specific mitochondrial and endoplasmic reticulum responses in non-small cell lung cancer cells

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KEYWORDS Cannabidiol; mitochondria; endoplasmic reticulum; non-small cell lung cancer; apoptosis

Background: Cannabidiol (CBD), a non-psychoactive phytocannabinoid derived from Cannabis sativa, has emerged as a promising candidate in anticancer research. However, the intracellular mechanisms underlying its cytotoxic effects remain incompletely understood. Mitochondria and the endoplasmic reticulum (ER) are central regulators of cellular stress, calcium homeostasis, and apoptosis and may contribute to cell line-specific responses to CBD. We investigated the effects of CBD on mitochondrial and ER homeostasis in two molecularly distinct non-small cell lung cancer (NSCLC) cell lines, A549 and H1299.

Methods: A549 and H1299 cells were exposed to CBD and analyzed using live-cell imaging, transmission electron microscopy (TEM), flow cytometry, and molecular analyses. Mitochondrial morphology, membrane potential, reactive oxygen species (ROS) production, ER morphology, ultrastructural changes, and caspase activation were assessed following CBD treatment.

Results: CBD induced distinct organelle-centered responses in A549 and H1299 cells. A549 cells exhibited pronounced mitochondrial morphological alterations, early mitochondrial dysfunction, and increased oxidative stress, accompanied by caspase activation, consistent with mitochondrial involvement in the initiation of apoptosis. TEM further revealed marked mitochondrial ultrastructural alterations following CBD exposure. In contrast, H1299 cells displayed pronounced ER remodelling and vacuolization, while mitochondrial polarization was comparatively preserved during the early response. These findings indicate differential engagement of mitochondrial and ER stress pathways in response to CBD.

Conclusion: CBD induces cell line-specific organelle responses in NSCLC cells, with mitochondria predominantly affected in A549 cells and ER remodelling more prominent in H1299 cells. The distinct cellular responses highlight the importance of organelle crosstalk and cellular context in determining CBD-induced cytotoxicity and may provide a basis for developing metabolism- and organelle-informed strategies for cannabinoid-based cancer therapy.

Abstract

ABSTRACT #10 **POSTER**

The telomere social network: telomere clustering patterns in ALT positive cancers

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KEYWORDS ALT, cancer, single-cell, microscopy

In order to acquire replicative immortality, cancer cells have to overcome the continuous shortening of their telomeres. Most cancers solve this problem by the activation of telomerase. Yet, 5-10% of cancers, such as osteosarcoma and glioma, utilise a substitute pathway known as alternative lengthening of telomeres (ALT) which relies on homologous recombination and break induced repair. However, ALT mechanisms and activity through the cell cycle remain poorly understood. Therefore, we developed a method which allows us to follow active ALT sites in real time. Cell lines simultaneously expressing fluorescently labeled shelterin proteins and a ssDNA sensor, or a DNA helicase, were used in combination with live-cell imaging, allowing us to follow ALT activity at high spatio-temporal resolution. Live cell recordings of ALT active sites in the context of chromosomes, tracked via our FAST CHIMP pipeline, facilitated real time analysis of telomere clustering. Our results demonstrate that between 2 and 10 clusters form per cell, each containing 2-5 telomeres, and the clusters increase upon replication stress. Furthermore, by comparing mother and daughter cells, we see that some chromosomes are involved in ALT during two subsequent cycles. Ultimately, we believe our system will be adopted as a handy tool for further in depth investigation of the ALT mechanism.

Abstract

ABSTRACT #11 **POSTER**

Distinct role of HMGB1-ΔC in driving epithelial-to-mesenchymal transition (EMT) and invasion in 2D and 3D breast cancer model: resistance to metformin inhibition

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KEYWORDS HMGB1-ΔC, cell migration and invasion, metformin

Background: High-mobility group box 1 (HMGB1) is a multifunctional protein involved in inflammation and cancer progression through both nuclear functions and extracellular signaling. Although extracellular HMGB1 has been implicated in epithelial–mesenchymal transition (EMT), tumor cell plasticity, and invasion, the contribution of structural modifications of HMGB1 to these processes remains poorly understood. In particular, the biological significance of the C-terminal acidic tail has not been fully elucidated. We investigated the effects of an HMGB1 variant lacking the entire C-terminal acidic tail and assessed its signaling properties and response to metformin.

Methods: The biological effects of full-length HMGB1 and C-terminal tail-less HMGB1 were evaluated in 2D and 3D cancer cell models. RAGE receptor downregulation by siRNA transfection was used to assess the contribution of HMGB1–RAGE interactions to cellular migration and invasion, as evaluated by a wound-healing assay. Subsequently, metformin, a potential inhibitor of this process, was assessed. The IC50 concentrations of metformin were determined through MTT cytotoxicity assays. Anti-EMT properties of metformin were analyzed by assessment of EMT markers via Western blot and immunofluorescence techniques.

Results: C-terminal truncation substantially altered the biological activity of HMGB1 and promoted increased cellular plasticity and invasive properties. The tail-less HMGB1 variant induced EMT-associated phenotypic changes and activated downstream inflammatory signaling in a context-dependent manner. These effects were not strictly dependent on RAGE, suggesting the involvement of additional signaling mechanisms. Notably, metformin effectively suppressed cellular responses induced by full-length HMGB1, whereas its inhibitory effect was markedly reduced in response to the C-terminal tail-less variant. These findings indicate that structural differences in HMGB1 can significantly influence both its tumor-promoting activity and cellular sensitivity to pharmacological intervention.

Conclusion: Our findings identify the C-terminal acidic tail as an important regulatory determinant of HMGB1-mediated tumor cell plasticity and aggressiveness. Loss of this domain generates an HMGB1 variant with distinct signaling properties and reduced sensitivity to metformin. These results highlight the functional relevance of extracellular HMGB1 structural heterogeneity and suggest that HMGB1 variants may represent an important consideration for the development of HMGB1-targeted and combination therapeutic strategies.

Abstract

ABSTRACT #12 **POSTER**

Serum levels of Nε-(carboxymethyl)-lysine in chronic kidney disease and Type 2 diabetes mellitus

Mariyana Petkova[2], Evan Gatev[1], Roumyana Mironova[1], Siyana Ilieva[2], Simona Kuzmanova-Hrischeva[2], Simona Kerezieva[2], Teodora Ilieva[2], Bilyana Vasileva[2], Toshimitsu Niwa[3], Daniela Popova[2], Vasil Vasilev[2], Rositsa Tsekovska[1]

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KEYWORDS Advanced glycation end products; Nε-(carboxymethyl)-lysine; chronic kidney disease; type 2 diabetes mellitus

Background: Nε-(carboxymethyl)-lysine (CML) is a major advanced glycation end product formed through glycation, oxidation, and lipoxidation. Because free CML is primarily eliminated by the kidneys, circulating levels may reflect impaired renal clearance. In diabetes mellitus, chronic hyperglycemia enhances the formation of advanced glycation end products including CML and may contribute to their systemic accumulation. This study aimed to assess the relative contribution of chronic kidney disease (CKD) and type 2 diabetes mellitus (T2DM) to serum CML accumulation.

Methods: In this single-center cross-sectional study, 98 adults were assigned to three groups: CKD without diabetes (n = 22), CKD with T2DM (n = 55), and T2DM without CKD (n = 21). Serum CML concentrations were measured by competitive ELISA. Group differences were analyzed using the Kruskal–Wallis test followed by Dunn's pairwise comparisons, and associations between CML and clinical variables were evaluated by Spearman correlation analysis.

Results: Serum CML levels differed significantly among the three groups (p = 0.017). The highest median CML concentration was observed in patients with CKD without diabetes, followed by patients with CKD and T2DM, whereas the lowest levels were found in patients with T2DM without CKD. In non-diabetic CKD patients, serum CML correlated positively with albuminuria, proteinuria, and age. In patients with T2DM, serum CML showed negative associations with postprandial glucose and diabetes duration. Negative correlations of serum CML with fasting glucose and HbA1c were observed only in the T2DM group without CKD. These findings suggest that impaired renal clearance is a stronger determinant of circulating CML than glycemic status per se.

Conclusion: Renal dysfunction appears to contribute more strongly to circulating CML accumulation than hyperglycemia. Serum CML appears a potential marker of kidney dysfunction, particularly in patients with CKD without diabetes.

Abstract

ABSTRACT #13 **POSTER**

From genome to chromatin: identification of a de novo SATB2 variant by trio whole-genome sequencing

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KEYWORDS SATB2; chromatin organization; whole-genome sequencing; neurodevelopmental disorder

Background: SATB2 encodes a nuclear matrix-associated DNA-binding protein that functions as an organizer of higher-order chromatin and a regulator of developmentally controlled transcription. Experimental studies show that SATB2 modulates chromatin accessibility, enhancer–promoter interactions, and three-dimensional genome organization in cortical neurons and coordinates major transcriptional transitions during vertebrate embryogenesis. Heterozygous pathogenic variants in SATB2 cause SATB2-associated syndrome.

Methods: Trio whole-genome sequencing was performed on a child with developmental and motor impairment, congenital cleft palate, and inability to walk independently, together with both unaffected parents. Sequencing was performed on an Illumina NovaSeq 6000 platform, followed by individual bioinformatic analysis.

Results: A heterozygous canonical splice-acceptor variant, SATB2:c.474-2A>G, was identified in the proband. The variant was absent from both parental blood samples, supporting a de novo origin It is predicted to cause aberrant splicing and loss of SATB2 function and was classified as pathogenic ACMG and ACGS criteria. The finding established a diagnosis of SATB2-associated syndrome (Glass syndrome).

Conclusion: This case illustrates how trio whole-genome sequencing can connect a neurodevelopmental phenotype with disruption of a chromatin-organizing gene. Although patient-specific epigenomic effects were not assessed, the predicted loss of function is consistent with established SATB2-dependent chromatin mechanisms. To our knowledge, this represents the first molecularly confirmed case of SATB2-associated syndrome documented in Bulgaria, extending the documented national spectrum of rare neurodevelopmental disorders. The diagnosis enabled syndrome-specific follow-up and informed genetic counseling.

Abstract

ABSTRACT #14 **POSTER**

Soil Microbiome as a Link between Ecosystem and Human Health

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KEYWORDS Soil, heavy metals, microbial communities, metagenomics, predicted function

Background: Soil microbiomes play a central role in maintaining soil health and ecosystem resilience through nutrient cycling, plant growth promotion and pathogen regulation. As a microbial link connecting soil, plants, animals and humans, the soil microbiome represents an important component of the One Health approach. This study aimed to characterize the taxonomic composition and predicted functional profiles of microbial communities in Bulgarian agricultural soils contaminated with heavy metals (HMs), including Zn, Pb, Cd, As and Cu, and to assess the potential ecological risks associated with this contamination and its implications for soil health.

Methods: High-throughput sequencing of the 16S rRNA gene (V3-V4 region) for bacterial and the internal transcribed spacer (ITS) region for fungal communities was used to assess taxonomic composition, and PICRUSt2 was used to predict their functional profiles. Graphical representations were performed in R4.4.1.

Results: The soil microbial communities were predominantly represented by the phyla Pseudomonadota, Acidobacteriota and Actinomycetota among bacteria and Ascomycota among fungi. Members of Alphaproteobacteria, Acidobacteriae, Chloroflexia and Eurotiomycetes exhibited comparatively high tolerance to HMs. Functional predictions indicated the enrichment of pathways potentially involved in HM resistance, including metal efflux systems and detoxification mechanisms. Microbial community structure was significantly influenced by HM concentrations, with several bacterial and fungal taxa showing strong associations with metal contamination and soil physicochemical properties.

Conclusion: Chronic HM contamination shaped soil microbial communities, favoring the enrichment of HM-tolerant taxa and influencing their predicted functional potential. These findings provide further insight into microbial adaptation to chronic metal contamination in agricultural soils and highlight the potential One Health relevance of HM-driven changes in soil microbiomes, particularly given the possible co-selection and enrichment of antibiotic resistance genes (ARGs).

Acknowledgements: This study was financially supported by the Bulgarian Science Fund (Grant KP-06-N91/2).

Abstract

ABSTRACT #15 **POSTER**

Development and validation of purpose-built machine learning models for predicting drug-likeness rule violations in peptide molecules

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KEYWORDS Peptide-based drug candidates; Drug-likeness; Machine learning; Model development

Background: Peptide-based drug candidates frequently violate classical drug-likeness rules such as Lipinski's Rule of Five (Ro5) due to their high molecular weight, polarity, and structural flexibility. Extended frameworks, like Beyond Ro5 (bRo5) and the Muegge rule better accommodate peptide chemical space, but systematic in silico tools for their assessment remain limited. The study aimed to develop and validate original, purpose-built machine learning (ML) models for predicting Ro5, bRo5, and Muegge rule violations in peptide molecules.

Methods: We curated a training dataset of >300,000 drug- and non-drug-like molecules from PubChem, computed physicochemical descriptors (molecular weight, logP, hydrogen bond donors/acceptors, TPSA, rotatable bonds) with RDKit, and designed custom violation counters for Ro5, bRo5, and Muegge's rule. Using this dataset, we built and trained, from scratch, three original ML architectures: Random Forest (RF), multilayer perceptron (MLP), and k-nearest neighbors (k-NN) models, with hyperparameters optimised via grid search. The newly created models were then applied to predict rule violations for 26 structurally diverse, previously synthesised peptides (MW 318 – 1335 Da) and benchmarked against SwissADME, Molinspiration, and manually calculated reference values.

Results: The self-developed RF, MLP, and k-NN models all achieved excellent predictive performance (accuracy, precision, recall, F1-score 0.94 – 1.0 across rules), confirming successful model construction rather than reliance on pre-existing predictors. RF and MLP showed the highest stability for Ro5, while k-NN achieved the strongest agreement for bRo5. Prediction errors were limited to ±1 violation, concentrated in borderline structures near rule thresholds and in larger peptides exceeding the processing limits of external platforms.

Conclusion: The three newly developed, descriptor-based ML models (RF, MLP, k-NN) reliably capture drug-likeness patterns across peptide chemical space and complement one another. As original, purpose-built tools, they offer a fast, accurate, and platform-independent alternative to existing web-based calculators for the early-stage screening and prioritisation of orally viable peptide drug candidates.

Abstract

ABSTRACT #16 **POSTER**

Cannabidiol reshapes exosomal miRNA regulatory networks to enhance Imatinib mesylate response in chronic myelogenous leukemia

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KEYWORDS Exosomal miRNA; epigenetic regulation; cannabidiol; Imatinib; CML

Background: Chronic myelogenous leukemia (CML) is relatively rare hematologic malignancy driven by the BCR ABL1 fusion oncogene. Despite the success of tyrosine kinase inhibitors (TKIs), resistance or suboptimal response to Imatinib mesylate (IM) remains a clinical challenge. Epigenetic regulation through exosomal microRNAs (miRNAs) plays a key role in leukemic signaling, drug response, and intercellular communication. Cannabidiol (CBD), a phytocannabinoid with emerging immunomodulatory and epigenetic effects, has been proposed as a potential sensitizer to anticancer therapies. This study investigates whether CBD modulates exosomal miRNA networks to enhance Imatinib efficacy in CML.

Methods: CML cells were treated with CBD, IM, or their combination. Exosomes were isolated and subjected to small RNA sequencing. Differential expression analysis, miRNA–mRNA network reconstruction, and pathway enrichment (KEGG, GO) were performed to identify epigenetic regulatory nodes affected by treatment. Functional assays assessed apoptosis, oxidative stress, and drug response.

Results: CBD induced distinct exosomal miRNA signatures targeting pathways involved in chromatin remodeling, oxidative stress regulation, vesicular trafficking, and apoptosis. Combined CBD+Imatinib treatment produced synergistic modulation of key miRNA hubs associated with TKI resistance, including regulators of BCR ABL1 signaling and mitochondrial stress responses. Network analysis revealed that CBD reshapes exosomal epigenetic communication by suppressing oncogenic nodes and enhancing pro apoptotic cascades. Functionally, CBD increased Imatinib sensitivity and amplified apoptotic responses.

Conclusion: CBD acts as an epigenetic modulator of exosomal miRNA networks, shifting leukemic signaling toward a more drug responsive phenotype. These findings highlight a novel therapeutic strategy integrating miRNA based epigenetic modulation with targeted therapy. Exosomal miRNA signatures may serve as minimally invasive biomarkers for treatment response in CML.

Abstract

ABSTRACT #17 **POSTER**

Germline Whole-Exome Sequencing Reveals a Heterogeneous Genetic Landscape of Pancreatic Cancer in a Bulgarian Cohort

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KEYWORDS Pancreatic cancer, DNA repair, Whole-Exome Sequencing (WES)

Background: A proportion of pancreatic cancer cases is attributable to hereditary predisposition. Germline pathogenic variants in BRCA1, BRCA2, and mismatch repair genes associated with Lynch syndrome are established contributors to pancreatic cancer risk. Additional genes involved in DNA repair and rare hereditary cancer syndromes may also contribute to susceptibility, although their clinical relevance varies. This pilot study aimed to characterize germline variants associated with hereditary cancer predisposition in Bulgarian patients with pancreatic cancer.

Methods: The study included 21 patients with pancreatic cancer who provided informed consent. Germline DNA was subjected to whole-exome sequencing using the Illumina DNA Prep with Enrichment workflow on a NovaSeq 6000 platform. Variant annotation and prioritization were performed using VarSeq (Golden Helix). Variants were evaluated using a predefined 228-gene hereditary cancer panel.

Results: Fourteen germline variants were identified, including eight classified as pathogenic/likely pathogenic (P/LP) and six as variants of uncertain significance (VUS). P/LP variants were identified in BRCA1 (c.2019delA), CFTR (c.1210-7_1210-6delTT), FANCM (c.5791C>T), MITF (c.952G>A), NBN (c.657_661delACAAA), PIK3CD (c.2194C>T), SQSTM1 (c.1175C>T), and VHL (c.598C>T). Nine of 21 patients (42.9%) carried at least one P/LP variant. The CFTR variant was identified in two patients; one of these patients also carried an additional LP variant in PIK3CD. Six VUS were identified in ATM, BRCA2, CBL, NPM1, RYR1, and SAMD9.

Conclusions: This pilot study identified germline P/LP variants in a substantial proportion of pancreatic cancer patients undergoing hereditary cancer assessment, including variants in BRCA1 and several additional DNA repair- and hereditary cancer-associated genes. The findings support further investigation of the germline genetic landscape of pancreatic cancer in the Bulgarian population. Larger cohorts and careful clinical interpretation of individual genes and variants are required to better define their contribution to hereditary pancreatic cancer risk.

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Abstract

ABSTRACT #18 **POSTER**

Srcap Chromatin Remodeler is Required for Efficient Replication Dynamics in Mammalian Cells

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The SNF2-related chromatin remodeler Srcap is the principal ATPase responsible for the deposition of the histone variant H2A.Z at promoters and regulatory chromatin regions. Although this activity is known to modulate transcription, its contribution to DNA replication remains unexplored. Here we show that Srcap is required for efficient replication fork progression and origin firing in mammalian cells. Using RNA interference in human PC3 cells, we found that Srcap depletion leads to a ~25% reduction in fork elongation rate, decreased replication fork density, accumulation of the replication-stress marker γH2AX and reduced chromatin-bound H2A.Z. High-resolution expansion microscopy further revealed diminished intensity and increased spacing of replication foci, consistent with reduced origin activation. Transcriptomic analysis of published data identified broad downregulation of replication-associated genes. These data uncover a dual mechanism by which Srcap sustains replication efficiency—through both H2A.Z-dependent chromatin organization and transcriptional maintenance of the replication machinery. Our findings establish Srcap as a key coordinator of replication dynamics, with implications for genome stability.

Acknowledgements: This work was supported by Bulgarian National Science Fund grant # КП-06-Н71/6.

Abstract

ABSTRACT #19 **POSTER**

Improved Metagenomic DNA Extraction to Reveal Hidden Hydrogen-Consuming. Microorganisms in Crohn's Disease

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KEYWORDS Human gut microbiome, hydrogen-consuming microorganisms, obligate anaerobes, Crohn's disease

Background: Crohn's disease (CD) is a chronic inflammation of the gastrointestinal tract. It is considered to arise from complex interactions between the host immune system and the gut microbiome. Dysbiosis in patients with CD has been associated with altered microbial metabolism and increased production of molecular hydrogen (H2) and hydrogen sulphide (H2S). Microorganisms involved in the consumption of these gases may therefore play an important role in maintaining metabolic balance within the gut ecosystem. However, the diversity and functional significance of hydrogen-consuming microorganisms in the human gut remain incompletely understood. A recent analysis of 4,644 bacterial and archaeal species-level genomes from the Unified Human Gastrointestinal Genome Collection, together with 1,700 stool metagenomes from healthy and diseased individuals, identified only 151 potential hydrogen-consuming species. This raises an important question: does this number represent the true diversity of hydrogen-consuming microorganisms in the human gut, or are members of this metabolically important group being missed because of methodological limitations? One potential challenge is DNA extraction. Many microorganisms involved in hydrogen metabolism areobligate anaerobes or highly sensitive to oxygen exposure. We hypothesize that oxygen exposure during sample processing, storage, and metagenomic DNA extraction may contribute to DNA degradation in oxygen-sensitive microorganisms. This may occur through the oxygen-dependent activation of cellular nucleases and/or non-enzymatic pathways of DNA damage. Such degradation could result in the loss of genomic material before sequencing, limiting the ability of downstream sequencing technologies and bioinformatic approaches to recover the complete diversity of these microorganisms.

Methods and Results: To address this challenge, we developed a method for metagenomic DNA extraction from fish gut content that preserves both the quantity and integrity of high-molecular-weight (HMW) DNA. We are now applying this approach to human faecal samples. We aim to determine whether improved preservation of metagenomic DNA enables more comprehensive detection and genomic characterization of oxygen-sensitive, hydrogen-consuming microorganisms. We will also investigate differences between healthy individuals and patients with Crohn's disease.

Conclusions: By generating high-quality HMW DNA suitable for long-read sequencing, this approach may facilitate the recovery of more complete genomes and previously underrepresented microbial populations. Ultimately, this strategy could help illuminate the currently unresolved microbial diversity, or microbial "dark matter," particularly that associated with hydrogen metabolism in the human gut and its potential role in Crohn's disease.

Abstract

ABSTRACT #20 **POSTER**

Synthesis and analysis of short cationic antimicrobial peptides structural analogues of Lfcinb(20-30)

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KEYWORDS Cationic antimicrobial peptides, lactoferricin, antimicrobial resistannce

Drug resistance of microorganisms is one of the serious threats to human health. This necessitates the search for new antibiotics, substances that could enhance the antibiotic effects or reverse microbial resistance, even if they themselves do not have an antimicrobial effect. Some peptides have been shown to have antimicrobial activity and / or the ability to potentiate the effects of antimicrobial agents. In recent years, antimicrobial peptides have attracted considerable interest.

According to published data the 25-amino acids fragment 17-41 (LFcin B 17-41) of the mature bovine lactoferrin protein, known as bovine lactoferricin B, exhibits antimicrobial activity. It has been established, that its undecapeptide segment LFcinB 20-30, has similar activity to that of the 25-amino acids fragment. Even shorter derivatives of the lactoferricin's have also been shown to have good antibacterial activity. The active center of the bovine lactoferricin is a hexapeptide, corresponding to the 20-25 fragment (LFcin B 20-25).

These observations and our interest in the research work based on the synthesis of novel cationic peptides prompted us to undertake the synthesis of some short LFcin B (20-28) derivatives, that would possess antimicrobial activity with nearly no cytotoxicity to human cells.

The target peptides containing sequence RRWQWR or RRWQWRMKK were synthesized via SPPS-Fmoc/tBu strategy, where the Arg residue in different position was replaced with unnatural amino acids Cav, Agp and Agb.

All peptides were tested for their ability to inhibit the growth of E. coli (Gram negative) and S. aureus (Gram positive) bacteria. The results obtained were expressed as the minimal inhibitory concentration (MIC) in μM. Generally, all of the tested peptides displayed increases in antimicrobial activity. In addition, the cytotoxic activity of the compounds was investigated also.

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Abstract

ABSTRACT #21 **POSTER**

Effect of ART558 and Novobiocin on the response to 5-fluorouracil in colorectal cancer cells

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KEYWORDS Colorectal cancer, Novobiocin, 5-fluorouracil

Colorectal cancer (CRC) is the second leading cause of cancer-related deaths worldwide. Therapeutic approaches for CRC employ DNA damaging agents, such as 5-fluorouracil (5-FU). The functional status of DNA mismatch repair (MMR) has been established as a molecular marker influencing treatment response for CRC. MMR deficiency is clinically equivalent to a high microsatellite instability (MSI) phenotype, while tumors with functional MMR correspond to a microsatellite stability (MSS) phenotype. Induction of MSI has been observed in cells with non-functional MMR, because of DNA double-strand break (DSB) repair involving DNA polymerase theta (POLQ). Thus, the aim of the present work was to study the involvement of POLQ in repair of replication stress-induced DSBs in CRC cell lines with different MMR status. For this purpose, MMR proficient (HT29) and deficient (HCT116) cell lines were treated with 5-FU alone, or in combination with the POLQ inhibitors - Novobiocin (NVB) and ART558. Cell cycle distribution was assessed by FACS analysis, alterations in the replication fork dynamics by DNA Fiber assay, R-loops accumulation by immunofluorescence, and DSBs induction by detection of γH2AX foci. After 24 h treatment with 5-FU in the HT29 cell line, cell cycle arrest in the G1/S phase was observed, in contrast to HCT116, where cells accumulated in G2. While formation of γH2AX foci increased after treatment with 5-FU and 5-FU+NVB at 24 h in HT29, in HCT116 such an increase was observed after 48 h for the combined treatment, and after 72 h for 5-FU alone. Our data showed that the effects of POLQ inhibition on repair of 5-FU-induced DNA lesions differ between HCT116 (MSI) and HT29 (MSS) cell lines, which could reflect the involvement of distinct repair mechanisms.

Financial support: This research was supported by the Bulgarian National Science Fund, Grant KP-06-H61/2.

Abstract

ABSTRACT #22 **POSTER**

Exploring the genetic architecture of coronary artery disease in a Bulgarian population

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KEYWORDS Coronary artery disease; genetic variants; next-generation sequencing; lipid metabolism; Bulgarian population

Background: Coronary artery disease (CAD) has a substantial genetic component, with heritability estimated at 40–60%. Its genetic architecture is complex, involving multiple variants across biological pathways regulating lipid metabolism, vascular function, and inflammation. Characterizing population-specific patterns of genetic variation may provide further insight into individual susceptibility to CAD. This study aimed to explore common cardiovascular disease-related genetic variants and their associations with CAD in a Bulgarian case–control cohort.

Methods: The study included: 50 patients with non-ST-elevation acute coronary syndrome (NAP), 50 patients with stable angina pectoris (SAP), and 50 controls. Targeted next-generation sequencing was performed using a cardiovascular gene panel. Common genetic variants (minor allele frequency >0.05) were assessed, and their distribution between CAD patients and controls was evaluated. Association analysis was performed using Fisher's exact test with Haldane–Anscombe correction.

Results: Potential associations with CAD were identified predominantly in genes involved in lipid and triglyceride metabolism, including PCSK9 (rs35574083, p=0.0003), APOB (rs373477107, p=0.00011), CETP (rs5883, p=0.0012), APOE (rs147610191, p=0.001), and ABCG5 (rs56204478, p=0.012). Additional associations involved genes related to vascular structure and function, CASQ2 (rs72703671) and COMT (rs925920723), and cardiac structure and function, TTN (rs13388274) and DPP (rs572667303). Notably, the PCSK9 variant rs509504 showed a pronounced frequency gradient across the clinical groups, being highest in NAP patients (62.5%), followed by SAP patients (43.75%), and markedly lower in controls (3.12%).

Conclusion: These preliminary findings support a multifactorial genetic architecture of CAD in the Bulgarian population, involving variants in genes related to lipid metabolism, vascular function, and cardiac homeostasis. The pronounced differences in the frequency of PCSK9 rs509504 between NAP, SAP, and control groups highlight PCSK9 as a potentially important contributor to CAD susceptibility and clinical phenotype. Larger studies are needed to confirm these associations.

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Abstract

ABSTRACT #23 **POSTER**

Rahman Syndrome Mutation in Linker Histone H1.4 Is Associated with Replication Stress and Altered Transcriptional Regulation in Mammalian Cells

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Linker histone H1.4 contributes to chromatin organization and the regulation of DNA replication and transcription. However, the consequences of H1.4 dysfunction for these processes remain elusive. Here, we investigated the effects of the Rahman syndrome-associated H1.4 mutation in mouse embryonic fibroblasts (MEFs). DNA fiber analysis revealed increased replication fork stalling and asymmetry in homozygous mutant cells. Immunofluorescence analysis of γH2AX and comet assay indicated an associated DNA damage response, consistent with increased replication stress. Analysis of Rpb1 (the major RNAPII subunit) C-terminal domain phosphorylation revealed reduced Ser2 phosphorylation in homozygous mutant cells, suggesting altered regulation of transcriptional pause release. Proximity ligation assays further demonstrated increased proximity between Ser5-phosphorylated Rpb1 and proliferating cell nuclear antigen (PCNA), indicating accumulation of replication forks at initiating RNAPIIs. These observations raise the possibility that impaired transcriptional pause release contributes to replication fork instability through transcription–replication conflicts, which may contribute to Rahman pathogenesis.

Acknowledgement: This work is supported by grant КП-06-Н71/6 of the Bulgarian National Science Fund

Abstract

ABSTRACT #24 **POSTER**

From mixed DNA to interpretable DNA profiles: improving touch DNA analysis by removing degraded DNA – A pilot study

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KEYWORDS Touch DNA, cell-free DNA, STR profiling, forensic genetics

Background: Touch DNA analysis is an important forensic tool but frequently generates complex or uninterpretable STR profiles due to the coexistence of recently deposited biological material and degraded DNA from previous contacts. Conventional extraction methods maximize DNA recovery without distinguishing intact cellular DNA from degraded cell-free DNA (cfDNA). Intact genomic DNA is associated with histones and other structural proteins, forming nucleoprotein complexes that sediment during centrifugation, whereas degraded cfDNA remains in the supernatant. We hypothesized that separating these fractions before DNA extraction would reduce background DNA and improve STR DNA profile interpretation.

Methods: Preliminary experiments identified donors with comparable shedding characteristics and distinguishable STR profiles. A mobile phone initially handled by one donor was subsequently touched by a second donor. Biological material was collected using a saline-moistened swab and suspended in physiological saline. After centrifugation, the pellet and supernatant fractions were separated. DNA was extracted using the Chelex method, with ethanol precipitation applied to the supernatant before extraction. STR amplification was performed using the AmpFlSTR® NGM™ PCR Amplification Kit, followed by fragment analysis on the 3130xl HID Genetic Analyzer. Profiles were analyzed using GeneMapper® ID-X v1.3.

Results: The pellet fraction yielded an interpretable mixed STR profile representing the two contributors. In contrast, the supernatant produced a more complex profile containing additional allelic peaks of similar intensity, consistent with degraded historical DNA, making interpretation considerably more difficult.

Conclusion: Centrifugation-based fractionation reduces historical DNA background and enriches recently deposited biological material, improving the interpretability of touch DNA profiles. This simple, inexpensive approach requires no specialized equipment and warrants validation on larger forensic sample sets.

Abstract

ABSTRACT #25 **POSTER**

Comparison of Transcriptomes of Linker Histone and HMG Yeast Chromatin Protein Knockouts

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KEYWORDS Chromatin, transcriptome, linker histone, HMG protein

Chromatin is widely implicated in the initiation and progression of various pathologies, although direct evidence linking specific chromatin proteins to disease development remains limited. The yeast Saccharomyces cerevisiae is an established model for genetic and epigenetic research and has contributed substantially to our understanding of chromatin biology.

Hho1p and Hmo1p are yeast chromatin proteins homologous to human linker histone H1 and HMGB1, respectively. They function as chromatin architectural factors by binding nucleosomes, bending DNA, stabilizing specific DNA structures, and contributing to higher-order chromatin organization. In addition to their architectural roles, both proteins participate in fundamental nuclear processes, including transcription, DNA repair, and recombination.

Whole-transcriptome RNA-seq profiles of hho1Δ and hmo1Δ mutants were analysed using DESeq2 differential-expression statistics. Shared, mutant-specific, and growth-phase-dependent gene responses were compared using Venn/Euler and inversion analyses. Functional and regulatory interpretation included GO over-representation analysis, KEGG pathway/module mapping, hierarchical clustering, TFLink regulon enrichment, and integration with curated protein complexes and physical/genetic interaction networks.

Deletion of either HHO1 or HMO1 affected the expression of numerous genes, with up to one-third of the transcriptome showing altered transcription. The extent of transcriptional changes varied across growth phases, with minimal effects during early and exponential growth and maximal effects during quiescence. Interestingly, HMO1 deletion affected substantially more genes than HHO1 deletion. Comparative analysis revealed distinct effects of the two proteins on gene groups involved in metabolism, energetics, nuclear organization, and DNA-related processes.

Linker histone and HMG protein in Saccharomyces cerevisiae together with their architectural role function as important fine gene activity regulators.

This work is supported from BNSF by Grant KP-06-H81/3.

Abstract

ABSTRACT #26 **POSTER**

Unveiling the antitumor potential of novel benzodioxin-annulated naphthalimides in lung cancer cells

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KEYWORDS Naphthalimide; benzodioxin; DNA intercalation; lung carcinoma

Background: 1,8-Naphthalimides represent a well-established class of DNA-intercalating heterocycles with potent antiproliferative activity. To enhance the therapeutic potential of the cytotoxic naphthalimide derivatives mitonafide and amonafide, a novel series of benzodioxin-naphthalimide hybrids was designed, combining the naphthalimide scaffold with the DNA-intercalating tricyclic dibenzodioxin chromophore into a single molecular entity. Among this series, selected candidates underwent further biological characterization as potential DNA-targeting anticancer leads.

Methods: The antiproliferative and cytotoxic properties of the novel benzodioxin-naphthalimide derivatives were evaluated in human non-small cell lung carcinoma cell lines (A549, NCI-H1299) and non-malignant MRC-5 lung fibroblasts. Studies on these molecules included assessment of DNA synthesis inhibition (EdU incorporation), induction of DNA damage (γH2AX), cell-cycle profiling, apoptosis analysis, and clonogenic survival assays following transient drug exposure.

Results: Benzodioxin-1,8-naphthalimides showed greater cytotoxic potency than mitonafide and other classical intercalating agents, with mitonafide-based derivatives more toxic than their amonafide-based counterparts. These compounds exhibited pronounced, selective cytotoxicity in tumor cells relative to MRC-5 controls. The candidates strongly inhibited DNA synthesis and induced robust γH2AX-marked DNA damage, with cell-cycle analyses revealing accumulation in G1 and G2/M phases and cell-line-dependent response patterns. These effects were followed by activation of apoptotic pathways, and clonogenic assays confirmed that even brief exposure to the lead structures caused sustained, irreversible loss of proliferative capacity.

Conclusion: Benzodioxin-1,8-naphthalimides appear to act through a replication stress-driven pathway consistent with interference in DNA-associated processes during S phase, resulting in potent, selective anticancer activity. While the precise molecular initiating event remains to be defined, these conceptually novel hybrid molecules represent promising DNA-targeting agents for continued preclinical development.

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Notes

Notes