ncRNA 2024 Conference

Non-coding RNA World 2024: Exploring Mechanisms, Designing Medicines

07–09 October 2024 | Basel, Switzerland

Program and Abstract Book

Organizers

MDPI
Academic Open Access Publishing
since 1996

Sponsors

NEW ENGLAND Biolabs

GO BEYOND RNA
ARRAYSTAR INC

Partnering Societies

The Epigenetics Society
Est. 1994

SFG

LS² Life Sciences Switzerland

SUSSEX RNA

Media Partners

cancers

biomolecules

genes

cells

viruses

epigenomes

biology

life

sciforum.net/event/ncRNA2024
#ncRNA2024

Non-coding RNA World 2024: Exploring Mechanisms, Designing Medicines

The memox Basel Main Station
Basel, Switzerland
7–9 October 2024

Conference Chairs

Prof. Dr. Rory Johnson
Dr. Ana Eulalio

Conference Committee

Prof. Dr. Gaetano Santulli
Prof. Dr. Francisco J. Enguita
Prof. Dr. Eleonora Leucci
Prof. Dr. Ulf Andersson Vang Ørom
Dr. Florent Hubé
Prof. Dr. Simon Conn
Dr. Laura Poliseno
Prof. Dr. Ioana Berindan-Neagoe
Prof. Dr. Clément Carré
Prof. Dr. Nicola Amodio

Organised by

Conference Secretariat

Mr. Timothy Lee
Ms. Mina Marjanović
Ms. Surayya Rehman

Contents

Welcome from the Chairs

Dear Colleagues,

We are delighted to announce the upcoming conference, Non-coding RNA World 2024: Exploring Mechanisms, Designing Medicines (ncRNA 2024), which will be held from 07–09 October 2024.

Following our successful conference ncRNA 2021, we now propose meeting you in the beautiful city of Basel, Switzerland.

The field of RNA continues on its meteoric ascent, with non-coding RNAs in the vanguard. The field continues to grow, with new transcripts discovered wherever one looks in molecular biology, physiology, disease, and across the tree of life. Discoveries are propelled, in part, by dramatically advancing technologies that can now sequence full-length single RNA molecules at single-cell and sub-cellular resolutions. The sequencing of many thousands of species' genomes will further drive evolutionary and informatic efforts to map, catalog, and model ncRNAs. And, of course, our power to manipulate and engineer RNAs using synthetic oligonucleotides and genome-editing technologies opens breathtaking vistas for medicine and biotechnology, as recognized by the 2023 Nobel Prize won by Karikó and Weissman.

ncRNA 2024 will bring together the international ncRNA community to share and explore the latest advances in our field. The conference will cover a broad range of topics, from basic biology to medical and technological applications. The sessions will take molecular biology through the topics of disease mechanisms, therapies, technologies, and bioinformatic approaches. Each session will be anchored by leading international speakers. We are also committed to showcasing the work of early-stage researchers, and the majority of the oral presentations will be selected from submitted abstracts. Crucially, the program will provide plenty of opportunities to casually network and mingle via poster sessions and refreshment breaks.

We look forward to welcoming you to Basel in October 2024. The city, with its picturesque autumnal scenery, coupled with its renowned cultural and scientific institutions, will create an inspiring atmosphere for our conference.

Ana Eulalio and Rory Johnson
ncRNA 2024 Chairs

Conference Chairs

Prof. Dr. Rory Johnson
School of Biology and Environmental Science, University College Dublin

Dr. Ana Eulalio
Faculty of Natural Sciences, Department of Life Sciences, Imperial College London

Keynote Speakers

Prof. Dr. Mauro Giacca
School of Cardiovascular and Metabolic Medicine and Sciences, King's College, London, UK

Prof. Dr. Ling-Ling Chen
Shanghai Institute of Biochemistry and Cell Biology (Center for Excellence in Molecular Cell Science), Chinese Academy of Sciences, China

Invited Speakers

Prof. Dr. Gunter Meister

Department of Biochemistry I of the University of Regensburg, Germany

Dr. Danny Incarnato

Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, The Netherlands

Prof. Dr. Yuanchao Xue

Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

Dr. Ainara Castellanos

Department of Genetics, Physical Anthropology and Animal Physiology of the University of the Basque Country, Spain

Dr. Marc Friedländer

Quantitative RNA Biology Group, Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Sweden

Prof. Dr. Cynthia Sharma

Department of Molecular Infection Biology II, Institute of Molecular Infection Biology, University of Würzburg, Bavaria, Germany

Dr. Alisha Jones

Department of Chemistry, New York University, New York, NY, USA

Prof. Dr. Roland Rad

Institute for Molecular Oncology and Functional Genomics at the Technical University of Munich, Germany

Dr. Sébastien Pfeffer

Architecture et Réactivité de l'ARN, Institut de Biologie Moléculaire et Cellulaire du CNRS, University of Strasbourg, France

Dr. Joanna Jachowicz

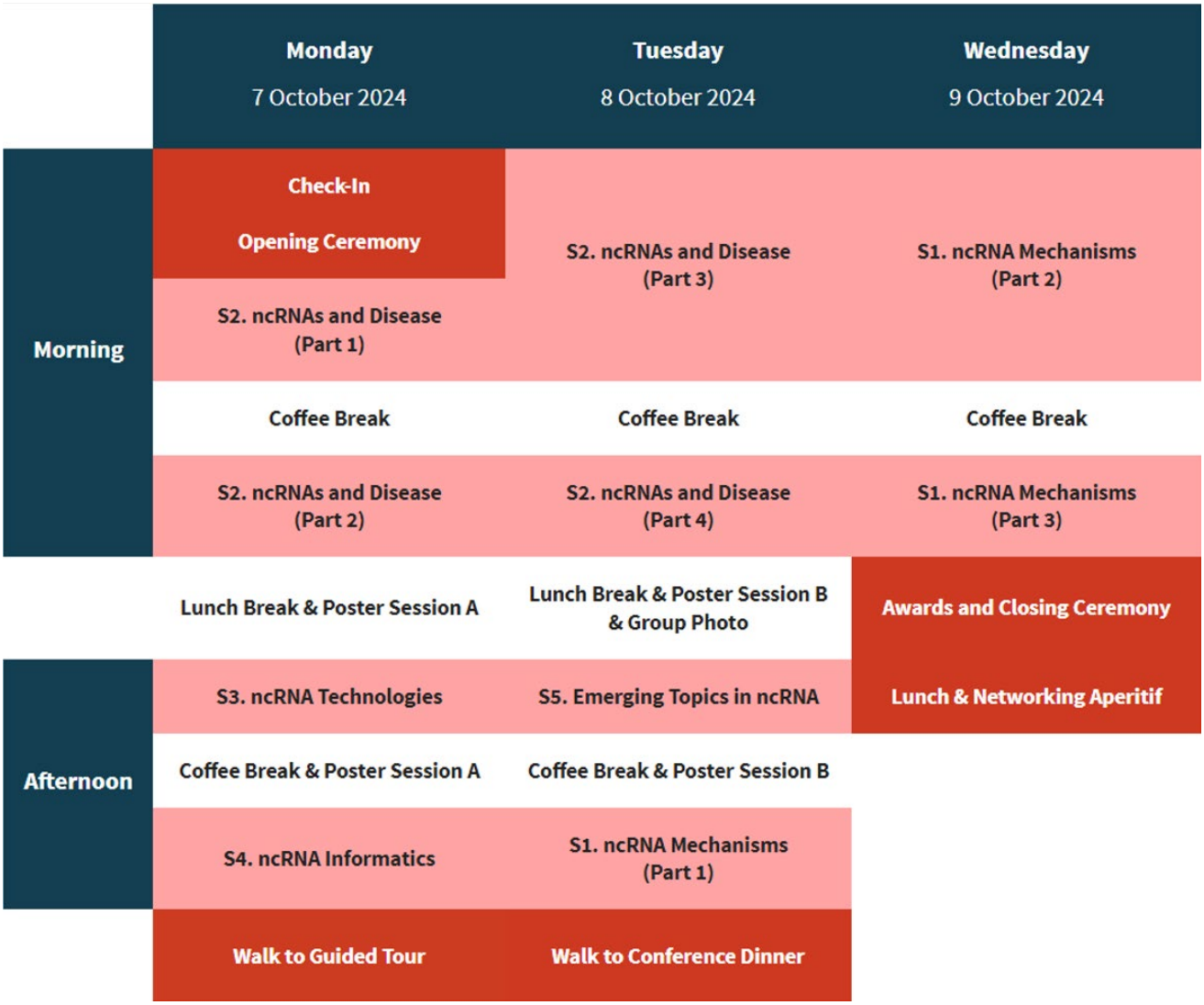
Institute of Molecular Biotechnology, Vienna Biocenter, Austrian Academy of Sciences, Austria

Abridged Program

Monday, 7 October 2024
8:00 am – 6:15 pm CET (Check-In: 8:00 am CET)

Tuesday, 8 October 2024
9:00 am – 6:30 pm CET (Conference Dinner: 19:00 pm CET)

Wednesday, 9 October 2024
9:00 am – 1:20 pm CET



[Conference Program]

General Information

Non-coding RNA World 2024: Exploring Mechanisms, Designing Medicines will be held at The memox Basel Main Station, Basel, Switzerland, on 7–9 October 2024. ncRNA 2024 will bring together the international ncRNA community to share and explore the latest advances in our field. The conference will cover a broad range of topics, from basic biology to medical and technological applications. The sessions will take molecular biology through the topics of disease mechanisms, therapies, technologies, and bioinformatic approaches. Each session will be anchored by leading international speakers. We are also committed to showcasing the work of early-stage researchers, and the majority of the oral presentations will be selected from submitted abstracts. Crucially, the program will provide plenty of opportunities to casually network and mingle via poster sessions and refreshment breaks.

Conference Venue

The memox Basel Main Station
Peter-Merian-Strasse 80, 4052 Basel

Registration Desk

The desk for registration, information and distribution of documents will be open from 8.00h on 7 October 2024.

Certificate of Attendance

Participants of the event will be able to downlod an electronic Certificate of Attendance by accessing their dashboards on Sciforum.net once the event is concluded. The certificates will be found under the “My Certificates" category.

Disclaimer

Delegates will receive a name badge at the Information Desk, upon registration. The badge must be worn prominently in order to gain access to the congress area during the event. Admission will be refused to anyone not in possession of an appropriate badge.

Insurance

The organizers do not accept liability for personal accidents, loss, or damage to private property incurred as a result of participation in *Non-coding RNA World 2024: Exploring Mechanisms, Designing Medicines*. Delegates are advised to arrange appropriate insurance to cover travel, cancellation costs, medical, and theft or damage of belongings.

Basel

Sitting on the banks of the Rhine River, Basel's history can be traced back to Roman times, making it a city steeped in tradition. The medieval period witnessed Basel's rise as a significant cultural and economic hub in Central Europe. The Old Town, with its cobbled streets and ancient buildings, showcases the city's grandeur from the 13th to the 15th centuries.

The 20th century witnessed transformative urban development across Basel, with a notable landmark being the Kleinbasel district. This area features striking architectural gems that reflect Basel's unique blend of tradition and modernity. Notably, the renowned Swiss architecture firm Herzog & de Meuron contributed to the city's contemporary skyline.

In 2008, Basel gained international recognition as a hub for contemporary art and culture with the inauguration of the Fondation Beyeler. This museum, designed by Renzo Piano, encapsulates Basel's commitment to both preserving its rich heritage and embracing contemporary artistic expressions.

Basel is a haven for art enthusiasts, boasting world-class museums like the Kunstmuseum and the Vitra Design Museum. The city offers a delightful array of open-air markets, dining establishments, boutiques, and historical churches. It is remarkably pedestrian-friendly, and a well-structured tram system efficiently connects residents and visitors to the city's many attractions.

For a complete overview, see wikitravel.org or visit www.basel.com.

The memox Basel Main Station

The memox Basel Main Station venue is located right next to Basel SBB station, within the Peter Merian Haus. Thanks to its central location, the building is optimally connected to the public transport network and is thus easily accessible by bus, tram, and train. Huge window fronts and an impressive atrium provide plenty of natural daylight in the venue and create a great atmosphere.

Address: Peter-Merian-Strasse 80, 4052 Basel

MDPI, the Multidisciplinary Digital Publishing Institute, is a publisher of fully peer-reviewed, Open Access journals with a focus on robust and rapid editorial processes. Our aim is to ensure that high-quality research is verified and made available to the research community as quickly as possible. MDPI stands at the forefront of the Open Access movement, having launched its first online journal *Molecules* in 1996. Today, MDPI is a leader in Open Access, publishing more than 400 journals across all research disciplines and offering publishing-related initiatives to scholars:

• Sciforum - A platform for academic communication and collaboration where scholars can set up scientific conferences.

• Preprints - A multidisciplinary not-for-profit platform for rapid communication of research results before peer-review.

• JAMS - A complete manuscript submission system that incorporates all steps from initial submission to publication, including peer-review.

• IOAP – An Institutional Open Access Program that supports its members with access to MDPI's submission system, publication data and administrative processes relating to Article Processing Charge (APC) management.

If you would like more information about open access or any of our services listed above, be sure to talk to us at the MDPI booth. See you there!

Contact persons during the event

Timothy Lee
Email: timothy.lee@mdpi.com

Mina Marjanović
Email: mina.marjanovic@mdpi.com

Surayya Rehman
Email: surayya.rehman@mdpi.com

Emergency Information

All emergencies in Switzerland: 112 (no area code needed)
Ambulance and health emergencies: 144
Fire brigade: 118
Switzerland National Police: 117

Abstracts — Talks

S1. ncRNA Mechanisms

Non-coding RNAs and Their Regulatory Functions During Early Mammalian Development

Joanna Jachowicz

Institute of Molecular Biotechnology, Vienna Biocenter, Austrian Academy of Sciences, Austria

Although thousands of non-coding RNAs are encoded in mammalian genomes, their mechanisms of action are largely uncharacterized. One such ncRNA Xist mediates chromosome-wide gene silencing on one of the two X chromosomes to achieve gene expression balance between males and females. However, how a limited number of Xist molecules can mediate robust silencing of a significantly larger number of target genes (~1 Xist RNA: 10 gene targets) while maintaining specificity exclusively to genes on the X within each cell, is unclear. I will present our recent observations uncovering a spatial amplification mechanism that allows Xist to achieve these two essentials but countervailing regulatory objectives: chromosome-wide gene silencing and specificity to the X. I will discuss how this spatial amplification mechanism may be a more general mechanism by which other non-coding nuclear RNAs can balance specificity to, and robust control of, their regulatory targets. Lastly, I will show how our newly developed method RNA-DNA SPRITE can help to understand the 3D nuclear localization of these RNAs and identify their genomic targets at the genome-wide scale.

PAPγ Associates With the PAXT Nuclear Exosome to Control the Abundance of PROMPT ncRNAs

Xavier Contreras[1], David Depierre[2], Charbel Akkawi[1], Marion Helsmoortel[1], Marina Srbic[1], Alexandre Heurteau[2], Olivier Cuvier[2], Rosemary Kiernan[1]

[1] Gene Regulation Lab, Institute of Human Genetics, Université de Montpellier, Montpellier, France
[2] CBI, MCD Unit, Toulouse, France

Pervasive transcription of the human genome generates an abundance of RNAs that must be processed and degraded. The nuclear RNA exosome is the main RNA degradation machinery in the nucleus. However, the nuclear exosome must be recruited to its substrates by targeting complexes such as NEXT or PAXT. Using proteomic analysis, we identify additional subunits of PAXT, including many orthologs of MTREC found in S. pombe. In particular, we show that polyA polymerase gamma (PAPγ) associates with PAXT. Genome-wide mapping of the binding sites of ZFC3H1, RBM27 and PAPγ shows that PAXT is recruited to the TSS of hundreds of genes. Loss of ZFC3H1 abolishes the recruitment of PAXT subunits, including PAP to TSSs and concomitantly increases the abundance of PROMPTs at the same sites. Moreover, PAPγ, as well as MTR4 and ZFC3H1, is implicated in the polyadenylation of PROMPTs. Our results thus provide key insights into the direct targeting of PROMPT ncRNAs by PAXT at their genomic sites.

Identification of Functional Sequence Motifs by Intra-Transcript Enrichment

Amit Felach[1], Nisrine Lahoud-Jeries[1], Haim Bar[2], Assaf C Bester[1]

[1] Faculty of Biology, Technion-Israel Institute of Technology, Haifa, Israel
[2] Department of Statistics, University of Connecticut, Storrs, Mansfield, CT, USA

Long non-coding RNAs (lncRNAs) play pivotal roles in various biological processes, yet understanding their sequence-to-function relationships is challenged by low conservation and complex modular structures. Inspired by human language, where specific words contextualize text, we developed SEEQ (Sequence Enrichment Extraction for Quantitative motif identification). This innovative computational tool detects intra-transcript sequence enrichment without alignment, enhancing the identification of functional elements in lncRNAs by searching for sequence similarities within and across transcripts. It clusters related motifs to form consensus sequences, facilitating the functional classification and identification of scaffold lncRNAs.

The application of SEEQ has unveiled a range of previously unidentified sequences and structures that significantly influence the subcellular localization of RNAs. Our analysis highlights distinct nuclear-enriched motifs that are more prevalent than their cytoplasmic counterparts, suggesting combinatorial effects of specific sequence-driven mechanisms that regulate RNA localization. This underscores the importance of whole-transcript analysis in understanding cellular distribution dynamics. Furthermore, SEEQ has been instrumental in identifying repetitive sequences within transcripts that play crucial roles in RNA transport and retention in the nucleus.

Additionally, SEEQ's rapid and adaptable analysis has identified multiple lncRNAs with unique sequence structures critical for RNA–protein interactions and biological functions. For example, SNHG14 is characterized by a complex pattern of 26 repeated motifs, which are essential for its RNA–protein interactions and overall functionality.

This research underscores the critical role of modular sequence motifs in studying lncRNAs and establishes a robust framework for predicting RNA functions based on sequence data. By revealing novel sequence motifs that influence subcellular localization, our study not only extends the understanding of RNA biology but also showcases the potential of language-based models in deciphering ncRNA functions. These advancements could revolutionize the methodology for studying and predicting RNA functions, opening new avenues for research in this dynamic field and offering a promising future for RNA research.

Biophysical Analysis of a Structurally Unique Human Alu RNA

Daniel Gussakovsky[1], Mira J.F. Brown[1], Higor Sette Pereira[2], Markus Meier[1], G. Pauline Padilla-Meier[1], Evan P. Booy[1], Jörg Stetefeld[1], Trushar R. Patel[2], Sean A. McKenna[1]

[1] Department of Chemistry, University of Manitoba, Winnipeg, MB, Canada
[2] Department of Chemistry and Biochemistry, Alberta RNA Research and Training Institute, University of Lethbridge, Lethbridge, AB, Canada

Alu RNA are transcription products of Alu elements which make up ~10% of the human genome. Alu RNA are implicated in a variety of human disease states by disrupting translation as non-coding Alu RNA and by introducing dysregulating splice sites within mRNA as intronic Alu RNA. There are over 1 million copies of Alu elements, with 99% of the elements being unique in terms of sequence. The diversity of Alu RNA is poorly understood, with previous structural analyses of Alu RNA consistently describing a pseudoknot structure capable of interacting with the protein heterodimer SRP9/SRP14. Herein, we present a case study of a structurally unique Alu RNA that lacks a pseudoknot and poorly interacts with SRP9/SRP14. We probed left-arm Alu monomers, the simplest possible non-coding human Alu RNA, and identified Expressed in Brain 120 RNA (EB120 RNA). The expression levels of EB120 RNA were assessed in 18 different human cell lines and tissues. In vitro-transcribed EB120 RNA was subjected to a variety of biophysical analyses and compared to Alu RNA BC120. Significant differences in conformation between EB120 and BC120 RNA were elucidated by SAXS. Structure prediction of EB120 RNA depicted a structure lacking a pseudoknot. A variety of SRP9/SRP14 pulldowns were performed and a significantly lower enrichment of EB120 RNA compared to BC200 and BC120 RNA is reported. These results provoke a consideration of the breadth of Alu RNA diversity, and we will discuss the impact mutations may have on generalized Alu RNA function.

Investigation of lncRNA Multifunctionality Through Splicing and Structural Dynamics

Alisha Jones

Department of Chemistry, New York University, New York, NY, USA

Over the past 15 years, long noncoding RNAs (lncRNAs) have emerged as critical regulators of biological function. They are transcripts that are classified based on their lengths (>500 nucleotides) and a lack of any protein-encoding ability. Similar to other RNA pol II transcripts, lncRNAs are 5' capped, spliced, and polyadenylated. Few studies have been conducted to identify the cis and trans regulatory factors that govern how lncRNAs are spliced. We carried out bioinformatic and experimental analyses to identify the structures and protein interactions of pre-spliced lncRNAs that influence their processing via the spliceosome. We will report on the RNA-binding proteins that primarily regulate lncRNA splicing, the structures adopted by pre-spliced lncRNAs, as well as the types of splice events more common to lncRNA transcripts. This work ultimately sheds light on the genesis of lncRNA isoforms and offers potential avenues for therapeutically targeting lncRNA isoforms that are implicated in disease.

Enhancer-Promoter RNA Maps Link Risk Variants to Disease Genes

Yuanchao Xue

Key Laboratory of RNA Biology, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

Enhancers are key noncoding DNA elements that control spatiotemporal gene expression programs by engaging cognate promoters through long-range chromosomal looping. Emerging evidence has shown that active enhancers and promoters are bidirectionally transcribed to generate vast enhancer RNA (eRNA) and promoter upstream antisense RNA (uaRNA). However, the functionality of those noncoding RNA transcripts and how they modulate transcription is poorly understood. We recently developed several RNA in situ conformation sequencing technologies, including RIC-seq and CRIC-seq, for the global profiling of RNA-RNA spatial interactions. We found that the functional connectivity of enhancers and promoters can be assigned using their pairwise-interacting RNAs. Importantly, these eRNA-uaRNA interactions are mediated by diverse RNA-binding proteins and appear required for long-range chromatin looping and transcriptional activation. Here, we analyze the largest RIC-seq and CRIC-seq datasets generated from multiple cell lines and identify tens of thousands of enhancer and promoter RNA interactions. These comprehensive enhancer-promoter RNA interaction maps enable us to unravel the enhancer and promoter communication principles and link many risk variants to disease genes. In this meeting, I would like to share our recent findings on how enhancers communicate with promoters via RNA-RNA interactions and how those RNA sequence variants that disrupt enhancer-promoter circuitry cause disease.

Long Non-coding RNA NRAD1 Regulates miRNA Biogenesis in Triple-Negative Breast Cancer

Hannah Faulkner Cahill[1], Justin Brown[2], Marie-Claire Wasson[2], Meghan McLean[2], Mattie Leslie-Toogood[2], Raj Pranap Arun[2], Jaganathan Venkatesh[2], Patrick Murphy[3], Paola Marcato[2, 4, 5]

[1] Dalhousie University, Halifax, NS, Canada
[2] Department of Pathology, Dalhousie University, Halifax, NS, Canada
[3] Department of Biology, University of Prince Edward Island, Charlottetown, PEI, Canada
[4] Department of Microbiology & Immunology, Dalhousie University, Halifax, NS, Canada
[5] Nova Scotia Health Authority, Halifax, NS, Canada

Triple-negative breast cancer (TNBC) is an aggressive breast cancer subtype associated with distinct gene expression profiles and worse patient outcomes, and needs new therapies. Increased understanding of the factors that lead to aberrant gene expression in TNBC will lead to novel treatment solutions. Non-coding RNAs, both microRNAs (miRNAs) and the long non-coding RNAs (lncRNAs) that regulate miRNAs, are critical contributors to the altered gene expression associated with TNBC progression. Although most lncRNA studies identify specific competitive endogenous miRNA interactions, our work instead suggests that TNBC-promoting lncRNA, a non-coding RNA in the aldehyde dehydrogenase 1A pathway (NRAD1), alters miRNA biogenesis, resulting in an altered miRNA landscape in TNBCs. We performed gene array, proteomics, and miRNA microarray analyses of TNBC cells, with or without NRAD1 knockdown. NRAD1 altered the expression of hundreds of genes and proteins connected to cancer-promoting pathways. This was reflected in the miRNA array, which showed altered abundances in hundreds of miRNAs by NRAD1. MiRNA target analyses predicted miRNA targets among the NRAD1-regulated genes and qPCR was conducted to validate hits. The most significantly upregulated miRNA upon NRAD1 knockdown in both cell lines is miR-4485-3p, which is thus far minimally studied in cancer but predicted to target genes regulated by NRAD1. Luciferase reporter assays failed to show direct binding interactions between NRAD1 miR-4485-3p, suggesting alternative mechanisms of miRNA regulation. The proteomics data suggest that NRAD1 regulates key proteins involved in miRNA biogenesis, which could explain the altered miRNA levels post NRAD1 knockdown. Overall, this work reveals new mechanistic information into how dysregulated lncRNA-miRNA networks result in the aberrant gene expression of TNBCs. Identifying key TNBC-promoting lncRNA-miRNA-mRNA axes that alter gene expression could result in the development of nucleotide-based therapies targeting these molecules.

The Role and Mechanisms of Subcellular Localization Regulation of TUG1 lncRNA

Luise Elektra-Keller, Rebeca Cordellini, Josep Biayna Rodriguez, Gabrijela Dumbović

Goethe University Frankfurt, Frankfurt, Germany

It is widely known that RNA can play a central role in biology. The dynamic regulation of subcellular RNA localization is a key component regulating RNA function, critical for biological processes ranging from organismal development to cellular activity. LncRNAs can act through a myriad of mechanisms in virtually all subcellular compartments. While the mechanisms regulating RNA localization have been found for some transcripts, new aspects of RNA localization regulation continue to emerge.

The highly conserved lncRNA locus TUG1 is a perfect example of an lncRNA with multiple functionalities within the nucleus and the cytoplasm. Our recent studies showed that intron retention influences the spatio-temporal dynamics of TUG1 RNA in a cell-type-specific manner. This is due to a fraction of TUG1 transcripts retaining introns, which are therefore retained in the nucleus. Nevertheless, the molecular mechanism and biological role of TUG1 RNA localization regulation remain unknown. We will present our recent findings on the complexity of RNA localization regulation, using single-molecule RNA imaging, CRISPR/Cas9, and RNA–protein interaction analyses as our methodologies. Our study demonstrates the complex interplay between splicing, RNA-binding proteins (RBPs), and RNA stability in guiding TUG1 RNA to subcellular compartments and regulating its molecular functions.

Biogenesis, Function and Potential Application of Circular RNAs

Ling-Ling Chen

[1] Key Laboratory of RNA Innovation, Science and Engineering, CAS Center for Excellence in Molecular Cell Science, Shanghai Institute of Biochemistry and Cell Biology, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China
[2] New Cornerstone Science Laboratory, Shenzhen, China
[3] Key Laboratory of Systems Health Science of Zhejiang Province, School of Life Science, Hangzhou Institute for Advanced Study, University of Chinese Acad-emy of Sciences, Hangzhou, China

Circular RNAs are covalently enclosed single-stranded transcripts with unique biogenesis, stability and conformation, thereby playing distinct roles in modulating cellular functions and also possessing great potential for developing circular RNA-based modalities. In this talk, I will discuss recent progress in our understanding of the conformation and turnover of circular RNAs. I will also discuss our recent efforts in developing circular RNA-based aptamers that can effectively suppress the excessive activation of dsRNA-activated Protein Kinase R (PKR) in different pathophysiological contexts.

Regulation of Small RNA-Guided Gene Silencing Pathways

Gunter Meister

Department of Biochemistry I of the University of Regensburg, Germany

Gene expression is regulated at many post-transcriptional steps of RNA maturation. Various classes of non-coding RNAs have been identified that affect gene expression including microRNAs, lncRNAs or circular RNAs. Furthermore, N6-methyladenosine (m6A) on cytoplasmic mRNAs recognized by the reader proteins of the YTH family also regulated gene expression through translation and mRNA degradation. All these pathways play critical roles in diverse cellular and physiological processes and have thus been associated with cancer development and progression.

Small RNAs such as microRNAs, siRNAs and piRNAs bind to a member of the Argonaute protein family and guide them to complementary target RNAs, which are subsequently degraded. Argonaute proteins recruit addition factors (such as members of the TNRC6 protein family) to facilitate downstream gene silencing processes, often organized in cellular condensates. Regulation of these processes is only poorly understood. Furthermore, small RNAs can also be transferred from cell to cell and even from one species to another in a process known as cross-kingdom RNAi. In our work, we have generated tools to study small RNA populations in cells and in addition found several regulatory pathways ranging from small RNA loading to protein phosphorylation and degradation.

Transforming Growth Factor β (TGFβ)-Induced Long Non-coding RNA LINC00313 Activates Wnt Signaling and Promotes Cholangiocarcinoma

Panagiotis Papoutsoglou[1,2], Raphaël Pineau[2], Raffaële Leroux[2], Corentin Louis[2], Anaïs L'Haridon[2], Dominika Foretek[1], Antonin Morillon[1], Jesus Banales[3,4], David Gilot[2,5], Marc Aubry[2], Cédric Coulouarn[2]

[1] ncRNA, Epigenetic and Genome Fluidity, CNRS UMR3244, Sorbonne University, PSL University, Institut Curie, Research Centre, Paris, France
[2] Inserm, Univ Rennes, Oncogenesis, Stress, Signaling (OSS) Laboratory, UMR_S 1242, Eugene Marquis Center, Rennes, France
[3] Department of Liver and Gastrointestinal Diseases, Biogipuzkoa Health Research Institute, Donostia University Hospital, CIBERehd, Ikerbasque, San Sebastian, Spain
[4] Department of Biochemistry and Genetics, School of Sciences, University of Navarra, Pamplona, Spain
[5] Mechanistic & Structural Biology, Discovery Sciences, R&D, AstraZeneca, Mölndal, Sweden

Cholangiocarcinoma is a devastating liver cancer characterized by high aggressiveness and therapy resistance, resulting in poor prognosis. Long non-coding RNAs and signals imposed by oncogenic pathways, such as transforming growth factor β (TGFβ), frequently contribute to cholangiocarcinogenesis. Here, we explore novel effectors of TGFβ signalling in cholangiocarcinoma. LINC00313 is identified as a novel TGFβ target gene. Gene expression and genome-wide chromatin accessibility profiling reveal that nuclear LINC00313 transcriptionally regulates genes involved in Wnt signalling, such as the transcriptional activator TCF7. LINC00313 gain-of-function enhances TCF/LEF-dependent transcription, promotes colony formation in vitro and accelerates tumour growth in vivo. Genes affected by LINC00313 over-expression in CCA tumours are associated with KRAS and TP53 mutations and reduce overall patient survival. Mechanistically, ACTL6A and BRG1, subunits of the SWI/SNF chromatin remodelling complex, interact with LINC00313 and affect TCF7 and SULF2 transcription. We propose a model whereby TGFβ induces LINC00313 in order to regulate the expression of hallmark Wnt pathway genes, in co-operation with SWI/SNF. By modulating key genes of the Wnt pathway, LINC00313 fine-tunes Wnt/TCF/LEF-dependent transcriptional responses and promotes cholangiocarcinogenesis.

A Novel lncRNA Alters Breast Cancer Risk by Modulating Interferon Signaling Through BHLHE40 and RIG-I

Stacey Edwards[1, 2, 3], Haran Sivakumaran[1], Maina Bitar[1, 2], Jonathan Beesley[1], Michelle Wykes[1], Juliet French[1, 2, 3]

[1] *QIMR Berghofer Medical Research Institute, Brisbane, Australia*
[2] *The University of Queensland, Brisbane, Australia*
[3] *Queensland University of Technology, Brisbane, Australia*

Genetic variants at 3p26 are associated with increased breast cancer (BC) risk. Using targeted RNAseq, we identified a novel lncRNA (called LncB) whose expression is associated with the BC risk variants. LncB is a 1.1 kb, three-exon lncRNA that is primarily expressed in estrogen receptor-positive (ER+) breast tumours and functions both in cis and in trans. The LncB promoter sits within an 11 kb super-enhancer, which is restricted to ER+ breast tumours. We used promoter capture HiC to show that LncB physically interacts with BHLHE40, a transcription factor that regulates cytokine production. CRISPR interference of the LncB promoter ablated the super-enhancer, reduced chromatin looping between LncB and BHLHE40, resulting in decreased BHLHE40. RNAseq on CRISPRi-LncB cells showed an enrichment in interferon (IFN) signaling pathways. LncB primarily localised to the cytoplasm; thus, we explored a possible trans-acting function. RNA pull-down and mass spectrometry identified RIG-I (a cytosolic RNA sensor) as an LncB-binding protein. Mechanistically, LncB directly interacts with RIG-I, promoting tumour-intrinsic type I IFN signaling and cytokine production in ER+ BC cells. LncB-conditioned media incubated with stimulated human peripheral blood mononuclear cells showed increased IFN-gamma and granzyme B produced from CD8+ T cells and natural killer cells, supporting an anti-tumour immune response. These findings emphasize the complex regulatory mechanisms underlying GWAS risk regions, provide further evidence that lncRNAs are key modulators of the tumor immune response and identify lncB as a potential RNA-based therapeutic in ER+ breast cancer.

S2. ncRNAs and Disease

RNA Therapies for Cardiac Regeneration and Precise Gene Editing

Mauro Giacca

School of Cardiovascular and Metabolic Medicine & Sciences, King's College London, United Kingdom

Cardiac disorders are common, lethal and expensive. Heart failure, which is a common consequence of most cardiac conditions, including cardiomyopathy of ischemic or hereditary origin, now affects over 1-3% of the global adult population, has a 5-year mortality of 50-75% and absorbs 2-3% of national health expenditures in high-income countries. Cardiomyocyte death is not offset by new cell generation after birth, as the regenerative capacity of the adult human myocardium is less than 1% per year over a lifetime. No drug exists for myocardial survival or regeneration, nor any gene editing therapy for the correction of genetic defects. More in general, no biological therapy has ever been developed for any primary cardiac condition.

A main goal of my laboratory is to develop RNA therapeutics to treat cardiac disease, with the objectives of preventing cardiomyocyte loss after damage, stimulating cardiac regeneration and achieving precise gene editing for the correction of hereditary cardiac mutations. Using whole genome siRNA and miRNA libraries, over the last few years we performed a series of high throughput screenings for non-coding RNAs that prevent cardiomyocyte death, stimulate cardiomyocyte proliferation and promote homology directed repair and prime editing using CRISPR/Cas9 tools. We have identified a few miRNAs that are very effective at stimulating re-entry of cardiomyocytes into the cell cycle. Once administered to mice or pigs after myocardial infarction, these miRNAs promote clinically relevant cardiac regeneration and remuscularisation of the infarcted hearts. Other miRNAs stimulate precise gene editing by reawakening expression of the homologous recombination machinery in post-mitotic, adult hearts or stimulating prime editing. We achieve expression of these miRNAs either using AAV vectors or by delivering their synthetic mimics to the heart using lipid nanoparticles (LNPs) generated with the SNALP technology, which is amenable to clinical translation in patients.

Exploiting the Dark Side of the Genome for Breast Cancer Stratification and Therapy

Nehad Alajez

Qatar Biomedical Research Institute (QBRI), Ar-Rayyan, Qatar

Triple-negative breast cancer (TNBC) presents a major clinical challenge due to its aggressive nature and the scarcity of targeted treatment options. Long non-coding RNAs (lncRNAs) have recently emerged as pivotal regulators in cancer pathogenesis, yet their expression profiles and functions in TNBC remain largely unexplored. In this study, our objective is to unravel the transcriptional landscape of lncRNAs across breast cancer subtypes, with a focus on their potential utility as disease biomarkers and therapeutic targets. Our findings delineate distinct expression profiles of lncRNAs in various breast cancer subtypes, suggesting a novel lncRNA-centric approach for TNBC classification.

To elucidate the contributions of lncRNAs to TNBC pathogenesis, we conducted comprehensive functional profiling using a CRISPR deletion (CRISPR-del) approach, highlighting the promise of RNA-based therapeutics as a novel treatment avenue for TNBC. To encompass both annotated and novel lncRNAs, we compiled a comprehensive reference assembly by integrating annotations from Gencode, MiTranscriptome, and BigTranscriptome. Subsequently, we constructed a customized paired-guide RNA (pgRNA) library targeting a total of 1,029 expressed lncRNA transcription start sites (TSSs), with a depth of ~10 pgRNAs per target to mitigate potential off-target effects. Leveraging the dual-excision CRISPR-Cas9 deletion system in a high-throughput format in MDA-MB-231 and BT-549 TNBC models, we identified numerous lncRNAs critical for TNBC cell proliferation and chemoresistance, elucidating their roles in TNBC pathogenesis. Validation using antisense oligonucleotides (ASOs) confirmed the significance of selected lncRNAs on TNBC cell growth and proliferation.

Further investigation into the expression patterns of two novel lncRNA candidates (candidate 385 and candidate 389) across a large TNBC cohort (n=360) revealed differential expression across TNBC molecular subtypes and their association with pathways pivotal for tumor progression, including cell motility, extracellular matrix organization, and immune response. Loss-of-function studies in conjunction with transcriptomic profiling provided mechanistic insights into their plausible regulatory roles, highlighting their potential utilization as therapeutic targets in TNBC. In summary, our data provide a comprehensive lncRNA dependency map in TNBC, underscoring the significance of lncRNAs in TNBC biology, and validated two novel candidates (candidate 385 and candidate 389) as promising targets for further investigation and therapeutic intervention.

LncRNA GRASLND Affects Melanoma Cell Growth, Phenotypic Switching, and Immunogenicity by Modulating dsRNA Innate Immunity Sensor PKR

Jochen Imig[1], Kim Denise Fischer[1], Shashank Tiwari[1], Beatrice Thier[2], Lin Christina Qiu[1], Annette Paschen[2]

[1] Chemical Genomics Centre of the Max Planck Society, Max Planck Institute of Molecular Physiology, Dortmund, Germany
[2] Department of Dermatology, University Hospital Essen, Essen, Germany

Melanoma is one of the most common cancers, and its incidence has been rising since the past decade. Patients at advanced stages of the disease have very poor prognoses. Nowadays, the standard-of-care for advanced melanoma is resection followed by immune checkpoint inhibition-based immunotherapy. However, a substantial fraction of patients either do not respond to or develop resistances towards this treatment. Melanoma displays a remarkable plasticity, enabling it to transition into dedifferentiated cell states and phenotypical switching, which contributes to T-cell and therapy resistance.

In that sense, novel approaches to overcome resistance and to better understand the underlying mechanisms are an urgent need. In this context, functional players and promising targets might be found in the poorly addressed class of long non-coding RNAs (lncRNAs). Here, the lncRNA GRASLND is investigated for its roles in melanoma pathogenesis, its correlation with IFNg signaling, immunogenicity, and cellular phenotype.

TCGA meta-analysis of a clinical dataset unveils that GRASLND is highly overexpressed in melanoma. It is associated both with poor survival and with a differentiated phenotype, but it is lowly expressed in immunological “hot” tumors. Additionally, we show that GRASLND knockdown by RNAi reduces melanoma cell growth and leads to phenotypic switching towards a dedifferentiation of certain melanoma cell lines while increasing the migrative capacities of cells. Transcriptome analysis upon GRASLND repression revealed a restoration of T-cell activation genes and the IFNg signaling gene signature but a reduction in PD-L1 mRNA. Most importantly, GRASLND repression reestablished MHC-I melanoma cell surface expression under IFNg treatment, making it an attractive candidate for therapeutic exploitation. Mechanistically, we show that GRASLND associates with the dsRNA innate immunity sensor PKR and represses downstream ATF4 signaling pathways, related autophagy and apoptosis, and the C-Jun pathway, leading to cytokine secretion.

We hypothesize an adaptive resistance mechanism of melanoma cells, which evades the immune system by overexpressing the lncRNA GRASLND via the suppression of IFNγ signaling. Together, targeting GRASLND's interaction with PKR may hold great promise forovercoming immunotherapy resistance in melanoma patients.

RNA-Based Regulation in Stress Response and Virulence Control of Pathogenic Epsilonproteobacteria

Cynthia M. Sharma

Department of Molecular Infection Biology II, Institute of Molecular Infection Biology, University of Würzburg, Bavaria, Germany

Non-coding RNAs play central roles in diverse physiological processes in all kingdoms of life. Our research focuses on RNA-based regulation in bacterial pathogens, using the gastric pathogen Helicobacter pylori and the related food-borne pathogen Campylobacter jejuni as models. We are especially interested in the molecular mechanisms and physiological roles of small, regulatory RNAs (sRNAs) and RNA binding proteins (RBPs) in bacterial stress response and virulence control. In this talk, I will describe multiple examples of the diverse mechanisms and functions of sRNAs in Helicobacter and Campylobacter. For example, our work on sRNAs in these bacteria revealed unexpected connections between so-called phase-variable control at simple sequence repeats and post-transcriptional regulation. On the one hand, we identified two sRNAs from H. pylori and C. jejuni that target length-variable G-repeats in mRNAs and thereby regulate genes involved in host colonization and antibiotic sensitivity [1,2]. On the other hand, we also identified a sRNA that is itself transcriptionally regulated by a phase-variable repeat and acts as a central repressor of the major virulence factors of H. pylori [3]. Furthermore, we recently discovered two sRNAs involved in flagella biogenesis of C. jejuni [4]. Biogenesis of the complex flagellar motility machinery requires a strict hierarchical transcriptional control of the individual components. In contrast, little is known about post-transcriptional control of flagella biogenesis via sRNAs. Here, we characterized two sRNAs with opposing effects on the flagellar cascade in C. jejuni. We demonstrate that CJnc230 sRNA is processed from the mRNA encoding the flagellar structural hook protein FlgE and acts as an enhancer of flagellar length and motility. In contrast, overexpression of CJnc170 sRNA, which is expressed downstream in the transcriptional cascade, represses flagellar length and motility. This interplay of two sRNAs likely fine-tunes flagellar biosynthesis through balancing of the hierarchically expressed components. Such post-transcriptional regulation of flagellar biogenesis via sRNAs is emerging as an additional layer of control in this process and seems to be more widespread among bacteria. Besides the characterization of single sRNA targets, we are also employing and developing genome-wide approaches, such as direct RNA-RNA ligation methods or RBP capture methods, to globally identify cellular interaction partners of RNAs and to further decipher the complex RNA-based networks as well as their underlying molecular mechanisms in bacteria.

References

[1] S.R. Pernitzsch, *et al.*, *Nature Communications* (2021) 12, 4433
[2] S. Svensson & C. Sharma, *eLife* (2021) 10:e69064
[3] S.K. Eisenbart *et al.*, *Molecular Cell* (2020) 80(2):210-226
[4] F. König, et al., *Nature Communications* (2024) 15(1):5240

Beyond the Silence: Decoding MicroRNA-198 Dynamics in Wound Healing

Prabha Sampath

*A*STAR Skin Research Labs (A*SRL) – Biopolis, Singapore*

Wound healing requires a regulated integration of complex biological events, including cell migration, proliferation and extracellular matrix remodelling, globally stimulated by transforming growth factor-b1 (TGF-b1) and other growth factors. Gene expression in this regenerative process is extensively regulated by non-coding microRNAs (miRNAs). We identified a post-transcriptional switch that dictates the spatiotemporal and mutually exclusive expression of two alternative gene products from a single transcript. In normal skin, expression of exonic microRNA-198 (miR-198), located in the 3'-untranslated region of follistatin-like 1 (FSTL1) messenger RNA, switches to expression of the linked open reading frame of FSTL1 upon wounding and orchestrates wound re-epithelialization. However, in chronic diabetic foot ulcers (DFUs), the dual-state molecular switch is defective, resulting in sustained expression of miR-198, a microRNA which inhibits keratinocyte migration. Modulation of the defective switch and abrogation of miR-198 expression locally in DFUs can lead to effective keratinocyte migration and wound re-epithelialization and accelerate wound healing. Towards this, we designed sequence-specific antimiR oligonucleotides (AMOs-198) with a judicious combination of chemical modifications which enhance cellular uptake and target the specificity and metabolic stability of AMOs-198 to effectively target miR-198. With the depletion of anti-migratory miR-198, we observed the restoration of all pro-migratory targets of miR-198, which promote the effective migration of keratinocytes towards the wound edge, leading to wound closure. DFUs are an escalating medical issue globally and a devastating complication of diabetes resulting in lower extremity amputations. We envisage the development of an early-intervention therapeutic to accelerate the healing of DFUs, mitigate complications and prevent lower extremity amputations.

AC011294.3 Is a New Enhancer-Associated lncRNA Decreasing the Sensitivity of Marginal Zone Lymphoma Cells to Bruton's Tyrosine Kinase Inhibition by Modulating RNA Editing Enzymes

Sara Napoli[1], Luciano Cascione[1, 2], Sunandini Ramnarayanan[3], Francesca Guidetti[1], Filippo Spriano[1], Alberto Jesus Arribas[1], Andrea Rinaldi[1], Rory Johnson[3], Francesco Bertoni[1, 4]

[1] *Institute of Oncology Research, Faculty of Biomedical Sciences, USI, Bellinzona, Switzerland*
[2] *SIB Swiss Institute of Bioinformatics, Lausanne, Switzerland*
[3] *Conway Institute for Biomolecular and Biomedical Research, University College Dublin, Ireland*
[4] *Oncology Institute of Southern Switzerland, Bellinzona, Switzerland*

Marginal zone lymphomas (MZLs) account for approximately 10% of all B-cell lymphomas. Pharmacological inhibition of Bruton's tyrosine kinase (BTK) has been one of the most successful targeted approaches developed for MZL patients. Three BTK inhibitors (ibrutinib, zanubrutinib, and orelabrutinib) have indeed been approved by different regulatory agencies for treating MZL patients.

The occurrence of resistance to anti-cancer agents is a common phenomenon. The resistance to BTK inhibitors in lymphomas, including MZL, is not often driven by somatic mutations affecting BTK itself or genes involved in the same signaling cascade. Stable non-genetic resistance is acquired via dynamic transcriptional adaptation. Enhancers, key regulators of cell differentiation, can help malignant cells evade therapeutic challenges by activating alternative transcriptional programs.

Most active enhancers are transcribed in long noncoding RNAs (lncRNAs) called enhancer RNAs (eRNAs). They play a regulatory role in sustaining the correct chromatin conformation required to transactivate promoters. eRNAs can occasionally be stabilized and, acting at distal loci in trans, may become e-lncRNAs, relevant regulators for the biology of cancer cells. Here, we studied the role of eRNAs in resistance to ibrutinib in MZL cells.

We defined active super-enhancers and their corresponding eRNAs expressed in MZL and built an eRNA-focused CRISPRi library to investigate their function. AC011294.3 emerged as one of the top hits among molecules involved in resistance to BTK inhibition. AC011294.3 is known to regulate the expression of ADAR1 and ADAR2, enzymes responsible for A-to-I RNA editing. Its silencing improved ibrutinib efficacy and altered the expression of enzymes involved in RNA metabolism and splicing, suggesting a hierarchical regulation of RNA stability in the therapeutic evasion of tumors. Moreover, the alteration in RNA editing, as a consequence of e-lncRNA silencing, re-activated the antigen presentation capability of tumor cells, suggesting a role of AC011294.3 also in tumor immune escape.

In conclusion, a CRISPRi screen identified a new e-lncRNA with a role in modulating the response to BTK inhibition in MZL and associated with post-transcriptional modification of the tumor genetic program.

Deciphering Cell-Specific lncRNA Targets in Colorectal Cancer: Integrated Single-Cell Transcriptomics and CRISPR-Cas9 Screens

Ante Mihaljevic[1, 2], Philip Daniel Rubin[1, 2], Ezgi Laeci[1, 2], Panagiotis Chouvardas[3], Roberta Esposito[1,2]

[1] Department of Medical Oncology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland
[2] Department for BioMedical Research, University of Bern, Bern, Switzerland
[3] Department for BioMedical Research, Urology Research Laboratory, University of Bern, Bern, Switzerland

Long non-coding RNAs (lncRNAs) represent a burgeoning category of genes with profound and diverse implications in human cancers. Their cell-type-specific expression renders them promising candidates for precision therapies. However, their functional roles across cancer cell subtypes remain elusive, often overshadowed by the predominant focus on protein-coding genes. In this study, we employed an integrative approach, combining single-cell RNA sequencing (scRNA-seq) and CRISPR-Cas9 technology to explore the contributions of lncRNAs to the heterogeneity of one the greatest cancer killers: colorectal cancer (CRC).

Re-analysing 49,436 single cells from 29 CRC patients, we scrutinized the cell-type-specific expression patterns of both protein-coding and lncRNA genes, estimating their expression distribution using the Gini coefficient across various cellular compartments. Remarkably, lncRNAs exhibited significantly higher Gini coefficients compared to protein-coding genes. Among these, we pinpointed 996 lncRNAs significantly enriched in epithelial cells, forming the basis for our CRC Epithelial LncRNAs CRISPRi (CELC) library. Leveraging CRISPR-dCas9 technology, we screened the CELC library in multiple CRC cell lines. From promising preliminary data, we identified 11 oncogenic epithelial lncRNAs.

Subsequent proof-of-concept experiments, encompassing viability and migration assays, validated the oncogenic potential of two lncRNA candidates: CASC19 and LINC00460. Recognizing the imperative of mirroring in vivo conditions, we progressed to validate our findings in patient-derived organoids (PDOs), pioneering the CRISPRi technology into PDOs. These 3D tumor organoids faithfully recapitulate tumor complexities, heralding a transformative approach in cancer research. We could confirm that CASC19 plays a role in PDO survival and also sensitizes the organoids to standard-of-care treatments.

Our work underscores the cell-type specificity of lncRNAs, highlighting their promise as therapeutic targets. Furthermore, by introducing CRISPR technology into PDOs, we amplify the exploration of lncRNAs in clinically relevant 3D cancer models, potentially paving the way for novel therapeutic interventions.

The Paradox of Inflammation-Related lncRNA LOC339803: Harming the Gut, Protecting the Brain

Ainara Castellanos

Department of Genetics, Physical Anthropology and Animal Physiology of the University of the Basque Country, Spain

Long noncoding RNAs (lncRNAs) are known to regulate various cellular mechanisms, with cell type specificity being one of their principal characteristics. LncRNA *LOC339803* is located in an inflammation-related region associated with diverse disorders such as inflammatory bowel disease, psoriasis, or multiple sclerosis. Notably, one of the disease-associated SNPs within the lncRNA is closely located to an m6A motif, which we found exhibits allele-specific methylation in different cell types.
The functional characterization of this lncRNA revealed that it induces inflammation in intestinal epithelial cells. We demonstrated that in intestinal cells, this lncRNA is methylated and located in the nuclear compartment, where it interacts with a protein complex composed of the m6A reader protein YTHDC1 and other chromatin repressors to inhibit the expression of *COMMD1*. A decrease in COMMD1 levels induces NFkB, which activates an inflammatory cascade. Conversely, *LOC339803* exhibits an anti-inflammatory role in neurons. In these cells, *LOC339803* interacts with the HK2 protein in the cytoplasmic compartment, maintaining mitochondrial integrity and preventing inflammatory reactions. Interestingly, this lncRNA is barely methylated in neurons, making its function m6A-independent. An analysis of *LOC339803* in the intestine of patients with intestinal inflammatory disorders revealed an induction of its expression levels. However, this lncRNA is downregulated in the demyelinated grey matter of multiple sclerosis patients. These results in human samples confirm its dual role in inflammatory processes.
Overall, we identified a cell-type specific mechanism of an inflammatory disease-associated lncRNA and demonstrated that certain lncRNAs can present tissue-specific epitranscriptomic modifications that dictate their cellular location and function, participating in the development of different inflammation-mediated diseases.

Deciphering the Connection Between Neurodegeneration and Cancer via lncRNAs: A Role for MINCR

Cecilia Pandini[1], Ilaria Cassotti[1], Giulia Pagani[1], Alessandro Macchia[1], Stella Gagliardi[2], Paolo Gandellini[1]

[1] *Department of Biosciences, University of Milan, Milan, Italy*
[2] *Molecular Biology and Transcriptomics Unit, IRCCS Mondino Foundation, Pavia, Italy*

Recent research has underscored the pivotal role of lncRNAs in both cancer and neurodegeneration, two significant health challenges worldwide. For instance, the dysregulated expression of MALAT1 and HOTAIR has been implicated in both conditions. Notably, the MYC-induced Long Non-Coding RNA (MINCR) was identified as upregulated in various cancers and conversely downregulated in Amyotrophic Lateral Sclerosis (ALS) patients, standing out as a promising candidate in elucidating the interconnectedness between these diseases.

Here, we reannotated bona fide MINCR alternative isoforms by analyzing data from the PolyA site database, the FANTOM CAGE project, and long-read sequencing and then through PCR validation. Their localization was assessed in prostate cancer and neuroblastoma cell lines, revealing cell type-dependent and isoform-specific subcellular localization. MINCR functional characterization was carried out using transient and stable approaches. Modulation of MINCR RNA level was obtained through gapmer and mixmer Antisense Oligonucleotides (ASOs) specifically designed on the reconstructed annotation. Stably overexpressing and CRISPR-Cas9-mediated knockout cell lines were created. RNA sequencing and phenotypic characterization (e.g. cellular growth for cancer and spheroid formation and differentiation for neurodegeneration) of transiently and stably over-/downexpressing cells were used to reveal the pathways governed by MINCR.

In prostate cancer, the amplification of chromosome 8q24, a region called a “gene desert” because of the presence of almost only lncRNAs, is associated with disease severity. MINCR, among these lncRNAs, exhibits overexpression in prostate cancer, particularly in metastatic cases, and correlates positively with cell cycle-related genes, suggesting its involvement in disease progression. MINCR silencing by ASO impacts cell cycle-related pathways, leading to decreased cell proliferation of different prostate cancer cell lines.

In contrast, altered expression of MINCR in an ALS cellular model leads to modifications in pathways related to apoptosis and neuronal differentiation.

Further exploration of MINCR isoforms and cellular functions holds promise for the development of novel therapeutic approaches for both cancer and neurodegenerative disorders.

Insight Into Mbnl1-Dependent Splicing Perturbation and Circular RNA Dysregulation in the Dysmyelinated Mouse Brain Afforded by a RNA–Protein Interactome Study

Marta Sztachera[1], Weronika Wendlandt-Stanek[1], Remigiusz A. Serwa[2], Luiza Stanaszek[3], Monika Piwecka[1]

[1] Department of Non-coding RNAs, Institute of Bioorganic Chemistry PAS, Poznan, Poland
[2] Proteomics Core Facility, IMol Polish Academy of Sciences, Warsaw, Poland
[3] NeuroRepair Department, Mossakowski Medical Research Institute, Warsaw, Poland

RNA-binding proteins (RBPs) are indispensable players involved in the entire life cycle of RNA. In neural cells, RBPs control a wide range of processes critical for neuronal maturation or synaptic plasticity. Malfunctions of RBPs, their mutations, mislocalization and distorted interaction with target RNAs have been associated with the pathogenesis of neurological and neuromuscular disorders. However, relatively little is known about whether and how RBPs are involved in the regulation of gene expression in myelin disorders, namely dys- or demyelination. To fill this gap, we applied an RNA–protein interactome capture method called XRNAX to shed light on the RNA-bound proteome in the brain of a mouse model of dysmyelination (shiverer, Shi). First, we adapted the XRNAX method to capture RNA-bound proteins in the tissue context, i.e., in the wild-type (WT) mouse brain. Second, we performed XRNAX on the Shi brain. We observed that sets of canonical RBPs involved in alternative splicing and RNA granule formation have altered patterns of interaction with RNA in Shi as compared to the WT brain. In reference to alternative splicing, we showed that Muscleblind Like Splicing Regulator 1 (Mbnl1)-mediated alternatively spliced exons of its brain-enriched target genes, such as Picalm, Add3, Mbnl2 and Sorbs1, are included or excluded in different proportions in the dysmyelinated brain as compared to WT. We also found a different exon utilization in Mbnl1 itself, which has been associated with nuclear the retention of the Mbnl1 protein and likely involves Mbnl1 circular RNA de-regulation. We also observed Mbnl2 circRNA deregulation in Shi brain samples. Our study provides a foundation for exploring mRNA–protein and ncRNA–protein interactions and their impact on myelin disorders.

Identifying Gene Modulators and Novel Compounds of T-cell–Tumor Cell Interactions to Enhance Immune-Checkpoint Inhibition Therapies

Ramiro Martinez, Chiara Finocchiaro, Louis Delhaye, Wim Trypsteen, Eric James De Bony, Pieter Mestdagh

University of Ghent, UGENT, Ghent, Belgium

The adaptive and innate immune systems collaborate to detect and eliminate tumor cells, with T lymphocytes playing a crucial role. Cancer cells develop mechanisms weakening T-cell-mediated anti-tumoral responses due to genetic heterogeneity. Checkpoint inhibition therapies (ICTs), including immune checkpoint inhibitors (ICIs) against PD-1/PD-L1 and CTLA-4, yield sustained anti-tumor responses in diverse cancer subtypes. Despite achievements, ICT efficacy is limited. For metastatic melanoma, 40–60% of patients lack a therapeutic response, and responders experience tumor relapse within two years. Strategies enhancing ICTs and understanding immune evasion mechanisms are crucial.

Emerging evidence highlights a prominent involvement of long non-coding RNAs (lncRNAs) in cancer development and progression. Moreover, some lncRNAs have been shown to actively regulate T-cell activation or evasion. Our research focuses on identifying immuno-modulating lncRNAs and compounds in T-cells and tumor cells. We employ an antigen-specific Jurkat-derived cell line (2D3) expressing GFP linked to an NFAT promoter and a MART-1-specific TCR, detecting immune cell activation via MART-1 TCR-antigen interactions. 2D3 was also transduced with dCas9-KRAB-MECP2 for CRISPRi purposes on lncRNAs.

To ascertain immuno-modulating lncRNAs in T-cells, we designed a custom sgRNA library targeting highly expressed lncRNAs selected via RNA sequencing on 2D3 and donor samples. Pooled screening infecting 2D3s with the sgRNA library and co-culturing with the melanoma cell line MALME-3M was performed, and promising hits are currently being validated.

Relevant lncRNAs on the tumor side were elucidated using a novel high-throughput lentiviral-based screening platform developed during this project. Silencing target lncRNAs in melanoma cell lines through dCas-KRAB-MeCP2 prior co-culture enabled hit identification, and we are also in the validation stage of HITs.

Finally, we integrated a screening arm for potential compounds modulating T-cell–tumor cell interactions. A co-culture setup in 384-well plates screens a library of 3000 compounds on SK-MEL-5 melanoma cells known for complex immune evasion. Our findings will hopefully provide valuable insights into cancer immune resistance mechanisms and potential therapeutic targets, including lncRNAs and compounds.

Modulation of Antiviral Innate Immunity by the Human Dicer Protein

Sébastien Pfeffer

RNA Architecture and Reactivity, Institute of Molecular and Cellular Biology of the CNRS, University of Strasbourg, France

In mammals, the co-existence of RNAi and the type I interferon response in somatic cells begs the question of their compatibility and relative contribution during viral infection. Previous studies provided hints that both mitigating co-factors and self-limiting properties of key proteins such as Dicer could explain the apparent inefficiency of antiviral RNAi. Indeed, the helicase domain of human Dicer limits its processing activity and acts as an interaction platform for co-factors that could hinder its function. We studied the involvement of several helicase-truncated mutants of human Dicer in the antiviral response. We show that all deletion mutants display an antiviral phenotype against alphaviruses and an enterovirus. While only one of them, Dicer N1, is antiviral in an RNAi-independent manner, they all require the expression of PKR to be active. To elucidate the mechanism underlying the antiviral phenotype of Dicer N1 expressing cells, we analyzed their transcriptome and found that many genes from the interferon and inflammatory response were upregulated. We could show that these genes appear to be controlled by transcription factors such as STAT-1, STAT-2, and NF-kB. Finally, we demonstrated that blocking the NF-kB pathway in Dicer N1 cells abrogated their antiviral phenotype. Our findings highlight the crosstalk between Dicer, PKR, and the IFN-I pathway, and suggest that human Dicer may have repurposed its helicase domain to prevent basal activation of antiviral and inflammatory pathways.

Targeting the MYC-Dependent Long Non-coding RNA MB3 Regulates the TGF-β and OTX2 Pathways in Group 3 Medulloblastoma

Alessia Grandioso[1], Paolo Tollis[2], Francesca Pellegrini[1], Jessica Rea[2], Francesco Migliaccio[3], Annamaria Carissimo[3], Daniela Trisciuoglio[1], Alessandro Palma[1], Monica Ballarino[1], Giada Tisci[1], Elisabetta Falvo[4], Pierpaolo Ceci[4], Pietro Laneve[4]

[1] *Sapienza University, Rome, Italy*
[2] *IIT- Center for Life Nano Science, Rome, Italy*
[3] *CNR-National Research Council, Naples, Italy*
[4] *CNR-National Research Council, Rome, Italy*

Medulloblastoma (MB) stands as the most prevalent malignant pediatric tumor affecting the central nervous system. Categorized into four primary subgroups, each with distinct biological and clinical features, MB presents unique therapeutic implications. Group 3 (G3) and 4 (G4) MBs collectively account for the majority of cases (60%) and exhibit the highest degree of aggressiveness. However, these subgroups remain poorly characterized from a biochemical perspective. Compared to normal human cerebella, the MYC-dependent lncRNA MB3 was found to be induced in G3-derived cell lines expressing the proto-oncogene and upregulated in G3-MB primary tumors. Consistently, LncMB3 is downregulated upon MYC inhibition and in vitro studies reveal its potent anti-apoptotic activity.

To dissect the molecular network through which LncMB3 operates in MB carcinogenesis, we initiated the identification of its target genes by analyzing, through RNA-sequencing, the alteration in the transcriptome following LncMB3 knockdown in G3 MB cell lines, achieved through transfections of antisense Locked Nucleic Acid (LNA) oligonucleotides (GapmeRs). Additionally, synergistic effects between GapmeRs and classical chemotherapeutics administrations were studied. Our validations provide substantial evidence that the TGF-β pathway is regulated by LncMB3 in G3 MB cells, acting via modulation of the chromatin protein HMGN5. TGF-β downregulation leads to deregulation of pivotal nodes in the downstream gene cascade, including OTX2, BCL-2L1 and PARP-1. The apoptotic effect obtained with GapmeR can be enhanced by the usage of classical chemotherapeutics which act on the same pathway. These results suggest that GapmeRs can serve as therapeutic molecules, leading us to develop engineered human ferritin delivery methods.

In conclusion, we assessed the molecular network underlying lncMB3 downregulation, implemented its effects on apoptosis through the use of chemotherapeutics and generated new tools able to deliver GapmeRs. This study may serve as a catalyst for new therapies involving non-coding RNA-based precision medicine.

The Role of the Long Non-coding RNA Charme in the Phenotype and Function of Resident Cardiac Mesenchymal Stromal Cells

Erica Floris[1], Claudia Cozzolino[1], Valeria Taliani[2], Giulia Buonaiuto[3], Antonella Bordin[1], Alessandro Palma[3], Vittorio Picchio[4], Carmine Nicoletti[5], Monica Ballarino[3], Isotta Chimenti[1], Francesca Pagano[6]

[1] Department of Medical Surgical Sciences and Biotechnologies, Sapienza University of Rome, Latina, Italy
[2] EMBL, Genome Biology Unit, Heidelberg, Germany
[3] Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Rome, Italy
[4] Department of Angio Cardio Neurology, IRCCS Neuromed, Pozzilli, Italy
[5] DAHFMO-Unit of Histology and Medical Embryology, Sapienza University of Rome, Rome, Italy
[6] Institute of Biochemistry and Cell Biology, National Council of Research (IBBC-CNR), Monterotondo, Italy

Background: Charme is a murine lncRNA expressed in striated muscles, presenting a human orthologue with 45% identity. Charme-/-mice display incomplete muscle differentiation and an altered cardiac phenotype with increased wall thickness due to cardiomyocyte hyperplasia and present impaired cardiac function at adult age (Taliani, Buonaiuto, eLife 2023). Cardiac mesenchymal stromal cells (CMSCs) play key roles in tissue pathophysiology by maintaining the extracellular matrix (ECM) and by paracrine action.

Purpose: To elucidate the role of Charme in the phenotype, paracrine function, and activation of resident CMSCs.

Results: Charme-/-hearts showed a reduced content of collagen-I (0.16±0.05 vs WT:0.83±0.18 normalized OD, N=4, p0.005). Charme-/-CMSCs revealed a reduced expression of genes involved in "Extracellular matrix organization" (GO:0030198) and "Cardiac muscle tissue development" (GO:0048738), enhanced spheroid growth (123.6±11.9 vs WT: 51.0±6.4 spheroids/well; N=8, p0.0001), clonogenesis efficiency (2.7±0.5-fold; N=7, p0.05), migration ability (68.5±2.7% vs WT: 80.2±0.7% wound area; N=3, p0.005), and reduced ECM digestion capacity (74.03±2.31% vs WT 56.15±5.65% collagen area at 96h, N=3, p0.05). Charme-/-CMSCs showed reduced lin-/Sca1+/CD90+ primitive mesenchymal cells (7.4±1.6% vs WT:56.9±6.5%; N=6, p0.05). Their secretome was depleted of cytokines involved in the PI3K-Akt signalling pathway (FDR0,0005), myoblast differentiation (GO:1901741, GO:0045663; FDR0.05), and mediated reduced Akt-phosphorylation in neonatal rat cardiomyocytes (0.08±0.01 vs WT:0.23±0.12 normalized OD; N=2), as well as reduced tube formation in an angiogenesis assay (fold change vs WT: 0.39±0.07 meshes, 0.60±0.06 nodes; N=3, p0.01). Stimulation with TGFb-1 revealed lower expression of fibroblast activation markers in Charme-/- compared to WT CMSCs (aSMA fluorescence intensity: 0.5±0.1 vs

0.8±0.1, N=3, p0.05; collagen I/III mRNA expression ratio: 1.3±0.1 vs 2.2±0.4 N=3, p0.01), and their secretome was less effective in sustaining cardiomyocyte survival (1.7±0.1 vs WT:1.9±0.1 normalized absorbance; N=5, p0.005).

Conclusions: Charme-/-CMSCs show a less mature phenotype associated with ECM alteration, reduced activation, and impaired cardioprotective paracrine functions, suggesting a key role of Charme in cardiac stroma physiology.

Identification of Host microRNAs Regulating the Infection of Macrophages by Invasive and Non-invasive Salmonella Strains

Susana Costa[1], Caio Franco[1], Miguel Mano[1], Ana Eulalio[1,2]

[1] Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal
[2] Imperial College London, London, United Kingdom

MicroRNAs play a crucial role in modulating numerous biological processes, including hostbacteria interactions. They have been shown to regulate the immune response to infection, but pathogens can also subvert microRNA expression to favour infection.

Salmonella enterica serovar Typhimurium (henceforth Salmonella) is a facultative intracellular bacterium that causes gastroenteritis in humans, and is amongst the most lethal food-borne pathogens. A crucial feature of Salmonella's pathogenicity is its ability to interact with a variety of phagocytic and non-phagocytic cells, being able to survive, replicate and persist inside these cells. However, significantly less is known about Salmonella's interaction with macrophages than that with epithelial cells.

High-throughput screening using microRNA mimics libraries has emerged as a sophisticated methodology to systematically identify relevant factors involved in bacterial infection. In this study, we performed two microscopy-based genome-wide screenings using a library of 2,588 microRNA mimics to identify microRNAs regulating the infection of macrophages by two Salmonella strains, specifically an invasive (D23580) and a non-invasive (SL1344) strain. Although invasive Salmonella strains present a higher tropism for macrophages than non-invasive strains, we found that infection by the two strains is regulated by a largely overlapping subset of microRNAs. Time-course experiments revealed that the microRNAs mostly affect bacterial replication at intermediate times post-infection. A comparison of the screening results obtained in macrophages with those from a previous screening performed in epithelial cells showed no correlation. This reveals a high degree of selectivity in the pathways affecting Salmonella infection in the two cell types.

The data obtained from this large-scale study constitute a valuable resource for further investigations aimed at uncovering previously unknown molecular players/pathways relevant to Salmonella–macrophage interactions.

MicroRNA-Mediated Control of Cardiac Fibrosis: Unraveling Key Players and Mechanisms Through Functional Genomics Screenings

Flavia Rodrigues[1], Mariana Bexiga[1], Ana Sofia Varanda[1], Ana Eulalio[1, 2], Miguel Mano[1, 3]

[1] Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal
[2] Imperial College London, London, United Kingdom
[3] King's College London, London, United Kingdom

Cardiac fibrosis is a maladaptive remodeling process of the myocardium which results from an over-activation of cardiac fibroblasts, excessive deposition of extracellular matrix proteins (particularly collagen), and scar formation, ultimately leading to a stiffening of the heart wall and compromising heart function. A deeper knowledge of the molecular and cellular processes underlying fibrosis is crucial to identify novel targets for therapeutic interventions.

To systematically identify microRNAs that modulate cardiac fibrosis, we performed a series of high-content microscopy functional screenings using a genome-wide library of microRNA mimics (2,588 mature microRNAs). We focused on key phenotypes relevant to cardiac fibrosis, specifically human primary cardiac fibroblast proliferation, myofibroblast differentiation, and deposition of fibrillary collagen.

We identified 134 microRNAs that strongly modulate myofibroblast differentiation (4-fold change) and a high number of microRNAs that block cardiac fibroblast proliferation (166 microRNAs) or that strongly modulate collagen deposition (4-fold change; 227 and 96 microRNAs in unstimulated and stimulated conditions, respectively).

Clustering performed on the global analysis of the phenotypes led to the selection of 92 microRNAs. We confirmed the phenotypes in human cardiac fibroblasts from different donors and in fibroblasts isolated from different tissues, including the aorta, skin, and lung. Based on these data, we selected eight microRNAs for detailed mechanistic characterization—seven of these microRNAs strongly block collagen deposition in stimulated conditions while exhibiting differential effects on cardiac fibroblast differentiation and/or tissue-specific activities and one microRNA increases collagen deposition. Transcriptomic analysis and additional mechanistic studies showed that three of these microRNAs target collagen directly, while the others act through alternative mechanisms.

Overall, our work establishes microRNAs as powerful modulators of multiple processes critical to cardiac fibrosis and offers a unique opportunity for testing different anti-fibrotic approaches based on the differential modulation of these processes by microRNAs.

S3. ncRNA Technologies

RNA Insights from Extinct Animals and Single Cells

Vaishnovi Sekar[1], Emilio Mármol-Sánchez[1,2], Panagiotis Kalogeropoulos[1], Bastian Fromm[3], Nikolay Oskolkov[4], Zoé Pochon[2,5], Franziska Bonath[1], Love Dalén[2,6,7], Inna Biryukova[1], Marc R. Friedländer[1]

[1] Science for Life Laboratory, Department of Molecular Biosciences, The Wenner-Gren Institute, Stockholm University, Stockholm, Sweden
[2] Centre for Palaeogenetics, Stockholm, Sweden
[3] The Arctic University Museum of Norway, UiT - The Arctic University of Norway, Tromsø, Norway
[4] Department of Biology, National Bioinformatics Infrastructure Sweden, Science for Life Laboratory, Lund University, Lund, Sweden
[5] Department of Archaeology and Classical Studies, Stockholm University, Stockholm, Sweden
[6] Department of Bioinformatics and Genetics, Swedish Museum of Natural History, Stockholm, Sweden
[7] Department of Zoology, Stockholm University, Stockholm, Sweden

Experimental microRNA target detection methods such as CLIP have over the last decade yielded many important biological insights. Yet these methods require millions of cells to work, and thus cannot be used to address questions related to targeting in single cells or in complex tissues. We have recently developed a new method - agoTRIBE - which fuses an Argonaute protein to the RNA editing domain of ADAR2, allowing us to detect microRNA targets by nucleotide conversions in single cells.

In a related project, we have applied sensitive methods to profile RNA in desiccated specimens of extinct animals such as the Tasmanian tiger. The authenticity of the sequences is supported by clade-specific molecules and microRNA and mRNA profiles that resemble the modern counterparts of the sampled tissues. In unpublished results, we extend our findings to a mummified ~30,000 year old woollen mammoth, allowing us a glimpse at gene expression in the Pleistocene.

Investigating the Sequence–Function Relationship in Long Noncoding RNAs

Sarang Bhutada[1], Ines Bravo[1], Tina Uroda[1], Hugo Guillen-Ramirez[2], Rory Johnson[1]

[1] School of Biology and Environmental Science, University College Dublin, Dublin, Ireland
[2] Department of Visceral Surgery and Medicine, University of Bern, Inselspital, Bern University Hospital, Bern, Switzerland

Long noncoding RNAs (lncRNAs) are speculated to be modular molecules, similar to proteins, composed of a series of functional 'elements' that collectively define the activity. However, the majority of lncRNAs are uncharacterized, and key questions about lncRNA elements are still unanswered: How many elements are there? How is function encoded in these elements? To address these questions, we performed a high-throughput CRISPR deletion screen, coupled with cell proliferation as phenotypic output, targeting over 900 exonic regions in more than 300 lncRNAs across four different cell lines. The hits from the screen, referred to as 'functional exons', significantly reduced proliferation in a cell-line-specific manner. The expression levels of the functional exons were higher compared to non-functional exons in all the cell lines, supporting the relevance of the screen results. To gain mechanistic insights into these functional exons, we examined the enrichment for known DNA and RNA sequence elements. The analysis revealed a significant overlap of functional exons with RNA elements like RNA binding protein sites and conserved RNA secondary structures compared to non-functional exons, establishing mechanistic hypotheses for further validation. In summary, our study enables us to unravel lncRNA sequence–function codes by creating a comprehensive map of functional lncRNA elements and forms the basis for predicting novel functional regions.

Unraveling the Role of Cancer-Associated Small Open Reading Frames Using an in Vivo Single-Cell CRISPR Screen

Fabiola Valdivia-Francia, Umesh Ghoshdastider, Peter F. Renz, Chenke Shi, Katie Hyams, Clara Duré, Daniel Spies, Mark Ormiston, Ataman Sendoel

Institute for Regenerative Medicine, University of Zurich, Zurich, Switzerland

Recent genome-wide searches using ribosome profiling and proteomics have led to the discovery of previously unannotated small open reading frames (sORFs). Although a growing number of sORFs encode microproteins, the function of most sORFs still remains enigmatic. To gain a comprehensive understanding of the role of sORFs, specifically in cancer, we tested the tissue-wide function of 300 novel sORFs using an in vivo single-cell CRISPR screening strategy in mice. We exploited a modified CROP-seq vector to simultaneously activate Cas9 and oncogenic Pik3caH1047R in the mouse epidermis. Our approach linked pooled CRISPR screenings with single-cell transcriptomic readout to assess sORF-dependent signaling pathways and alterations across all epidermal cell populations. We identified dozens of sORFs, both upstream ORFs (uORFs) and new sORFs that either influenced cell proliferation or induced strong transcriptional changes. We further characterized a subset of sORFs via a validation screen to verify their phenotypic behavior both in vivo and in vitro using different cancer cell lines. We confirmed both proliferation and transcriptional changes for select sORFs and are now focusing on the functional characterization of select sORFs identified across our screens. These findings provide a basis for investigating the molecular mechanisms of how the expression of selective sORFs impacts cellular function and fuel tumor growth. Understanding the tissue-wide molecular function of sORFs in tumorigenesis will elucidate potential new oncogenic drivers and therapeutic targets that can be exploited for future cancer therapies.

Expanding the lncRNA GENCODE Universe: Unlocking the Non-coding Genome Vault and Unraveling Genome Complexity

Sílvia Carbonell-Sala[1], Tamara Perteghella[1, 2], Gazaldeep Kaur[1], Carme Arnan[3], Emilio Palumbo[1], Andrea Tanzer[1], Adam Frankish[4], Roderic Guigó[3, 5], The GENCODE Consortium[6, 7, 8, 9, 10, 11, 12]

[1] *Centre for Genomic Regulation, Computational Biology and Health Genomics, Barcelona, Spain*
[2] *Pompeu Fabra University, (MELIS), Barcelona, Spain*
[3] *Centre for Genomic Regulation (CRG), The Barcelona Institute of Science and Technology, Barcelona, Spain*
[4] *European Bioinformatics Institute, (EBI-EMBL), Cambridge, United Kingdom*
[5] *Pompeu Fabra University (UPF), Barcelona, Spain*
[6] *EMBL European Bioinformatics Institute, Cambridge, UK*
[7] *Centre for Genomic Regulation (CRG), Barcelona, Spain*
[8] *Massachusetts Institute of Technology (MIT), Boston, MA, USA*
[9] *Yale University, New Haven, CT, USA*
[10] *University of California, Santa Cruz, CA, USA*
[11] *Spanish National Cancer Research Centre (CNIO), Madrid, Spain*
[12] *Stanford University, Silicon Valley, CA, USA*

Accurate mapping of genes and transcripts, especially long non-coding RNAs (lncRNAs), is crucial for understanding genome function. The GENCODE consortium employs manual, computational, and experimental methods to refine genome annotation and produce the Ensembl/GENCODE geneset. This comprehensive annotation covers protein-coding and non-coding regions, including alternative splicing and pseudogenes, essential for global biomedical research. Recent efforts have prioritized creating high-quality transcript models for lncRNAs. In this report, we reveal outcomes from GENCODE Phase 3, focusing on mapping the unexplored portion of human and mouse transcriptomes suspected of non-coding transcriptional activity and utilizing Capture Long-Read Sequencing (CLS). Through CapTrap-Seq-CLS, we captured full-length transcripts from diverse adult and embryonic tissues of both species. Using PacBio and ONT sequencing technologies, our curated data revealed a doubling of annotated lncRNAs, a significant milestone in GENCODE's history, emphasizing the importance of lncRNAs in genome regulation and function. Moreover, reanalysis of short-read RNA-Seq samples with the latest GENCODE annotation, including the largest addition to the lncRNA Gencode catalog, revealed spatial expression patterns across brain regions and significant associations with neurodegenerative diseases, particularly Alzheimer's disease. This underscores lncRNAs' critical role in neurological function and disease pathology. Additionally, identifying long transcripts harboring orphan miRNAs enriches our understanding of the regulatory landscape, adding complexity to gene expression regulation. Furthermore, discovering new lncRNAs impacting regulatory regions has profound implications for classifying ENCODE candidate cis regulatory regions, enhancing our ability to

decipher gene regulation networks. These findings advance our understanding of genome complexity and provide insights into the non-coding molecular mechanisms.

Developing RNA Ligands for Glutamate Ion Channels and Testing Them in ALS Animal Models

Li Niu, Megumi Akamatsu

Department of Chemistry, University at Albany, State University of New York, Albany, NY, USA

Amyotrophic lateral sclerosis (ALS) is the most common adult-onset motor neuron disease, characterized by progressive neurodegeneration of both upper and lower motor neurons. In the motor neurons of sporadic ALS patients, RNA editing at the glutamine/arginine (Q/R) site of the GluA2 subunit of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, which is a subtype of glutamate ion channels, is defective or incomplete. As a result, AMPA receptors containing the abnormally expressed, unedited isoform of GluA2 are highly Ca2+-permeable, and are responsible for mediating abnormal Ca2+ influx, thereby triggering motor neuron degeneration and cell death. Thus, blocking the AMPA-receptor-mediated abnormal Ca2+ influx is a potential therapeutic strategy for the treatment of sporadic ALS. Here, we present the development of RNA aptamers using SELEX to target the AMPA subtype of glutamate receptors, and them them for the efficacy and safety of these RNA molecules on the phenotype of AR2 mice. This is a mouse model where AMPA receptor expression is dysregulated due to incomplete Q/R editing, and thus AR2 mice serve as the ideal animal model for testing our RNA aptamers as a potential drug candidate for ALS treatment. A 12-week, continuous, intracerebroventricular infusion of aptamers to AR2 mice reduced the progression of motor dysfunction, normalized TDP-43 mislocalization, and prevented the death of motor neurons. Our results demonstrate that the use of AMPA-receptor-selective RNA aptamers as a novel class of AMPA receptor antagonists is a promising strategy for the development of an ALS treatment approach. Our work also involves in the design of these RNA ligands using a combination of SELEX, deep sequencing, and a computational/mutational approach to produce highly potent and highly selective RNA ligands.

S4. ncRNA Informatics

Transcriptome-Scale Mapping of RNA Secondary Structure Ensembles in Living Cells

Ivana Borovská[1], Edoardo Morandi[1], Chundan Zhang[1], Michael T. Wolfinger[2,3,4], Daphne A.L. van den Homberg[5], Willem A. Velema[5], Danny Incarnato[1]

[1] Department of Molecular Genetics, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Groningen, The Netherlands
[2] Department of Theoretical Chemistry, University of Vienna, Vienna, Austria
[3] Research Group Bioinformatics and Computational Biology, Faculty of Computer Science, University of Vienna, Vienna, Austria
[4] RNA Forecast e.U., Vienna, Austria
[5] Institute for Molecules and Materials, Radboud University Nijmegen, Nijmegen, the Netherlands

Mapping of RNA structures in living cells by chemical probing has gained substantial popularity over the last decade. Although RNA can theoretically sample multiple alternative conformations for long time chemical probing data has been erroneously interpreted as a function of a single predominant RNA conformation. Thanks to recent combined experimental and computational advances it is now becoming possible to grasp the true extent of the RNA structural heterogeneity in living cells. During this talk I will discuss some of these advances and recent findings they enabled in the context of viral and bacterial RNAs.

Prediction of Protein–RNA Interactions From Single-Cell Transcriptomic Data

Jonathan Fiorentino[1], Alexandros Armaos[2], Alessio Colantoni[1,3], Gian Gaetano Tartaglia[1,2]

[1] Center for Life Nano- and Neuro-Science, RNA Systems Biology Lab, Italian Institute of Technology (IIT), Rome, Italy
[2] Centre for Human Technologies (CHT), RNA Systems Biology Lab, Italian Institute of Technology (IIT), Genova, Italy
[3] Department of Biology and Biotechnologies "Charles Darwin", Sapienza University of Rome, Rome, Italy

Proteins are crucial in regulating every aspect of the RNA lifecycle, yet understanding of their interactions with coding and non-coding RNAs remains limited. Experimental studies are typically restricted to a small number of cell lines and a limited set of RNA-binding proteins (RBPs). Although computational methods based on physico-chemical principles can predict protein–RNA interactions accurately, they often lack the ability to consider cell-type-specific gene expression and the broader context of gene regulatory networks (GRNs). Here, we assess the performance of several GRN inference algorithms in predicting protein–RNA interactions from single-cell transcriptomic data, and propose a pipeline, called scRAPID (single-cell transcriptomic-based RnA Protein Interaction Detection), that integrates these methods with the catRAPID algorithm, which can identify direct physical interactions between RBPs and RNA molecules. Our approach demonstrates that RBP–RNA interactions can be predicted from single-cell transcriptomic data, with performances comparable or superior to those achieved for the well-established task of inferring transcription factor–target interactions. The incorporation of catRAPID significantly enhances the accuracy of identifying interactions, particularly with long non-coding RNAs, and enables the identification of hub RBPs and RNAs. Additionally, we show that interactions between RBPs can be detected based on their inferred RNA targets. The software is freely available at https://github.com/tartaglialabIIT/scRAPID.

Unraveling the Regulatory Landscape of RNA–Chromatin Interactions During Human Monocyte to Macrophage Differentiation

Laura Carpen[1], Aslihan Karabacak Calviello[1], Rodrigo Pracana[1], Masaki Kato[2], Hazuki Takahashi[2], Piero Carninci[1]

[1] *Human Technopole, Milan, Italy*
[2] *RIKEN Center for Integrative Medical Sciences, Yokohama, Kanagawa, Japan*

Recent studies in RNA biology have clarified that many RNAs undertake a variety of diverse functions beyond carrying genetic information from DNA to protein. Emerging evidence shows that RNAs, especially long non-coding RNAs (lncRNAs), play pivotal roles in regulating gene expression and chromatin organization by interacting with other RNAs, proteins, or chromatin. However, the full extent of these interactions and their impact on chromatin regulation, especially within the dynamic context of cellular differentiation, remain largely unexplored. Here, we map genome-wide RNA–chromatin interactions in a time course of differentiation of human monocytes into macrophages. Our study reveals a dynamic landscape of lncRNA interactions over the course of differentiation. Notably, we identify differential lncRNA–chromatin interactions enriched at key cis-regulatory elements, including promoters of macrophage-related genes. These interactions are associated with binding sites for transcription factors with defining roles in macrophage biology, revealing potential physical or functional links between transcription factors and lncRNAs, shedding light on their collaborative roles in gene regulation. Integration with 3D chromatin conformation datasets uncovers lncRNA–chromatin interactions associated with functional DNA loops, such as promoter–enhancer hubs, providing insights into the structural roles of lncRNAs in shaping three-dimensional nuclear organization. Overall, our findings elucidate the dynamic regulatory network governed by lncRNAs during cellular differentiation, expanding our understanding of RNA-mediated chromatin regulation. By uncovering novel regulatory mechanisms and functional links, our study contributes to the broader effort to decode the complexity of gene regulation and expand the repertoire of regulatory RNAs.

Exploring Structural Conservation in lncRNAs Beyond Sequence Alignment

Nicolás Aira, Uciel Chorostecki

International University of Catalunya, Barcelona, Spain

Long non-coding RNAs (lncRNAs) have emerged as critical regulators in various biological processes, primarily through interactions with RNA-binding proteins (RBPs). The misregulation of lncRNAs can contribute to complex human diseases, including cancer, neurological disorders, inflammatory responses, and roles in immunity. Additionally, lncRNAs' ability to form complex secondary structures, which are more conserved than primary sequences, suggests that they have the ability to mediate their molecular functions. The surge in available RNA structural data emphasizes the pressing need for innovative methods to efficiently compare and identify similar structures, especially in the context of tools that may face challenges in these tasks.

We propose using a Siamese Convolutional Neural Network (SCNN) to compare RNA secondary structures. SCNNs, used in face recognition and sequence comparison applications, measure the similarity between two input samples. Trained on an in silico dataset, our model shows promising results. Its key advantage is eliminating the need for sequence alignment, allowing for the comparison of diverse structures, which traditional methods struggle with due to computational costs. Preliminary tests demonstrate the model's efficiency, especially in handling RNA molecules that differ significantly in size and sequence.

Our initial results using an RNases P dataset show that the SCNN effectively discerns structural similarities within the same family and distinguishes between various classes of RNases P. The contrast in distance scores between random sequences and RNAse P pairs validates the model's capability to identify structural similarities in RNA sequences. The model's versatility extends to handling diverse scenarios, including instances where short structural motifs exhibit different arrangements in functionally homologous RNAs, exemplified by the observed functional redundancy in roX lncRNAs. Thus, we want to use our method to explore the conservation of secondary structures in lncRNAs. Through our approach, we aspire to shed light on the structural conservation of lncRNAs, contributing to a deeper understanding of their functional implications.

Pipeline for lncRNA-Targeted Antisense Oligonucleotides Design—From Sequence to Therapy

Artem Baranovskii, Lambert Moyon, Andreina Tirabassi, Annalisa Marsico

Helmholtz Center Munich, Institute of Computational Biology, Munich, Germany

Long non-coding RNAs (lncRNAs) are an abundant class of transcripts second in number only to protein-coding genes. The functions of lncRNAs include regulating gene expression both in cis and in trans, operating on gene-specific and general transcription levels alike. Owing to their central role in transcriptional regulation, lncRNAs are implicated in some diseases and suspected to be involved in many others, thereby being a natural target for the new generation of RNA therapeutics, such as antisense oligonucleotides (ASOs).

ASOs are short oligomers, usually 16 to 22 nucleotides long, that bind to their target transcripts by complementarity. Their functions are multiple: degradation, splice-switching, and blockade of RBP access to the target transcript. Optimization of their chemical properties and molecular vehicles allows ASOs to be tailored for long-term and tissue-specific efficacy.

Nevertheless, the design of ASOs against lncRNAs of interest is obstructed by the inherent qualities of the latter: poor conservation, and the sheer size and number of putative transcripts generated from a single lncRNA gene.

In our work, we propose a unified framework for ASO design targeting putative lncRNAs based on in silico sequence annotation that addresses the aforementioned challenges in two steps: first locating the functional elements within the target sequence and then scanning for candidate ASOs within identified subsequences. Identification of the subsequences can be readily achieved in silico via the application of deep learning models trained on large-enough corpora of genomic data. Our framework enables the large-scale exploration of the putative impact of ASOs on target gene expression associated with their post-transcriptional processing. Furthermore, high-impact ASOs are filtered based on predicted off-targets, cross-reactivity, and robustness to population genetic variations, with the ultimate goal of automating the process from target sequence identification to candidate suggestions.

S5. Emerging Topics in ncRNA

TBA

Roland Rad

Institute for Molecular Oncology and Functional Genomics at the Technical University of Munich, Germany

A Missing Piece of the Regulatory Puzzle: Epitranscriptomic Profiling of Sense and Natural Antisense Transcripts of a Clinical Plasmodium Vivax Isolate With Severe Malaria

Priyanka Roy[1], Pallavi Gupta[2, 3, 4], Ishaan Gupta[2], Tanmaya Mahapatra[5], Dinesh Gupta[6], Sanjay Kumar Kochar[7], Dhanpat Kumar Kochar[7], Ashis Das[1]

[1] Molecular Parasitology and Systems Biology Lab, Department of Biological Sciences, Birla Institute of Technology and Science (BITS), Pilani, India
[2] Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology (IIT), New Delhi, India
[3] Australian Institute of Bioengineering and Nanotechnology (AIBN), The University of Queensland (UQ), St Lucia, Australia
[4] University of Queensland – IIT Delhi Research Academy, New Delhi, India
[5] Department of Computer Science and Information Systems, Birla Institute of Technology and Science (BITS), Pilani Campus, Pilani, India
[6] Translational Bioinformatics Group, International Centre for Genetic Engineering and Biotechnology, New Delhi, India
[7] Department of Medicine, S.P. Medical College, Bikaner, India

Background: Globally, half of the world's population is at risk of malaria caused by Plasmodium vivax. The highest prevalence of P. vivax malaria burden lies in the South-East Asia and Western Pacific regions. Recent advances in transcriptomics have identified non-coding RNAs, especially Natural Antisense Transcripts (NATs) as potential 'non-conventional' drug targets. We have previously reported the prevalence of NATs in clinical isolates of P. vivax that exhibits altered patterns of gene expression in severe malaria, but the regulation at the post-transcriptional level is not well understood. There is no information on RNA methylation and alternative splicing events from any clinical isolate in P. vivax.

Method: To this end, we identified mRNA modifications (both Sense and NATs) in the P. vivax transcriptome using Nanopore Direct RNA Sequencing and performed a comprehensive characterization of N6-methyladenosine(m6A) and 5-methylcytosine(m5C) modifications along with splicing events to decipher the context-specific functions of these post-transcriptional modifications.

Result: The distinct RNA methylation profiles of m6A and m5C in the parasites suggest species-specific regulatory mechanisms. These modifications are unevenly present in the annotated regions (5'UTR, CDS & 3'UTR) of the mRNA, potentially influencing mRNA splicing, polyadenylation, mRNA export, stability and translation. Methylated NATs, splicing events and modified transcripts originating from mitochondrial genomes have been detected. We observe a striking overlap of differential methylation of isoforms with either of the modifications.

Conclusions: The correlation of these regulatory layers will decipher the post-transcriptional environment of malaria parasites in vivo and elucidate their inherent proteome plasticity, offering potential targets for therapeutic strategies against malaria.

Mechanistic Insights Into Long Non-coding RNAs Through High-Resolution Analysis of Tumour Mutations

Roofiya Koya[1, 2, 3], Sunandini Ramnarayanan[1, 2, 3,] Hugo A. Guillen-Ramirez[1, 2, 4, 5], Rory Johnson[1, 2, 5]

[1] School of Biology and Environmental Science, University College Dublin, Dublin, Ireland
[2] Conway Institute for Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland
[3] The SFI Centre for Research Training in Genomics Data Science, National University of Ireland, Galway, Ireland
[4] Department of Medical Oncology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland
[5] Department for BioMedical Research, University of Bern, Bern, Switzerland

Long non-coding RNAs (lncRNAs) are extensively involved in diverse regulatory roles and have numerous disease associations, particularly in cancer. Our lab recently demonstrated that tumour mutations can impact lncRNA activity to increase cancer cell fitness. lncRNAs exert their bioactivity through interactions with other biomolecules in the cell, including DNA, RNA, and proteins. These interactions are encoded in the lncRNA primary sequence in the form of sequence motifs and structures, here termed 'elements'. To date, the elements of just a handful of lncRNAs have been elucidated.

This study posits that tumour single-nucleotide variants (SNVs) can impact lncRNA activity by altering functional element activity, and hence provide mechanistic insights into cancer progression. We introduce Exinator-EL (Element), a pipeline designed to detect lncRNA elements with a significantly elevated mutational burden. Exinator-EL systematically estimates the mutational burden for each element type, with respect to a modified background estimation (non-element regions) and the determination of significance through Fisher's test. It also accounts for mutational signatures with a trinucleotide-aware empirical significance estimation.

We apply Exinator-EL to a catalogue of 270 classes of elements within 122 mutated cancer-driver lncRNAs identified using cohorts from the Genomics England (GEL) resource. Our analysis reveals a significantly higher burden of mutations within the elements of these cancer-driver lncRNAs compared to non-cancer-driver lncRNAs, with the elements Conserved RNA Structures and RNA Binding Protein Sites showing a two- to five-fold enrichment in mutations. These findings suggest that mutations in these elements alter lncRNA functionality, leading to abnormal cancer cell fitness. This comprehensive map of mutated lncRNA elements on both pan-cancer and individual cancer cohorts lays the groundwork for testable mechanistic hypotheses, advancing our understanding of how mutations affect these elements in both biological and clinical contexts.

AI-Based Functional Classification of lncRNAs

Andreina Tirabassi[1], Marc Horlacher[1], Lambert Moyon[1], Ronald Rad[2], Annalisa Marsico[1]

[1] Institute of Computational Biology - Helmholtz Center Munich, Munich, Germany
[2] Institute of Molecular Oncology and Functional Genomics, Rechts der Isar Hospital of the TUM, Munich, Germany

Long non-coding RNAs (lncRNAs) play crucial roles in cellular processes and diseases, particularly in transcriptional regulation, including scaffolding RNA-binding proteins. Despite their importance, a systematic functional classification of lncRNAs remains incomplete due to experimental challenges.

These challenges can be circumvented through in silico analyses made possible by deep learning algorithms, which can decipher functional elements within RNA sequences from sequence data alone. We aim to annotate lncRNAs with RBP-binding predictions using RBPnet [1], a convolutional neural network (CNN)-based method trained on CLIP experiment data, to predict read-count profiles for over 200 RNA-binding proteins.

We develop an approach leveraging RBPnet for RNA representation learning of all annotated human lncRNAs. By extracting embeddings of the sequences and analyzing the manifold and low-dimensional structures, we identify clusters and functional similarities based on protein binding; omics data, such as epigenetic marks; and clinical variants. We apply this method to the lncRNAs identified in a genome-scale lncRNA transcriptome screening performed with Cas13d/CasRx [2]; this dataset includes thousands of transcripts selected based on expression, evolutionary conservation, and tissue specificity.

This approach enhances our understanding of the functions of lncRNA and has applications in RNA-based drug discovery. By uncovering the dependencies and relationships between lncRNAs and RNA-binding proteins, we contribute to the development of new therapeutic targets and more effective RNA-based treatments. Ultimately, this method can unlock the full potential of existing RNA and lncRNA high-throughput data and annotations, helping to unravel their unknown functions.

References:

1. Horlacher, Marc, et al. “Towards in Silico CLIP-seq: Predicting protein-RNA Interaction via Sequence-to-signal Learning.” Genome Biology, 24(1), 8 (2023).
2. Montero, Juan J et al. “Genome-scale pan-cancer interrogation of lncRNA dependencies using CasRx.” Nature Methods, 21(4) (2024).

Elucidating the Role of RNase κ in Regulating the Generation of a Hidden Layer of the Transcriptome

Jeyashri Rengaraju[1,2], Keerthic Aswin[1,2], Babita Sharma[1,2], Ayesha Nasreen[1,2], Viivek T Natarajan[1,2]

[1] *CSIR – Institute of Genomics and Integrative Biology, New Delhi, India*
[2] *Academy of Scientific and Innovative Research (AcSIR), Ghaziabad, India*

RNase κ is an endoribonuclease that is ubiquitously expressed in diverse cells and tissues. It is known to be a V-ATPase-associated factor which impacts the entry and replication of pathogenic viruses by regulating endocytosis. Knowledge pertaining to endoribonuclease function of RNase κ is limited to Bombyx mori cells derived from the ovary, where it is involved in generating 2',3'-cyclic Phosphate-containing RNAs (cP-RNAs). cP-RNAs are precursors of piRNAs, a group of small non-coding RNAs which are known to regulate transposon expression. The biogenesis and function of piRNAs are studied primarily in germ cells due to their abundance, whereas their expression and role in somatic cells are still emerging. A prominent lacuna is related to the question of how a transmembrane protein which mediates endocytosis regulates piRNA precursor generation. Also, the substrates of RNase κ and its involvement in piRNA biogenesis in somatic cells are not known, as cP-RNAs lacking a 3'-OH cannot be captured with conventional mRNA sequencing strategies. Uncovering the role of this gene in cP-RNA generation might bring to light a novel regulatory transcriptome.

Single-cell sequencing analysis of neural crest cells from Danio rerio revealed that RNase κ is expressed in cells undergoing specification and in the progenitor population of melanophores. Knockdown of RNase κ by splice-block morpholino in Tg-Mitfa: GFP (transgenic) and ASWT (wild type) zebrafish resulted in a reduced number of mitfa+ cells and melanophores, respectively. Additionally, pigmentation in the melanophores reduced significantly in the morphant fishes. To understand the mechanism of action of RNase κ, the gene was knocked out in MNT-1 (pigmented human melanoma cell line). The knockout cells exhibited varying degrees of reduction in pigmentation attributed to the observed reduction in melanosome pH. Interestingly, the protein levels of pigmentation-regulating genes like MITF, Tyrosinase, and PMEL were observed to be reduced in the knockout cells, suggesting a role of RNase κ as a V-ATPase regulator beyond pH maintenance. Furthermore, piRNA sequencing was conducted to assess whether the regulation of pigmentation genes can be associated with the endoribonuclease function of RNase κ in piRNA biogenesis. The sequencing revealed the downregulation of certain piRNAs in the knockout population, hinting at an intriguing possibility of the presence of a novel layer of a hidden transcriptome that is generated by RNase κ.

Abstracts – Posters

A1. A High-Throughput CRISPRi Screening Platform to Unravel Functional Long Non-coding RNAs

Xin Wang[1, 2, 3], Eric J. de Bony[1, 2, 3], Wim Trypsteen[1, 2, 3], Ramiro Martinez[1, 2, 3], Nurten Yigit[1, 2, 3], Tim Van De Looverbosch[4], Winnok De Vos[4], Pieter Mestdagh[1, 2, 3]

[1] OncoRNALab, Center for Medical Genetics (CMGG), Ghent University, Ghent, Belgium
[2] Cancer Research Institute Ghent (CRIG), Ghent University, Ghent, Belgium
[3] Department of Biomolecular Medicine, Ghent University, Ghent, Belgium
[4] Laboratory of Cell Biology and Histology, Department of Veterinary Sciences and Health Sciences, University of Antwerp, Antwerp, Belgium

Long non-coding RNAs (lncRNAs) are a class of transcripts with lengths exceeding 200 nucleotides that do not encode proteins. Despite their crucial roles in cellular functions and biological processes, only a minority of the over 20,000 annotated lncRNAs have been functionally characterized. Here, we established a high-throughput, CRISPR-Interference (CRISPRi) arrayed screening platform with serial cellular and molecular phenotyping to systematically characterize lncRNA functions. We reasoned that the integration of a comprehensive cellular and molecular phenotype can increase the probability of uncovering cellular functions and pathways controlled by lncRNA transcripts.

Based on the lncRNA expression data of two cell lines, we selected 156 lncRNAs that are either specifically expressed in one cell line or expressed in both cell lines. SgRNAs targeting the TSS region of these lncRNAs were produced through an optimized in vitro transcription (IVT) process and transfected in both cell lines stably expressing a dCas9 protein. Following the transfection of the sgRNAs, cells were analyzed by high-content microscopy (Cell Painting) and were then lysed for 3'-end shallow RNA-Seq. Preliminary results from this screen will be presented.

This platform is applicable across different cell types and under varying cellular conditions, facilitating the exploration of cell-state- and cell-type-specific lncRNA functionality. Further analysis of the sequence and structural features of the functional lncRNAs identified through this screen may reveal structure–function relationships and could support the development of a functional prediction model.

A2. Cap-Trap Full-Length cDNA seq: A New Technology to Detect Full-Length Cap-Trapped RNAs

Chiara Medaglia[1], Hiromi Nishiyori Sueki[2], Rossana Piccioni[1], Sachi Kato[2], Diane Delobel[2], Ilaria Nisoli[1], Masayoshi Ito[2], Callum Parr[2], Chi Wai Yip[2], Piero Carninci[1, 2], Hazuki Takahashi[2]

[1] Human Technopole, Viale Rita Levi-Montalcini 1, Milan, Italy
[2] RIKEN, Center for Integrative Medical Sciences, Yokohama, Japan

Long-read sequencing strikingly improved the de novo assembly of native transcripts, allowing us to unbiasedly annotate new isoforms. Despite its many promises, this technology still fails to provide a reliable identification of transcription start sites and end sites of mRNAs. To overcome this gap, we developed Cap-trap full-length cDNA seq (CFC-seq), a method used to detect 5'capped full-length transcripts, which combines the cap-trapping strategy with the Oxford nanopore platform.
Upon ligation to a 3' linker, all mRNA transcripts are retrotranscribed with barcoded primers. Next, cDNA undergoes cap trapping, in which cap sites are trapped by streptavidin-coated magnetic beads. The library is then completed and amplified. Finally, ONT sequencing adapters are added, and the sample can be spotted on the flow cell. Previously developed cap analysis gene expression protocols use polyadenylation to capture non-poly(A) RNAs. These approaches rely on oligo dT retrotranscription and can be biased based on oligo dT internal priming. The 3' linker ligation strategy of CFC-seq overcomes this pitfall, allowing us to detect non-poly(A) RNA derived from confident transcription start sites with high accuracy and providing transcript models with a median length of >700 nt. We benchmarked the technology in human cell lines using both ONT and PacBio sequencing. In less than a week, CFC-seq allowed us to accurately capture 5' start sites and 3' end sites of both poly(A) and non-poly(A) RNA, paving the way for a more comprehensive annotation of capped transcriptome, including long non-coding and enhancer RNAs.

A3. MinION-Enabled microRNA Quantification in Liquid Biopsies

Anastassia Kanavarioti, M. Hassaan Rehman, Salma Qureshi, Aleena Rafiq, Madiha Sultan

Yenos Analytical LLC, El Dorado Hills, CA, USA

We developed an analytical platform for detecting and quantifying trace single stranded nucleic acids in liquid biopsies using a bracketing protocol designed to yield copy number with approximately ± 20% accuracy across all concentrations. The microRNAs (miRNAs) let-7b-5p, miR-15b-5p, miR-21-5p, miR-375-3p and miR-141-3p were measured in serum and urine samples from healthy subjects and patients with breast, prostate, or pancreatic cancer. Detection and quantification were amplification-free and enabled using osmium-tagged probes, each probe unique to its target miRNA, and the MinION, a commercially available nanopore-array detection device. Combined serum from healthy men (Sigma-Aldrich, St. Louis, MO, USA #H6914) was used as the reference. Total RNA isolated from biospecimens using commercial kits was used as the miRNA source. The unprecedented ± 20% accuracy led to the conclusion that miRNA copy numbers must be normalized to the same RNA content, which in turn illustrated (i) independence from age, sex, and ethnicity, as well as (ii) equivalence between serum and urine. miR-15b was confirmed to be cancer-independent. Serum samples from cancer patients were purchased from bloodbanks, selected for stage I or II, pretreatment, and inclusive regarding age, sex, and ethnicity. miR-21-5p, miR-375-3p and miR-141-3p copies in all three cancer indications were approximately 1.8-fold overexpressed, exhibited zero overlap with healthy samples and a p-value of 1.6×10^{-22}, tentatively validating each miRNA as a multi-cancer biomarker.

A4. ncRNA Informatics for Drug Discovery

Michiaki Hamada

Waseda University, Shinjuku, Japan

In the current landscape of drug discovery, the traditional approach of targeting proteins with small-molecule compounds has reached its limits, presenting significant challenges in creating new and effective drugs. In this poster, we introduce two promising research directions based on RNA, namely "RNA aptamer drug discovery" and "RNA-targeted drug discovery," which hold great potential for addressing the current bottlenecks in drug development.
Firstly, we highlight our efforts in "AI-driven RNA aptamer drug discovery," which focuses on the efficient creation of aptamers using cutting-edge artificial intelligence techniques. We showcase our developed AI model, RaptGen [Iwano et al. Nature Computational Science, 2023], which has demonstrated remarkable success in generating high-affinity aptamers against specific targets. Our achievements in creating aptamers against viral infections [Adachi et al. Biochemistry, 2024] underscore the potential of this approach in tackling pressing global health challenges.
Secondly, we introduce an integrated database for RNA-targeted drug discovery, designed to facilitate the identification and selection of suitable drug target elements. This comprehensive resource contains a wealth of valuable information, including RNA secondary structures, RNA modifications, and binding sites for RNA-binding proteins (RBPs). By consolidating these diverse data points into a single, easily accessible platform, we aim to expedite the drug target discovery process and enable researchers to make more informed decisions when designing RNA-targeted therapeutics.

A5. Foundation Models for in Silico Probing of RNA Structures

Lambert Moyon, Tobias Bernecker, Annalisa Marsico

Computational Health Department, Helmholtz Center Munich, Munich, Germany

RNA sequences can fold through base pairings to form stable, functionally important structures that can be experimentally mapped and probed. Recent sequence-to-signal models have been trained to predict such experimental profiles in vitro for designed sequences. In parallel, Large Language Models have been successful in learning context-agnostic structure constraints from genic sequences across species. But no model exists for the in vivo probing of sequences.
We address this gap by applying transfer learning of the in vitro-trained models onto in vivo probing profiles in both mammalian and viral species. We apply different approaches of fine-tuning, notably using representations of genic sequences generated by Large Language Models as guides to the fine-tuning.
The resulting models allow for completing the probing profiles of sequences that were not detected in the original experiments, consequently enabling the prediction of context-specific RNA structures. These predictions can be used to further improve the characterization of the space of structures adopted by in vivo genic sequences. Finally, we explore the impact of non-coding mutations from the human population on the predicted probing profiles and associated structures.

A6. Learning and Memory in Prader–Willi Syndrome Through the Lens of *snord116* in Catecholaminergic Cells

Layla Skye Vasquez[1, 2, 3], Samantha Stack[1, 2, 3], William Taylor[1, 2, 3], Sooki Beeley[4], Brian Dias[1, 2, 5]

[1] *Developmental Neuroscience and Neurogenetics Program, The Saban Research Institute, Los Angeles, CA, USA*
[2] *Division of Endocrinology-Diabetes-Metabolism, Children's Hospital LA – Los Angeles, CA, USA*
[3] *Neuroscience Graduate Program, University of Southern California, CA, USA*
[4] *Psychology Undergraduate Program, University of Southern California, CA, USA*
[5] *Department of Pediatrics, Keck School of Medicine of USC – Los Angeles, CA, USA*

Prader–Willi Syndrome (PWS) results from genetic deletion of paternal alleles on chromosome 15 and is characterized by altered learning and memory. Of the paternal alleles deleted, several PWS cases have identified a microdeletion encompassing the small nucleolar non-coding RNA *snord116*. In mice, global knock-out of *snord116* has led to learning and memory disruptions; however, the effects of knocking-out *snord116* in specific neuronal populations remains unexplored. Catecholaminergic cells are ideal for investigating the contributions of *snord116* to learning and memory due to their involvement in these processes and their region-specific organization throughout the brain. We first examined the influence of knocking-out the paternal allele of *snord116* in all catecholaminergic cells of the mouse brain on learning and memory. Next, we targeted the catecholaminergic-dense locus coeruleus (LC), a brain region involved in learning and memory. Using a CRE-LoxP approach and an elaborate breeding strategy, we initially knocked-out paternal *snord116* in all catecholaminergic cells (Global-KO) of the mouse brain and measured learning and memory using auditory fear conditioning (AFC), extinction training, and extinction recall tests. Next, using intra-cranial infusions of CRE recombinase directly into the LC, we determined how knocking-out paternal *snord116* in the LC (LC-KO) influenced learning and memory. All animals acquired fear during auditory fear conditioning and extinguished their fear during extinction training. However, both the Global-KO and LC-KO animals exhibited alterations in memory recall during the extinction recall test. Specifically, the Global-KO and LC-KO males tended to freeze less compared to the control males, while the Global-KO and LC-KO females froze more compared to the control females. Our data suggest that knocking-out the paternal allele of *snord116* in all catecholaminergic cells and, more specifically, cells in the LC results in altered learning and memory. Our work begins to uncover cell- and region-specific contributions of *snord116* to PWS-associated deficits in learning and memory.

A7. Identifying the Role of Oncogenic Wnt-Associated Circular RNAs in Colorectal Cancer

Steven Eleonora

Department Center of Experimental and Molecular Medicine, Amsterdam University Medical Center, Amsterdam, The Netherlands

Colorectal cancer can be characterized by the sequential accumulation of mutations in driver genes involved in cellular proliferation. Early in the adenoma-to-carcinoma sequence, the majority of colorectal cancer patients acquire mutations in the Wnt signaling-associated APC gene. Even though this Wnt signaling pathway has been widely studied in the context of colorectal cancer, direct therapeutic targeting of its protein intermediates remains challenging. In contrast, non-coding RNAs have recently been shown to be highly tumor-specific molecules and to mediate oncogenic signaling through various mechanisms. The subgroup of circular RNAs, which are formed by an alternative splicing process called back-splicing, are rather unexplored molecules that have the potential to influence cellular signaling. Due to their ability to effectively bind miRNAs and RNA binding proteins and influence transcription, research into the function of circRNAs in aberrant cancer signaling has gained attention. Therefore, here, we attempt to identify functional circRNAs associated with the oncogenic Wnt signaling pathway. Using RNA sequencing, we identify multiple circRNAs whose expression levels are influenced upon ß-catenin knockdown. Using a cas13D-mediated CRISPR dropout screening approach, we specifically knock down circRNAs in a panel of CRC cell lines to evaluate their impact on tumor proliferation. We are currently investigating the mechanism in which the top candidate(s) from this CRISPR screening influence CRC proliferation. Further characterization of the oncogenic role of these circRNAs will ultimately provide insights into how we can use these molecules for novel, tumor-specific therapeutic strategies.

A8. A Long Non-coding RNA (lncRNA) Signature in Blood Defines Activity of the Oncogene Yes-Associated Protein (YAP) in Tumor Cells of Cancer Patients

Fabian Rose[1], Nada El-Ekiaby[2], Fabiola Pedrini[1], Lilija Wehling[1], Merna Hatem Mohamed Hatem[2], Sofia ME Weiler[1], Ahmed Ihab Abedelaziz[2], Kai Breuhahn[1]

[1] *Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany*
[2] *School of Medicine, NewGiza University (NGU), Giza, Egypt*

Body fluids from cancer patients inform alterations in tumor tissues; however, robust blood biomarkers used for detecting oncogene activity in tumor cells do not exist. Here, we aim to define a set of long non-coding RNAs (lncRNAs) that are transcriptionally regulated by Hippo pathway-controlled oncogenes, yes-associated protein (YAP) and WW domain containing transcription regulator 1 (TAZ). To identify a cancer-spanning lncRNA signature, which could serve as a liquid biopsy biomarker, we comparatively analyzed two cancer entities: hepatocellular carcinoma (HCC) and non-small cell lung cancer (NSCLC).

NGS data from HCC and NSCLC cells after YAP/TAZ silencing, ChIP-seq results, and cancer patient expression data derived from HCC and NSCLC patients were used to identify differentially regulated lncRNAs. A core lncRNA signature consisting of CYTOR, MIR4435-2HG, SNHG1, and SNHG17 defines HCC and NSCLC patients with poor overall survival. ChIP analysis revealed that signature lncRNAs are transcriptionally regulated by YAP and TEA domain family member (TEAD) transcription factor family members. Equally, in situ hybridization revealed that the CYTOR orthologue Morrbid is elevated in YAP^{S127A}-induced murine tumors and CYTOR/SNHG1 in human HCCs with nuclear YAP enrichment. In vitro, CYTOR, MIR4435-2HG, and SNHG17 support cancer cell viability and proliferation. The lncRNA signature characterizes HCC cells responding to YAP-directed pharmacological inhibition. Importantly, nuclear YAP positivity in HCC tissues significantly correlates with the lncRNA signature in corresponding serum samples. The signature in serum predicts YAP activity in HCC tissues with high specificity and sensitivity.

We demonstrate that lncRNA signatures in patient serum represent a robust proxy for the activity of druggable oncogenic transcriptional regulators in tumor cells. As the YAP-specific lncRNA signature is detectable in several tumor entities, it may serve as a pan-cancer biomarker for diagnostics and therapy.

A9. LncRNAs as Potential Targets for Multi-Tumor Therapies: Using scRNA-seq to Investigate the lncRNA Profile in the Tumor Microenvironment

Amanda Piveta Schnepper[1,2], Victória Larissa Schimidt Camargo[1], Caio Fernando Ferreira Mussatto[1], Fernando de Souza Leite[1], Iago Diogo Silveira[1], Sarah Santiloni Cury[1], Rory Johnson[2], Robson Francisco Carvalho[1]

[1] *São Paulo State University (UNESP), Institute of Biosciences, Botucatu, Brazil*
[2] *School of Biology and Environmental Science, University College Dublin, Dublin, Ireland*

Cancer affects around 20 million people worldwide, resulting in nearly 10 million deaths (GLOBOCAN, 2020). One of the main obstacles to achieving universal treatment is the diversity of cancer types and the heterogeneity of the tumor microenvironment. Single-cell RNA sequencing (scRNA-seq) technology can unveil gene expression patterns in diverse cell types. While this technology has been utilized to explore new targets for cancer treatment, its application, explicitly targeting long non-coding RNAs (lncRNAs), remains relatively unexplored. LncRNAs are RNA molecules with over 200 nucleotides that do not encode proteins but play crucial roles in various cellular mechanisms. In this context, we utilized scRNA-seq data to investigate the transcriptional profile of lncRNAs across ten different tumor types. For this purpose, scRNA-seq data for head and neck, lung, liver, prostate, breast, pancreas, neuroendocrine, colorectal, ovarian, and skin tumors were downloaded from the Curated Cancer Cell Atlas. The inclusion criteria were applied to select only untreated primary samples from the 10X platform with available count data. The count data were processed using the Seurat (v4.0.6) package (R v4.2.2). The identities of cell types were determined based on the expression of canonical markers. Hierarchical clustering of expression values revealed that lncRNAs grouped into five distinct clusters were associated with different cell types independent of cancer types: malignant cells, endothelial cells, fibroblasts, macrophages, dendritic cells, and immune cells. Furthermore, comparing malignant cells from all tumor types with other cell types enabled the identification of 25 lncRNAs positively correlated with malignant cells. Among them are lncRNAs FAM201A, VPS9D1-AS1, BOK-AS1, LINC00668, and PCAT7, all of which have been previously implicated in cancer regulation in different cancer types. This comprehensive analysis across different tumor types, coupled with scRNA-seq technology, can aid in identifying more specific targets within malignant cells applicable to various cancer types.

A10. Long Noncoding RNA Profiling Identifies LINC00960 as Poor Prognostic Indicator Promoting Triple-Negative Breast Cancer Progression

Ramesh Elango[1], Nehad M Alajez[1,2]

[1] Translational Oncology Research Center (TORC), Qatar Biomedical Research Institute (QBRI), Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), Doha, Qatar
[2] College of Health & Life Sciences, Hamad Bin Khalifa University (HBKU), Qatar Foundation (QF), Doha, Qatar

Long noncoding RNAs (lncRNAs) are pivotal players in breast cancer pathogenesis, including the aggressive Triple-Negative Breast Cancer (TNBC) subtype. Analyzing the patterns of lncRNA expression in different subtypes of breast cancer holds promise for uncovering valuable insights into their potential roles as both biomarkers for disease and targets for therapy. In our study, we analyzed lncRNA expression in 96 breast cancer cases, revealing significant differences compared to normal breast tissue. We observed variations among breast cancer subtypes, including Hormone Receptor-positive (HR+), HER2-positive (HER2+), HER2+HR+, and TNBC, as well as in relation to tumor grade and patients' age at diagnosis. TNBC and HER2+ subtypes exhibited distinct clustering, while HER2+HR+ tumors clustered closer to HR+ tumors based on lncRNA profiles. Our analysis unveiled numerous lncRNAs enriched in TNBC, with a particular focus on the elevated expression of LINC00960, a finding substantiated by two additional datasets. Evaluating LINC00960 expression in an independent TNBC cohort (n=360) revealed its correlation with cell movement, invasion, proliferation, and migration functional categories. Depletion of LINC00960 notably reduced TNBC cell viability, colony formation, migration, and three-dimensional growth while increasing cell death. Transcriptomic profiling of LINC00960-depleted cells confirmed its tumor-promoting role, possibly through sponging hsa-miR-34a-5p, hsa-miR-16-5p, and hsa-miR-183-5p and subsequent upregulation of BMI1, KRAS, and AKT3 cancer-promoting genes. Our findings underscore the distinct lncRNA expression patterns in breast cancer subtypes and highlight the critical role of LINC00960 in TNBC progression, suggesting its potential as both a prognostic marker and therapeutic target.

A11. Oncogene-Induced Long Non-coding RNA (lncRNA) Signatures in Different Cancer Cells

Fabiola Pedrini, Stefania Bonusi, Peter Schirmacher, Kai Breuhahn

Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany

Long non-coding RNAs (lncRNAs) may be robust and sensitive markers for cancer patient stratification. The gold-standard method for biomarker identification compares samples from healthy and diseased individuals. However, this approach does not allow the definition of lncRNAs regulated by specific oncogenic transcriptional regulators, which may serve as therapeutic targets. This study investigates whether lncRNA signatures can serve as a proxy for the activity of the cancer-relevant β-catenin/transcription factor 4 (TCF4, TCF7L2) transcription factor complex in different tumor types.

To identify transcription factor-specific lncRNAs, β-catenin and TCF7L2 were separately inhibited by siRNAs in liver cancer (HCC, hepatocellular carcinoma) and lung cancer (NSCLC, non-small cell lung cancer) cells. After next-generation sequencing (NGS) analysis, the regulation of candidate lncRNAs was confirmed in different HCC and NSCLC cells by qPCR. Moreover, candidate lncRNAs were regulated after activating or silencing the β-catenin/TCF7L2 complex using LiCl or iCRT3, respectively. The binding of TCF7L2 to lncRNA gene promoters was confirmed using chromatin immunoprecipitation (ChIP) experiments and ChIP-seq data. Notably, the results illustrated a moderate overlap between β-catenin- and TCF7L2-regulated lncRNAs in both cancer types. In addition, no significant overlap between TCF7L2-regulated lncRNAs in HCC and NSCLC was detected. Based on these results, we created HCC- and NSCLC-specific lncRNA signatures for β-catenin and TCF7L2. The presence of individual lncRNAs and tumor-entity-specific lncRNA signatures was confirmed using human patient expression data (TCGA data for LIHC and LUAD, respectively).

In summary, we define cancer-type-specific lncRNA signatures that may be diagnostic tools for β-catenin and TCF7L2 activity in cancer cells. The minimal overlap between β-catenin- and TCF7L2-regulated lncRNA indicates the existence of alternative transcriptional complexes for both factors. Next, we plan to test whether lncRNA signatures can be detected in the serum of HCC and NSCLC patients.

A12. The Adrenergic-Specific lncRNA NESPR Regulates Neuroblastoma Cell Viability and Survival

Louis Delhaye[1, 2, 3, 4], Eric James de Bony[1, 2, 3], Dries Rombaut[1, 2, 3], Lisa Depestel[1, 2, 3], Eva D'haene[1, 3], Nurten Yigit[1, 2, 3], Delphine De Sutter[1, 4], Jasper Anckaert[1, 2, 3], Björn Menten[1, 3], Jo Vandesompele[1, 2, 3], Frank Speleman[1, 2, 3], Sven Eyckerman[1, 2, 4], Pieter Mestdagh[1, 2, 3]

[1] *Department of Biomolecular Medicine, UGent, Ghent, Belgium*
[2] *Cancer Research Institute Ghent (CRIG), UGent, Ghent, Belgium*
[3] *Center for Medical Genetics, UGent, Ghent, Belgium*
[4] *VIB-UGent Center for Medical Biotechnology, VIB, Ghent, Belgium*

Neuroblastoma is a childhood cancer of the sympathetic nervous system. Recent studies have shown that neuroblastoma tumors are composed of two cell identities, i.e., the adrenergic identity and the mesenchymal identity. Both identities are driven by a core regulatory transcriptional circuitry, which acts as an autoregulatory positive feedforward loop, to delineate the cell identity through the regulation of its target genes. We identified the long non-coding RNA NESPR to be specifically expressed in neuroblastoma cells of the adrenergic cell identity. NESPR expression correlates with several parameters of high-risk neuroblastoma and poor patient survival. We show that NESPR is contained within an insulated gene neighborhood with the adrenergic core regulatory transcription factor PHOX2B and that NESPR regulates PHOX2B expression in cis. The knockdown of NESPR decreased neuroblastoma cell proliferation and induced cell death, highlighting NESPR's importance in the survival of the adrenergic neuroblastoma cells.

A13. The lncRNA Charme, an Evolutionarily Conserved Regulator of Muscle Differentiation and Pathology

Giulia Buonaiuto[1], Marco Simula[1], Daniele Durante[1], Giulio Storari[1], Alessandro Palma[1], Pietro Laneve[2], Monica Ballarino[1]

[1] Department of Biology and Biotechnology "C. Darwin", Sapienza University of Rome, Rome, Italy
[2] Institute of Molecular Biology and Pathology, National Research Council (CNR), Rome, Italy

Introduction: Tissue-specific long noncoding RNAs (lncRNA) play essential roles in regulating cell growth, differentiation and physiology, although the knowledge concerning their mechanisms of action is still far from complete. Among them, nuclear lncRNAs are generally associated with chromatin and can contribute to the formation of higher-order three-dimensional structures. Their dysregulation has often been linked with several pathological states, including neuromuscular and cardiovascular diseases. As such, understanding their role in muscle development and pathologies is extremely important, particularly in cases when a clear genetic cause has not been identified.
Background: Our lab has recently identified Charme (Chromatin architect of muscle expression), a highly conserved and abundant lncRNA, specifically expressed in the nucleus of differentiated myotubes and cardiomyocytes (1,2). We found that Charme acts as an “architect” RNA to scaffold the formation of physiologically important ribonucleoprotein nuclear condensates. Consistent with a possible association with disease, the functional inactivation of Charme in mice leads to muscle hyperplasia and dilatative cardiomyopathy (3). Critically, no molecular analysis has yet been performed to dissect the role of Charme in humans.
Aim: Uncovering the role of the human syntenic *HSCHARME* locus in muscle physiology and disease.
Results: By applying comparative genomics to explore the evolutionary conservation of Charme, we discovered the human syntenic *HSCHARME* lncRNA. *HSCHARME* shares several features and a similar tissue specificity with its murine counterpart, which offers intriguing possibilities for ongoing studies. In skeletal muscle, we are studying the impact of *HSCHARME* ablation in both myogenesis and neuromuscular physiology and diseases. In the heart, preliminary data show that *HSCHARME* expression is significantly altered across patients affected by several cardiomyopathies and impinges on pathology-associated pathways.
1.Ballarino et al., *EMBO journal*, 2018. DOI: 10.15252/embj.201899697
2.Desideri, Cipriano et al., *Cell reports*, 2020. DOI: 10.1016/j.celrep.2020.108548
3.Taliani, Buonaiuto et al.,eLife, 2022. DOI:10.7554/eLife.81360

A14. Revealing Hidden Regulators in Lung Cancer: Using CRISPR to Discover lncRNA Targets for RNA-Based Therapies

Yifan Yu[1], Rory Johnson[1, 2, 3], Michela Coan[1, 2], Hugo Guillen-Ramirez[4], Sunandini Ramnarayanan[1, 2, 7], Ai-Ming Tham[1, 2], Nivedha Balaji[1], Jack Roban[1, 2], Bhavya Dhaka[1, 2], Clodagh Murphy[1, 8], Xingyu Guo[1], Ines Jover Bravo[1, 7]

[1] School of Biology and Environmental Science, University College Dublin, Dublin, Ireland
[2] Conway Institute for Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland
[3] FutureNeuro, SFI Research Centre for Chronic and Rare Neurological Diseases, Dublin, Ireland
[4] Department of Visceral Surgery and Medicine, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland
[7] Ireland The SFI Centre for Research Training in Genomics Data Science, Dublin, Ireland
[8] Cancer Biology and Therapeutics Laboratory, Conway Insititute, University College Dublin, Dublin, Ireland

Non-small-cell lung cancer (NSCLC) remains a leading cause of mortality, underscoring the urgent need for novel therapeutic strategies. Long noncoding RNAs (lncRNAs), with their unique expression patterns across cells, tissues, and patients, offer immense potential for targeted cancer therapies. This specificity facilitates the precise targeting of cancer cells while minimizing collateral damage to normal tissues. Antisense oligonucleotides (ASOs), which can modulate specific lncRNAs, emerge as accessible and cost-effective candidates for innovative cancer treatments.
Despite their promise, lncRNAs remain underexplored in therapeutic contexts. Our project seeks to close this gap by advancing precision oncology through the identification and validation of lncRNA therapeutic targets for NSCLC. We have developed a highly sensitive and specific pipeline to prioritize lncRNA hits from CRISPR perturbation screens. By computing efficacy and toxicity scores for each candidate, we integrated comprehensive data from four cancer cell models (both primary and metastatic) and two normal cell models, along with multiomics datasets including patient survival data and single nucleotide variants (SNVs). This robust approach identified seven high-priority cancer-promoting lncRNAs for subsequent ASO validation.
Our work exemplifies a precision medicine approach to addressing the critical need for effective NSCLC treatments. By unveiling the vast potential of lncRNAs and ASOs in precision oncology, we pave the way for novel therapeutic interventions that could significantly improve patient outcomes.

A15. Tocotrienol-Rich Fraction Modulates the Expression of lncRNAs in Triple-Negative Breast Cancer Cells

Jacelyn Lee, Ammu Radhakrishnan, Uma Devi Palanisamy, Kasthuri Bai Magalingam, Reyhaneh Farghadani

Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Subang Jaya, Malaysia

Triple-negative breast cancer (TNBC) is the most lethal breast cancer subtype, with the highest metastasis and recurrence rate. Conventional chemotherapy remains the standard treatment, but there is a lack of targeted therapies. In recent years, there has been a growing body of evidence indicating that long non-coding RNAs (lncRNAs) can regulate gene expression and play an important role in tumour progression. Tocotrienols (T3s) are natural vitamin E derivatives that have been reported to have potent anticancer activities in various cancers, including breast cancer. However, T3s' mechanism of action has yet to be fully characterised, and to date, and it is not known whether the molecular mechanism(s) through which T3 exerts its anticancer activities involve lncRNAs. In an effort to investigate this, we treated the human TNBC cell line MDA-MB-468 with palm-oil-derived tocotrienol-rich fraction (TRF) and used the Claroim D© microarray to identify differentially expressed lncRNAs. The expression of selected lncRNAs, including H19 and NEAT1, was also validated using qPCR. We report that in MDA-MB-468 cells, TRF modulates the expression of lncRNAs, most of which were uncharacterised transcripts. Bioinformatic analysis of lncRNAs with annotations suggests that lncRNAs modulated by TRF regulate cell cycle-related pathways, but further mechanism-of-action assays and in vivo studies are required.

A16. Prioritisation of Targets for Precision RNA Therapeutics via Cancer Genomics Data Integration

Jack Roban, Rory Johnson

[1] *School of Biology and Environmental Science, University College Dublin, Dublin, Ireland*
[2] *Conway Institute for Biomolecular and Biomedical Research, University College Dublin, Dublin, Ireland*

Cancer remains a leading cause of death worldwide despite advancements in conventional therapies, many of which target proteins. A new alternative comes from RNA therapeutics (RNATx), which targets RNA molecules using programmable oligonucleotides. Long non-coding RNAs (lncRNAs), cited as key regulators of cancer development and progression, exhibit huge potential as a promising source of targets for RNATx. Although there are estimated to be >100,000 lncRNAs in total, 1% have been functionally characterised, and the process is notoriously laborious. The consequent testing of poor-quality therapeutic targets has led to late-stage failures during clinical translation, resulting in huge losses of resources and time. My project aims to improve the selection of lncRNA targets for RNATx through a data-driven multi-omics approach focused on integrating diverse cancer genomic datasets. Specifically, I aim to prioritise lncRNA targets based on features including clinical data (gene expression, prognosis, mutations, germline variants) and prior links to cancer in literature. Machine learning algorithms will utilise and rank feature contributions, thereby prioritising lncRNAs implicated in both pancancer and specific cancer types. This novel method will expedite the lncRNA target selection process, enabling robust lncRNA prioritisation precision therapy development for unmet needs in oncology, greatly increasing both the speed and volume of RNATx which could reach the clinic/patient.

A17. Exploring the Interaction Between YAP/TAZ and NOTCH2 and Their Downstream lncRNA Machinery in Hepatic Stellate Cells

Nada El-Ekiaby[1], Amira Khaled Abdelhamid[1], Manon Ragheb[1], Jennifer Schmitt[2], Kai Breuhahn[2], Ahmed Ihab Abdelaziz[1]

[1] *School of Medicine, Newgiza University (NGU), Giza, Egypt*
[2] *Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany*

Liver fibrosis is regulated by hepatic stellate cells (HSCs), which produce extracellular material upon activation. The transcription co-activators yes-associated protein (YAP) and WW domain containing transcription regulator 1 (TAZ) have shown controversial data regarding their role in liver fibrosis, with some studies showing that they promote fibrosis[1,2], while others show that they alleviate it[3]. Hence, we aimed to investigate the role of YAP/TAZ in the development of liver fibrosis by identifying new interaction partners and their impact on the downstream long non-coding RNA (lncRNA) machinery in HSCs.

We show that YAP/TAZ silencing by siRNAs in the human HSC cell line LX-2 led to a marked increase in HSCs' activation marker alpha-smooth muscle actin (α-SMA), indicating that YAP/TAZ might act as repressors of HSC activation. We identified NOTCH2 as a novel YAP/TAZ interaction partner using a BioID assay and confirmatory proximity ligation assay. NOTCH2 inhibition phenocopied YAP/TAZ silencing with regard to α-SMA and YAP/TAZ target gene expression.

Because previous data illustrated the fibrogenic and anti-fibrogenic effects of lncRNAs, we investigated how the YAP/TAZ/NOTCH2 complex can control lncRNAs in LX2 cells. We performed next-generation sequencing (NGS) in LX2 cells after YAP/TAZ silencing and identified lncRNAs with binding sites for the main transcription factor activated by NOTCH2 intracellular domain, RBPJ, in their promoters using ChIP-seq data. Bioinformatic analysis revealed YAP/TAZ-regulated lncRNAs (n=13) that are also potentially regulated by NOTCH2. The hypoxia-inducible factor-1alpha antisense RNA-3 (HIF1A-AS3) triggered our interest as it controls the expression of pro-fibrotic HIF1A. We suggest that HIF1A-AS3 may play a role in the development of fibrosis, which is a common non-neoplastic liver disease. At the molecular level, this is regulated by the YAP/TAZ/NOTCH2 axis and can be targeted by new drugs currently being tested in clinical trials.

A18. Expression Profiling of Exosome-Derived microRNAs Involved in the Progression of Cutaneous Malignant Melanoma With Implications for Diagnosis and Treatment

Andreea-Daniela Lazar (Popa)[1], Sorina Dinescu[1,2]

[1] *Department of Biochemistry and Molecular Biology, University of Bucharest, Bucharest, Romania*
[2] *Research Institute of the University of Bucharest - ICUB, Bucharest, Romania*

Cutaneous malignant melanoma is an aggressive type of cancer with high mortality and poor prognosis. Its incidence has been increasing yearly and current therapeutic options remain insufficient. Understanding the mechanisms underlying melanoma progression is essential for proper diagnosis and for the development of efficient treatment. Recently, exosomes, as essential intercellular messengers with microRNA (miRNA) cargo, have been of particular interest, as they can modulate the tumor microenvironment and regulate the expression of a wide range of target genes, potentially playing a significant role in cancer invasion and metastasis. In this context, the purpose of our study was to investigate the expression of exosome-derived miRNAs involved in the progression of melanocytes to primary and metastatic cancer. Therefore, expression profiling of 84 miRNA molecules was performed on exosomes isolated from the culture media of melanocytes and tumor cell lines originated from primary and metastatic melanoma (lymph node and brain) using a qPCR array. The results revealed the overexpression of oncogenic miRNAs (e.g., miR-184, miR-572) and the underexpression of miRNAs with tumor-suppressing function (e.g., miR-34b-3p, miR-224-5p, etc.) between the different stages of melanoma and normal cells. Furthermore, some miRNAs, among them miR-143-3p, were found to be decreased during early tumor progression but increased in metastatic phases. Potential targets for the miRNAs of interest were highlighted through bioinformatic analysis, including KRAS, CDK4, and IGF1R, important markers involved in several signaling pathways that contribute to cell proliferation and survival. In conclusion, the delivery of miRNAs through exosomes can mediate the progression of melanoma, and continuous exploration could lead to improved diagnosis and development of efficient targeted therapy.

A19. Circular RNA Aptamers Ameliorate Phenotypes Relevant to Alzheimer's Disease by Targeting PKR

Xin Feng[1], Bo-Wen Jiang[1], Si-Nan Zhai[2, 3], Si-Kun Guo[1], Chu-Xiao Liu[1], Hao Wu[1], Bang-Qi Zhu[1, 4], Meng-Yuan Wei[1], Jia Wei[2], Li Yang[2], Ling-Ling Chen[1, 5, 6]

[1] Key Laboratory of RNA Innovation, Science and Engineering, CAS Center for Excellence in Molecular Cell Science, Shanghai Institute of Biochemistry and Cell Biology, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China
[2] Center for Molecular Medicine, Children's Hospital, Fudan University and Shanghai Key Laboratory of Medical Epigenetics, International Laboratory of Medical Epigenetics and Metabolism, Ministry of Science and Technology, Institutes of Biomedical Sciences, Fudan University, Shanghai, China
[3] Shanghai Institute of Nutrition and Health, University of Chinese Academy of Sciences, Chinese Academy of Sciences, Shanghai, China
[4] School of Life Science and Technology, Shanghai Tech University, Shanghai, China
[5] New Cornerstone Science Laboratory, Shenzhen, China
[6] Key Laboratory of Systems Health Science of Zhejiang Province, School of Life Science, Hangzhou Institute for Advanced Study, University of Chinese Academy of Sciences, Hangzhou, China

Circular RNAs containing short imperfect duplex (ds-cRNAs) robustly suppress double stranded RNA (dsRNA)-activated protein kinase R (PKR), but their potential biomedical application has remained unaddressed, in particular for detrimental diseases related to excessive PKR activation. Alzheimer's disease (AD) is a severe neurodegenerative disorder in need of new treatment whose pathogenesis is in part attributed to PKR-related neuroinflammation. Here, we delineated remarkably elevated neuroinflammation, accompanied by the progressive activation of PKR and PKR-related dsRNA pathways, both in hippocampus tissues and in microglia isolated from 5×FAD mice upon AD progression. The AAV-delivery of ds-cRNAs to neurons and microglia effectively dampened excessive PKR activity with little toxicity, alongside reducing neuroinflammation and amyloid-beta (Aβ) plaques, resulting in neuroprotection and enhanced spatial learning capability and memory in an AD model consisting of 5×FAD mice with PKR depletion. Additionally, synthetic ds-cRNAs also dampened excessive PKR activity and related inflammation and Aβ amyloid plaques in an acute neuroinflammation model consisting of mice stressed by EMCV. These findings suggest a therapeutic potential of ds-cRNA aptamers to mitigate PKR-related neuroinflammation and a potential new strategy for AD treatment.

B1. Deciphering the Non-coding RNA Landscape Associated With Endochondral Bone Formation Potential in Marrow Stromal Cells

Andrea Lolli[1], Encheng Ji[1], Janneke Witte-Bouma[1], Paul de Jongh[1], Remco Hoogenboezem[2], Eric Bindels[2], Marc Raaijmakers[2], Gerjo van Osch[3, 4], Niamh Fahy[5, 6], Eric Farrell[1]

[1] Department of Oral and Maxillofacial Surgery, Erasmus MC University Medical Center, Rotterdam, The Netherlands
[2] Department of Haematology, Erasmus MC University Medical Center, Rotterdam, The Netherlands
[3] Department of Orthopaedics and Sports Medicine and Department of Otorhinolaryngology, Erasmus MC University Medical Center, Rotterdam, The Netherlands
[4] Department of Biomechanical Engineering, University of Technology Delft, Delft, The Netherlands
[5] Department of Oral and Maxillofacial Surgery and Department of Orthopaedics and Sports Medicine, Erasmus MC University Medical Center, Rotterdam, The Netherlands
[6] Department of Applied Science, Technological University of the Shannon: Midlands Midwest, Limerick, Ireland

Endochondral ossification is the natural process in development and regeneration whereby cartilage is remodelled into bone. It is a promising avenue to treat bone defects in patients, and may be achieved by implanting cartilage generated by differentiated human marrow stem/stromal cells (hMSCs). Unfortunately, the lengthy in vitro period to chondrogenically differentiate hMSCs (≥3 weeks) hinders clinical translation. To aid the development of brief priming strategies, we set out to define the minimum priming duration required for hMSC-mediated bone formation. Furthermore, we elucidated the underlying changes in the non-coding (nc)RNA landscape, with the aim of gaining instrumental knowledge for the design of ncRNA-targeting tools to optimise bone formation.
hMSC aggregates/pellets were chondrogenically differentiated by exposure to Transforming Growth Factor (TGF)-β3 for 1, 3, 5, 7 or 21 days. After subcutaneous implantation in mice for 12 weeks, 7d- and 21d-primed pellets consistently led to bone formation (N=4 donors). Priming hMSCs for less than 7 days led to negligible bone formation. Longitudinal immunohistological analyses showed that 7d- and 21d-primed pellets comparably underwent mineralisation, vascularisation and remodelling into bone. However, µCT analyses demonstrated increased bone volume for 21d- vs 7d-primed pellets (~5-fold). To determine the ncRNA signature associated with bone formation capacity, we compared the transcriptome of 7d- and 21d-primed pellets with that of 3d-primed pellets via RNA-sequencing, and then focused on microRNAs. Among the 141 microRNAs differentially modulated in bone-forming samples, we selected miR-218-5p, miR-31-3p, miR-100-5p and miR-335-3p/5p as targets negatively associated with bone formation potential, and thus candidates for silencing strategies to stimulate bone formation.

To conclude, brief chondrogenic priming reliably leads to endochondral bone formation, but the bone volume is reduced. We identified a panel of ncRNAs to be targeted in combination with short priming to scale-up bone volume or speed-up bone formation. Our data raise exciting opportunities for developing clinically relevant tissue engineering approaches for bone defects.

B2. The Role of Parentally Provided RNAs in Transgenerational Thermal Tolerance in Zebrafish

Anastasiia Berezenko, Dragan Stajic, Irene Adrian-Kalchhauser

Institute for Fish and Wildlife Health, Vetsuisse Faculty, University of Bern, Bern, Switzerland

Non-genetic inheritance (NGI) is the inheritance of alterations in gene expression without changes in the underlying DNA sequence. NGI has been observed in a wide range of animal species and even in humans; it has been postulated to facilitate adaptation to rapidly changing conditions and is recognized as a key factor in a population's health.
Among the various factors involved in NGI, RNA plays a particularly interesting role. Maternally provided RNA orchestrates the early development of numerous species, including vertebrates, and paternally provided RNA has been shown to mediate stress phenotypes. However, the precise role of maternal and paternal RNAs in epigenetic inheritance during early development and their impact on further adaptation to changing environmental conditions remain unknown.
We study the response of parental RNA contributions in zebrafish with temperature as a stimulus. We investigate the molecular response of fish to heat at the level of paternal and maternal RNA and the physiological response to heat experienced by their parents. We then attempt to establish functional links between the two responses.
We want to understand which regulatory pathways and gene regulatory mechanisms are involved in NGI in fish, how they affect offspring behavior and physiology, and for how long parental exposures remain transmittable.

B3. Dynamics of lncRNA and miRNA Expression in the Postnatal Mouse Pituitary

Julian Zacharjasz[1], Michał Smuszkiewicz[1], Marta Sztachera[1], Ewelina Kałużna[1], Michał Szcześniak[2], Monika Piwecka[1]

[1] Department of Non-coding RNAs, Institute of Bioorganic Chemistry, Polish Academy of Sciences, Poznań, Poland
[2] Institute of Human Biology and Evolution, Faculty of Biology, Adam Mickiewicz University, Poznań, Poland

The pituitary gland (PG) is a neuroendocrine organ located at the base of the brain. Six types of endocrine cell populations within the PG produce and secrete hormones that are essential for the regulation of organismal metabolism, growth, reproduction, and stress responses. The regulation of hormone production and secretion on a cell-type basis remains elusive. We hypothesize that cell-type-specific ncRNAs are essential contributors in post-transcriptional gene expression regulation in the PG. Here, we present the results of miRNA and long non-coding RNA (lncRNA) profiling in the male murine PG from three postnatal stages of development: P1 (newborn), P30 (onset of puberty) and adult mice. We used a NanoString miRNA assay and total ribominus RNA sequencing to quantify global miRNA and lncRNA expression levels in addition to mRNA. We quantified the expression of 1310, 1284 and 1202 lncRNAs (threshold: 2 TPM in n=3 biological replicates per stage), and 279, 325 and 315 miRNAs (threshold: 26 counts in at least 2 biological replicates per stage) in PG from each developmental stage. We created a resource of stage-specific ncRNAs and recognized the dynamic expression patterns of lncRNAs and miRNAs in the PG during postnatal development. We observed that the majority of differentially expressed lncRNAs are increasing, whereas the majority of differentially expressed miRNAs are decreasing in PG during the progress of the murine lifespan. We are also analyzing the spatial expression pattern of lncRNAs and miRNAs in the PG of adult mice using the Visium Spatial Transcriptomics platform and RNAscope miRNA assays, respectively. In the future, we aim to explore the cell-type specificity of lncRNAs and miRNAs in pituitary gland endocrine and non-endocrine cells in more detail.

B4. Unraveling the Neurodevelopmental Regulatory Landscape of Antisense Long Non-Coding RNAs

Anastasia Casali[1], Jessica Severin[2], Hiromi Nishiyhori Sueki[2], Rodrigo Pracana[1], Hazuki Takahashi[2], Piero Carninci[1, 2]

[1] *Genomic Research Centre, Human Technopole, Milan, Italy*
[2] *RIKEN Center for Integrative Medical Sciences (IMS), Yokohama, Japan*

Long non-coding RNAs (lncRNAs) have emerged as pivotal regulators of gene expression, shedding light on the complexity of RNA-mediated processes. Natural antisense transcripts (NATs), RNA molecules transcribed from the opposite strand of the sense gene, represent a prominent class of regulatory lncRNA. While it is acknowledged that some NATs are involved in modulating the expression of their sense genes, the full extent of their regulatory network and their overall functional significance is not yet completely understood. This study aims to elucidate the molecular role and network features of NATs during neurodevelopment by investigating RNA–RNA interactions (RRIs) using an innovative technique based on direct RNA psoralen-based crosslinking called PARIS-seq. PARIS-seq enables the detection of RNA duplex formation, revealing direct RNA–RNA contacts without the involvement of intermediary proteins or proximity-based methods. Through this approach, we aim to unveil novel roles of antisense transcripts in regulation of gene expression, specifically during neurodifferentiation. Preliminary findings demonstrate that antisense transcripts can interact directly with other transcripts both locally (*in cis*) and distantly (*in trans*) from their transcription locus. This suggests that their interactions extend beyond their cognate sense genes, potentially participating in broader regulatory networks. Such insights will deepen our understanding of the intricate mechanisms governing gene expression during neurodevelopment.

B5. Mitochondrial Genome-Derived circRNAs: Neglected Epigenetic Regulators in Redox Biology

Nima Sanadgol[1], Cordian Beyer[1], Adib Zendedel[2]

[1] *RWTH Aachen University, Aachen, Germany*
[2] *University of Basel, Basel, Switzerland*

Mitochondria are vital for cellular activities, influencing ATP production, $Ca2^{+}$ signaling, and reactive oxygen species generation. It has been proposed that nuclear genome-derived circular RNAs (circRNAs) play a role in biological processes. For the first time, this study aims to comprehensively explore experimentally confirmed human mitochondrial genome-derived circRNAs (mt-circRNAs) via in silico analysis. We utilized wide-ranging bioinformatics tools to anticipate their roles in molecular biology, including miRNA sponging, protein antagonism, and peptide translation. Among five well-characterized mt-circRNAs, SCAR/mc-COX2 stands out as particularly significant with the potential to sponge around forty-one different miRNAs, which target several genes mostly involved in the endocytosis, MAP kinase, and PI3K-Akt pathways. Interestingly, circMNTND5 and mecciND1 specifically interact with miRNAs through their unique back-splice junction sequence. These exclusively targeted miRNAs (has-miR-5186, 6888-5p, 8081, 924, and 672-5p) are predominantly associated with the insulin secretion, proteoglycans in cancer, and MAPK signaling pathways. Moreover, all mt-circRNAs intricately affect the P53 pathway through miRNA sequestration. Remarkably, mc-COX2 and circMNTND5 appear to be involved in the RNA's biogenesis by antagonizing AGO1/2, EIF4A3, and DGCR8. All mt-circRNAs, except mecciND1, are involved with IGF2BP proteins and are crucial in redox signaling. Additionally, they all potentially generate at least one protein resembling the immunoglobulin heavy chain protein. Given P53's function as a redox-sensitive transcription factor and insulin's role as a crucial regulator of energy metabolism, their indirect interplay with mt-circRNAs could influence cellular outcomes. However, due to limited attention and infrequent data availability, it is advisable to conduct more thorough investigations to gain a deeper understanding of the functions of mt-circRNA.

B6. Exploring the Contribution of Novel Small ncRNAs in β-Cell Destruction During Type 1 Diabetes (T1D) Pathogenesis

Shagun Poddar[1], Flora Brozzi[1], Cecile Jacovetti[1], Cristina Cosentino[1], Claudiane Guay[1], Romano Regazzi[1,2]

[1] Department of Fundamental Neurosciences, University of Lausanne, Lausanne, Switzerland
[2] Department of Biomedical Sciences, University of Lausanne, Lausanne, Switzerland

Type 1 diabetes (T1D) results from the invasion of the islets of Langerhans by autoreactive immune cells that mediate the destruction of insulin-secreting β-cells. Non-coding RNAs (ncRNAs) are crucial regulators of biological functions and are involved in diabetes pathogenesis. Recent reports suggest that the immune cells invading the islets release extracellular vesicles (EVs) delivering a subset of ncRNAs to β-cells and contributing to diabetes development. To identify the ncRNAs transferred from immune cells to β-cells during the initial phases of T1D, the RNA of $CD4^+$ T lymphocytes of NOD Bdc2.5 mice, which develop T1D, was tagged by incorporation of 5-ethynyl uridine (EU). EU-labelled T cells were then injected into NOD-SCID mice to induce T1D development. Two days after injection, when the mice were still normoglycemic, β-cells were isolated by FACS and the EU-tagged RNA transferred from T cell to β-cells was purified on streptavidin beads. Bioinformatic analysis of the RNAs delivered to β-cells revealed an enrichment of small intron-derived RNAs (sinRNAs). An increased level of these sinRNAs in prediabetic NOD mice was confirmed by RT-PCR and their delivery via EVs from activated $CD4^+$ T lymphocytes to β-cells was confirmed in vitro. To assess the functional role of the sinRNAs transferred to β-cells during the prediabetic phases, these ncRNAs were directly overexpressed in dissociated islet cells. These experiments revealed that elevated levels of these sinRNAs promote β-cell apoptosis. Taken together, our data identify a subset of sinRNAs as novel players in the crosstalk between the immune system and insulin-secreting cells, suggesting a potential involvement of this novel class of ncRNAs in T1D pathogenesis.

B7. Beyond the Sequence—Structural Insights Into LncRNA Function in Trained Immunity

Amy Crawford, Alisha Jones

Department of Chemistry, New York University, New York, NY, USA

The molecular mechanisms underlying trained immunity are poorly understood, but increasing evidence implicates long non-coding RNAs (lncRNAs) in its regulation. The Upstream Master LncRNA of the Inflammatory Chemokine Locus (UMLILO) has been shown to regulate epigenetic priming of chemokine genes through interactions with the WD repeat-containing protein 5 (WDR5)-mixed lineage leukemia protein 1 (MLL1) complex. Exposure of monocytes to β-glucan stimulates increased transcription of UMLILO, which is transcribed near the promoters of chemokine genes. UMLILO recruits the MLL1/WDR5 complex to these gene promoters, where the methylation complex marks the genes for downstream transcriptional activation in response to future stimuli. WDR5, a highly conserved scaffold protein, is the key mediator that brings UMLILO and the rest of the methylation complex together, enabling tight control over the expression levels of pro-inflammatory genes. However, the molecular mechanism by which WDR5 brings these components together is still unclear. Using chemical probing experiments and in vitro binding assays, I have investigated the structural and molecular mechanisms behind how UMLILIO interacts with WDR5 to exert transcriptional control. This work serves the overall goal of enhancing our understanding of how lncRNAs regulate gene expression in cells.

B8. Characterizing the Structural Mechanisms of RBM15 With the Long Non-Coding RNA XIST

Bryan Eusse, Alisha Jones

Department of Chemistry, New York University, New York, NY, USA

The long non-coding RNA XIST (X-specific inactive transcript) is responsible for facilitating X-chromosome inactivation in placental female cells as a mechanism for dosage compensation. XIST has been shown to bind over 80 RNA-binding proteins, including the RNA-binding motif protein 15
(RBM15). RBM15 possesses three N-terminal RNA recognition motifs (RRMs), to facilitate binding with RNA, and a C-terminal SPOC domain, to mediate interactions with other proteins. Though RBM15 is known to interact with a ~450 nucleotide region located at the 5' end of XIST, the A-repeats, the
molecular and structural mechanisms by which RBM15 binds this region of the transcript have yet to be elucidated. Here, I describe the work I have carried out to investigate how RBM15 interacts with XIST to gain a more comprehensive understanding of the structure-function relationship that governs XIST's roles in cells.

B9. Defining a Long Non-coding RNA (lncRNA) Signature That Predicts Cholestasis-Associated Chronic Liver Diseases (CLDs)

Amruta Damle-Vartak[1], Elias Klossok[1], Camilla Maggi[2], Patrizia Birner[1], Peter Schirmacher[1], Kai Breuhahn[1]

[1] *Institute of Pathology, Heidelberg University Hospital, Heidelberg, Germany*
[2] *Department of Molecular and Translational Medicine, University of Brescia, Brescia, Italy*

Cholestasis is a clinically relevant complication characterized by the aberrant accumulation of bile acids (BAs) in the liver parenchyma. This permanent challenge causes hepatocellular damage and, eventually, chronic liver diseases (CLDs), such as primary biliary cholangitis and cirrhosis. In clinics, liver enzymes are measured, and imaging techniques focusing on macroscopic abnormalities are utilized to detect cholestasis. However, these methods fail to detect the early phases of cholestasis due to low sensitivity and robustness, poor structural resolution, and limited applicability. We propose that long non-coding RNAs (lncRNAs) are robust and sensitive biomarkers for detecting sub-toxic alterations in hepatocytes exposed to BA-associated cholestasis. To identify a panel of BA-regulated lncRNAs in an in vitro setup, hepatocyte-derived HepG2 cells were treated with sub-toxic concentrations of the BA chenodeoxycholic acid (CDCA, 10 µM) and a farnesoid-X receptor agonist (GW4064, 5 µM), which stimulated the production of BAs. Twelve-hour exposure to CDCA and GW4064 resulted in a robust induction of known cholestasis marker genes, such as organic solute transporter α/β, without affecting cell viability. Quadruplicate samples of total RNA after CDCA and GW4064 treatment for 12 hours were subjected to next-generation sequencing followed by bioinformatic analysis. Pathway enrichment analysis illustrated the regulation of protein-coding genes associated with BA synthesis and transport processes. In addition, we identified a signature of 14 lncRNAs that was consistently and significantly regulated after CDCA and GW4064 administration. A panel of XXX candidate lncRNAs, for which gene-specific primers could be designed, was confirmed by real-time PCR. Other BAs, such as taurocholic acid, equally induce the expression of the candidate lncRNAs. Notably, signature lncRNA gene promoters contained binding sites for transcription factors known to facilitate the cholestatic response in hepatocytes, including the peroxisome proliferator-activated receptor (PPAR) family.
The proposed BA-induced lncRNA signature may represent an easy-to-detect biomarker in the earliest phases of cholestasis. We currently investigate the molecular mechanism of lncRNA regulation in hepatocytes and the diagnostic potential of this signature in serum samples from patients with CLD.

B10. Identification of Long Noncoding RNA (lncRNA) Signatures in Meningioma: Path to Discovery of Potential Biomarkers and Therapeutic Targets?

Ritanksha Joshi[1], Jyotsna Singh[2], Vaishali Suri[2], Ritu Kulshreshtha[1]

[1] Department of Biochemical Engineering and Biotechnology (DBEB), Indian Institute of Technology Delhi (IIT Delhi), New Delhi, India
[2] All India Institute of Medical Sciences, New Delhi, India

Meningioma is one of the most common primary brain tumors, classified into three malignancy grades by the WHO. Surgical resection and radiotherapy are the mainstays of treatment and are often inadequate in tackling higher-grade tumors. Its complex histology, coupled with a lack of reliable genetic and epigenetic markers, impacts the accuracy of grading and prognosis. Distinct interactions between the coding (mRNAs) and noncoding (ncRNAs) transcriptomes have been shown to drive the regulation of gene expression at multiple levels, with a significant impact on disease development, including cancer. While long noncoding RNAs (lncRNAs) are emerging as important entities that influence cancer phenotypes through their interactions with DNA, RNA, and proteins, little is known about the dysregulated lncRNA signature in meningioma. In our study, through transcriptome sequencing of Indian meningioma patient tumor samples (N = 75) inclusive of the three WHO grades (Grade 1, 2, and 3) and those from healthy controls (N = 4), we characterized the expression profiles of lncRNAs, both across individual tumor grades and inter-grades. Several lncRNAs, such as H19 and MIR124-2HG, among others, showed significant dysregulation across different meningioma grades. The expression of some of these was further validated in a cohort of 30 patients and 5 healthy controls by RT-qPCR. The differentially expressed lncRNAs were predicted to impact key pathways such as mTOR signaling, cell cycle, and focal adhesion pathways, previously implicated in meningioma. Kaplan–Meier analysis revealed lncRNAs of prognostic relevance. LncRNA–mRNA expression correlation networks suggested probable functional roles of both well-characterized and novel unannotated lncRNAs. Through lncRNA–miRNA–mRNA ceRNA networks, key associations of lncRNAs with other miRNAs and mRNAs commonly dysregulated in meningioma and their regulatory axes were identified. Overall, our comprehensive analysis of lncRNA signatures in meningioma unveils important ncRNA–mRNA inter-relationships that may impact meningioma pathogenesis. Further functional validation of key lncRNAs may thus reveal potent biomarkers and therapeutic targets.

B11. Restoring Gut Barrier Integrity in Alcohol-Related Liver Disease Through Targeted Modulation of Long Non-coding RNAs

Maria-Emanuela Maxan[1, 2, 3], Antonio Riva[1, 3], Rocio Teresa Martinez-Nunez[3], Onofrio Zanin[3], Jasmohan S. Bajaj[4], Andrew Fagan[4], Peter Stärkel[5], Leila Gobejishvili[6], Luca Urbani[1, 3], Vishal C. Patel[1, 2, 3], Shilpa Chokshi[1, 3]

[1] Roger Williams Institute of Hepatology, Foundation for Liver Research, London, United Kingdom
[2] Institute of Liver Studies, King's College Hospital NHS Foundation Trust, London, United Kingdom
[3] School of Immunology & Microbial Sciences, Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom
[4] Division of Gastroenterology, Hepatology and Nutrition, Virginia Commonwealth University and Richmond VA Medical Center, Richmond, VA, USA
[5] Department of Hepatology and Gastroenterology, St. Luc University Hospital, Catholic University of Louvain, Brussels, Belgium
[6] Department of Physiology, Alcohol Research Center, University of Louisville, Louisville, KY, USA

Alcohol-related liver disease (ArLD) is a major cause of premature deaths with high mortality rates. Gut hyperpermeability and pathological bacterial translocation in ArLD drive systemic inflammation, disease progression and a profound susceptibility to infections, which are the predominant contributors to hepatic decompensation and mortality. Our ability to enhance gut barrier resilience in ArLD and other conditions associated with gut barrier dysfunction such as inflammatory bowel disease (IBD) remains limited due to a paucity of targeted treatments, exacerbated by an inadequate understanding of the underlying pathophysiological mechanisms. LncRNA modulation can improve gut hyperpermeability in experimental models of IBD. RNA interference therapeutics are being trialled in liver disease, suggesting their potential in ArLD.
Colonic tissue from 10 patients with alcohol-related cirrhosis (F=5; age=57.2±1.7; model for end-stage liver disease score=12.7±1.3) and 10 healthy participants (F=1; age=52.2±2.5) was profiled by microarray to identify differentially expressed mRNA and lncRNAs. We selected the top upregulated lncRNAs (based on p-value and log fold-change) with conservation in mice. We confirmed their annotation by GENCODE and their expression in intestinal organoids derived from healthy human control samples and in an intestinal epithelial barrier monolayer model (Caco2/HT29). We also tested them in colonic tissue from an alcohol-fed mouse model (versus control-fed) (n=6/group) and in duodenal biopsies from patients with alcohol use disorder (n=62) versus healthy participants (n=20).
A total of 8,400 coding genes were differentially expressed. Gene set enrichment analysis identified upregulated cell adhesion pathways in colonic tissue from ArLD patients, driven by tight and adherens junction alteration.
RT-qPCR was used to validate 8/13 selected coding genes (p=0.005). A previously uncharacterized lncRNA was also significantly upregulated in patients with alcohol use disorder (AUD) (p0.0001) and a mouse model of ArLD (p=0.0012).

This novel lncRNA may contribute to gut barrier dysfunction in ArLD and its downregulation could improve barrier function and improve clinical outcomes.

B12. Identification and Characterisation of Non-coding RNAs Involved in the Progression of Ductal Carcinoma in Situ (DCIS)

Magdalina Derlipanska[1], Kellie Dean[1], Sudipto Das[2]

[1] School of Biochemistry, University College Cork, Cork, Ireland
[2] School of Pharmacy and Biomolecular Sciences, Royal College of Surgeons in Ireland, Dublin, Ireland

Ductal carcinoma in situ (DCIS) is an early form of breast cancer, the most common cancer in women worldwide. DCIS can develop into potentially fatal invasive disease, but studies show that less than half of all cases progress if untreated[1,2]. Currently, most patients receive surgery, as there is no reliable way to distinguish which cases will become invasive. Our goal is to develop a molecular-based method to differentiate potentially invasive DCIS.

To do this, we identified non-coding ribonucleic acids (ncRNAs) that are involved in the development and progression of DCIS, and current work is focused on discovering their molecular functions.

Through target-capture RNA sequencing, we identified 34 ncRNAs overexpressed in high-grade DCIS cell lines (ETCC-006 and ETCC-010[3]), compared to normal-like breast cells. Next, we validated the specificity of the ncRNAs across a panel of cancer cell lines and confirmed elevated levels in patient DCIS samples (n = 14). From that, we selected two candidates for in-depth analysis.

Maternally expressed gene 3 (MEG3) is a tumour-suppressive long ncRNA that is downregulated in various cancers[4], in contrast to our findings in DCIS. Vault RNA 2-1 (vtRNA2-1) is an RNA that has been implicated in some cancers; however, its mechanism of action is not clear[5].

To determine why ncRNAs might be overexpressed, we looked at epigenetic regulation and found both to be differentially methylated in DCIS cells compared to normal-like cells. Through affinity chromatography, we identified 51 protein interactors of vtRNA2-1, and gene ontology/pathway analysis suggests that it may be involved in processes within the cytoskeleton. We intend to explore this further through immunocytochemistry.

Ongoing knockdown and overexpression experiments aim to characterise the involvement of both ncRNAs in fundamental cancer processes by monitoring the proliferation, migration and anchorage-independent growth of DCIS cells.

By discovering these ncRNAs, which are found in abundance in high-grade DCIS, but not in normal-like or invasive breast cancer cell lines, we believe these RNAs could be used as biomarkers to stratify patients. By elucidating the ncRNAs' cellular functions, we will better understand the processes that contribute to the transition of DCIS to invasive cancer, providing new targets to limit cancer progression.

1. Erbas, B., Provenzano, E., Armes, J. & Gertig, D. The natural history of ductal carcinoma in situ of the breast: a review. Breast Cancer Res. Treat. 97, 135–144 (2005).

2. Maxwell, A., et al. Unresected screen-detected ductal carcinoma in situ: Outcomes of 311 women in the Forget-Me-Not 2 study. Breast 61, 145–155 (2022).
3. Samson, J., Derlipanska, M., Zaheed, O. et al. Molecular and cellular characterization of two patient-derived ductal carcinoma in situ (DCIS) cell lines, ETCC-006 and ETCC-010. BMC Cancer 21, 790 (2021)
4. Zhou, Y., Zhang, X. & Klibanski, A. MEG3 noncoding RNA: a tumor suppressor. J. Mol. Endocrinol. 48, R45–R53 (2012).
5. Fort, R. S. & Duhagon, M. A. Pan-cancer chromatin analysis of the human vtRNA genes uncovers their association with cancer biology. F1000Res. 10, (2021).

B13. Remodelling of Neuronal RNA Metabolism in Prader–Willi Syndrome

Alexandra Lehmann[1, 2], Aleksandra Helwak[1, 2], Diego Oyarzún[1], David Tollervey[1, 2]

[1] *University of Edinburgh, Edinburgh, United Kingdom*
[2] *Wellcome Centre for Cell Biology, Edinburgh, United Kingdom*

Prader–Willi syndrome (PWS) is a neurodevelopmental disorder typically caused by deletions in the paternally imprinted genomic region at 15q11.2-q13 that includes multiple tandem copies of two human C/D box small nucleolar RNAs (snoRNAs), SNORD115 and SNORD116. The deleted regions range in size, the smallest removing only the SNORD116 cluster. These result in hypothalamic gene expression changes, impacting hunger regulation and causing early-onset hyperphagia. Direct targets and mechanisms of both snoRNAs remain unknown; however, they are linked to late neurodevelopmental stages.
We aimed to understand how neuronal RNA metabolism is remodelled during neuronal differentiation in a PWS model. Three questions were investigated: which snoRNAs show neuronal specific expression, how SNORD115 and SNORD116 loss alters the expression of other small RNAs during neurodevelopment, and what features result in snoRNA accumulation during neurodevelopment.
We addressed the first two questions by small RNA sequencing (RNAseq). This identified numerous snoRNAs with expression changes during neuronal differentiation. We compared the wildtype with two paternal-specific heterozygous deletion mutant cell lines lacking either SNORD115 or SNORD116 expression. Cells lacking either snoRNA showed altered levels of several other snoRNAs; the mechanism for this remains to be determined. The multiple tandem repeats in SNORD115 and SNORD116 clusters are related, but non-identical, and differences in relative accumulation were also observed.
Addressing the third question, we tested the role of Fibrillarin-like 1 (FBLL1), a largely uncharacterised but 83% identical paralog of the essential protein Fibrillarin (FBL). We discovered that during neuronal differentiation, FBL expression decreases, whereas FBLL1 expression increases. FBL is one of four proteins that associate with canonical C/D box snoRNAs to form small nucleolar ribonucleoproteins (snoRNPs) involved in RNA methylation and ribosome synthesis. We are currently testing the effects of FBLL1 gene knockout on the transcriptome and neuronal differentiation and will identify protein and RNA binding partners.

B14. BC120: a Novel Processed Alu RNA With Elevated Expression in Ovarian Cancer

Evan Paul Booy, Daniel Gussakovsky, Sean McKenna

Department of Chemistry, University of Manitoba, Winnipeg, Manitoba, Canada

The non-coding RNA BC200 is elevated in human cancers and is implicated in translation regulation as well as in survival and proliferation. Upon BC200 overexpression, we observed correlated expression of a second, smaller RNA species. This RNA is expressed endogenously and exhibits cell-type-dependent variability relative to BC200. Aptamer-tagged expression constructs confirmed that the RNA is a truncated form of BC200 and sequencing revealed a modal length of 120 nt. Thus, we refer to the RNA fragment as BC120. We present a methodology for the accurate and specific detection of BC120 and establish that BC120 is expressed in several normal human tissues and is also elevated in ovarian cancer. BC120 exhibits remarkable stability relative to BC200 and is resistant to knock-down strategies that target the 3' unique sequence of BC200. Combined knock-down of BC200 and BC120 exhibits greater phenotypic impacts than the knock-down of BC200 alone and overexpression of BC120 negatively impacts the translation of a GFP reporter, providing insight into a potential translational regulatory role for this RNA. The presence of a novel, truncated, and stable form of BC200 adds complexity to the investigation of this non-coding RNA that must be considered in future studies of BC200 and other related Alu RNAs.

B15. Plasma microRNAs Reflect the Severity of Acute Traumatic Spinal Cord Injury in Humans

Ruslan Klassen[1, 2], Sarka Benesova[1, 3], Eva Rohlova[1, 4], Ivan Arzhanov[5, 6], Ales Hejcl[5, 7], Lukas Valihrach[1], Nataliya Romanyuk[5]

[1] Laboratory of Gene Expression, Institute of Biotechnology of the Czech Academy of Sciences BIOCEV, Vestec, Czech Republic
[2] Department of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, UCT Prague, Prague, Czech Republic
[3] Department of Informatics and Chemistry, Faculty of Chemistry and Technology, UCT Prague, Prague, Czech Republic
[4] TATAA Biocenter AB, Gothenburg, Sweden
[5] Department of Neuroregeneration, Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic
[6] Department of Neuroscience, 2nd Medical Faculty, Charles University, Prague, Czech Republic
[7] Neurosurgery Department, Masaryk Hospital, J. E. Purkyně University, Ústí nad Labem, Czech Republic

Spinal cord injury (SCI) presents a significant challenge in clinical settings, resulting in permanent disability for over 180,000 individuals annually worldwide. The severity of the initial injury profoundly influences the subsequent secondary response, thereby shaping the selection of appropriate therapeutic interventions. However, current clinical measures for assessing SCI severity rely on functional tests that are not immediately applicable post-injury due to factors such as shock, concurrent injuries, and substance use. To address the need for a novel diagnostic indicator during the acute phase of SCI, we conducted comprehensive microRNA (miRNA) profiling in the blood plasma of 16 healthy individuals and 16 injured individuals. Blood samples were collected from injured patients at various intervals post-injury: 3, 12, and 24 hours, as well as 3 and 7 days. These patients underwent evaluations using ASIA scores and IMR assessments. MiRNA isolation was performed for all samples, with quality assessment based on hemolysis markers and the concentration of specific endogenous miRNAs. Through our miRNA profiling, we identified approximately 100 differentially expressed miRNAs (DEM) whose expression levels correlate with the severity of SCI. Moreover, approximately 30 DEM with log2 Fold Changes over 0.5 or -0.5 and adj P value less than 0.1 will be validated using RT-qPCR. These findings will be integrated with the individual medical histories of patients to establish robust diagnostic criteria for assessing SCI severity during the acute phase. Our data not only enhance the understanding of SCI pathophysiology but also facilitate the development of innovative diagnostic approaches.
Supported by NU21-08-00286 and MEYS – CZ.02.01.01/00/22_008/0004562.

B16. Interaction of SCoV-2 NSP7 or NSP8 Alone with NSP12 Causes the Constriction of the RNA Entry Channel: Implications for Novel RdRp Inhibitor Drug Discovery

Deepa Singh, Tushar Kushwaha, Krishna K Inampudi

Department of Biophysics, All India Institute of Medical Sciences, New Delhi, Delhi, India

The RNA-dependent RNA polymerase (RdRp) of SARS-CoV-2 is vital for replication and transcription, an appealing target for antiviral drugs. Non-structural protein 12 (NSP12) displays RdRp activity along with NSP7 and NSP8, forming the RdRp holoenzyme. Understanding NSP8 and NSP7's role in NSP12 polymerase activity regulation is vital for replication mechanisms and potential treatments. We used bacterial recombination to purify NSP12, NSP8, and NSP7, and this involved studying polymerase activity and RNA binding with diverse templates. NSP12 alone had 20-30% lower polymerase activity than the RdRp holoenzyme, which rose with the addition of NSP7 and NSP8. However, adding NSP8 or NSP7 alone halted RdRp activity, detaching the RNA--primer complex from NSP12. Computational analysis showed that NSP8 constricted the template RNA entry channel more than NSP7, likely causing RNA--template complex separation. NSP7 and NSP8 together boosted NSP12, but hindered it alone. Blocking NSP7/NSP8 binding to NSP12 could hamper viral RNA replication, suggesting antiviral drug development prospects.

B17. The Role of lncRNA-Encoded Micropeptides in the Radioresistance of Head and Neck Cancer

Katarina Vrbnjak[1], Peihua Zhao[1], Ruveyda Dok[2], Raj Nayan Sewduth[1], Sandra Nuyts[2, 3], Anna Sablina[1]

[1] Laboratory for Mechanisms of Cell Transformation, VIB-KU Leuven Center for Cancer Biology, Leuven, Belgium
[2] Laboratory of Experimental Radiotherapy, Department of Oncology, University of Leuven, Leuven, Belgium
[3] UZ Leuven, Radiation Oncology, Leuven, Belgium

lncRNA-encoded micropeptides are an emerging class of small proteins that have until now remained largely undetected. There is increasing evidence that a considerable subset of “non-coding” RNAs contains small open reading frames which are translated and involved in the regulation of various cellular functions. Here, we explored the role of the lncRNA-encoded peptidome in the radiation resistance of head and neck cancer (HNSCC). Radiotherapy is the primary non-surgical treatment modality for most patients with locally advanced HNSCC, but local control and five-year survival rates in patients with advanced-stage disease only remain at about 40%, often due to acquired radioresistance. Currently, there is an unmet need for cancer therapies that selectively sensitize HNSCC tissue to irradiation. We performed ribosome profiling of radiosensitive and radioresistant HNSCC cells, which revealed that nearly one-third of cytoplasmic lncRNAs in HNSCC cells were bound to ribosomes and displayed three-nucleotide periodicity. By employing a machine learning framework to identify lncRNAs with mRNA-like features, we generated a comprehensive map of the lncRNA-encoded HNSCC micropeptidome. To validate the translation capacity of mRNA-like lncRNAs, we generated a pooled library of sgRNAs targeting the stop codon of the putative micropeptides and used a CRISPR-based tagging system to endogenously tag predicted micropeptides with Clover. This approach allowed us to experimentally validate the translational capacity of a large pool of mRNA-like lncRNAs. We next determined a potential association of the identified micropeptides to radiation response and/or radioresistance in HNSCCs by integrating the results of multiple omics datasets and clinical data from radiation-treated HNSCC patients. Functional studies then confirmed the role of the two top-scoring micropeptides in the radioresistance of HNSCCs. Our results not only highlight the functional significance of lncRNA-encoded micropeptides in radiation response but also their therapeutic potential in the radiosensitization of HNSCC.

Academic Open Access Publishing
since 1996