ncRNA 2021

Noncoding RNA World:
From Mechanism to Therapy

21–23 JULY 2021 | ONLINE

Program Booklet

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Noncoding RNA World: From Mechanism to Therapy

Virtual
21-23 July 2021

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Welcome from the Chairs

Dear Colleagues,

It is a great pleasure to announce the upcoming conference “Noncoding RNA World: from Mechanism to Therapy”, to take place virtually (originally planned in Basel (Switzerland)) on 21-23 July 2021.

Vast numbers of new and mysterious ncRNAs continue to be discovered across the tree of life. Although many key questions remain to be answered, nevertheless there are grounds for great optimism for the relevance of ncRNAs to biology and medicine. Powerful new tools are being refined to detect and manipulate ncRNAs with confidence and accuracy, including chemical structure probing, spatially-resolved sequencing, and of course CRISPR-based technologies. At the same time, rapid strides are being made in translating these findings to the clinic, not least thanks to oligonucleotide drugs.

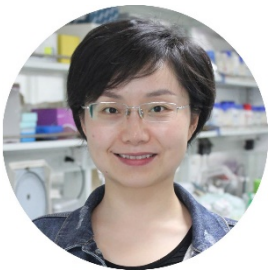
This meeting aims to bring together the community to discuss these latest advances, with sessions on molecular mechanisms, bioinformatics, biotechnology, disease and therapeutics chaired by leading researchers from across the globe.

In the interests of safety given the present COVID-19 situation, we have decided to bring the conference to a fully online format. The conference will proceed on the same dates with the same fantastic Speakers.

Importantly, we aim to select a high number of oral presentations from early-stage researchers, from their submitted abstracts.

We look forward to seeing you this July online!

Prof. Dr. Rory Johnson
Conference Chair



Prof. Dr. Ling-Ling Chen
Conference Chair

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Certificate of Attendance

Upon request, the participants of the event, who have registered on Sciforum, will receive an electronic Certificate of Attendance by email once the event is concluded.

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General Information

Non-Coding RNA is an international, peer-reviewed, open access journal on non-coding RNA research dealing with elucidating the structure, function and biology of regulatory non-coding RNAs. Non-Coding RNA is published quarterly online by MDPI. Cardiolinc is affiliated with Non-Coding RNA.

The CiteScore 2020 for /Non-coding RNA/ is 8.4, ranking 41/325 (Q1) in the category 'Genetics'.

Journal Webpage: https://www.mdpi.com/journal/ncrna

Program

Keynote Speakers

Dr. Piero Carninci
RIKEN Center for Integrative Medical Sciences, Deputy Director

Prof. Dr. Narry Kim
Center for RNA Research, Institute for Basic Science, Korea, School of Biological Sciences, Seoul National University, Korea

Invited Speakers

Prof. Dr. Irene Bozzoni
Sapienza, University of Rome, Rome, Italy

Prof. Dr. Janusz M. Bujnicki
Laboratory of Bioinformatics and Protein Engineering, International Institute of Molecular and Cell Biology, Poland

Prof. Dr. Sven Diederichs
Division of Cancer Research, Dept. of Thoracic Surgery, University of Freiburg, Freiburg, Germany
Division of RNA Biology & Cancer, German Cancer Research Center (DKFZ), Heidelberg, Germany

Prof. Dr. Shinichi Nakagawa
RNA Biology Laboratory, Faculty of Pharmaceutical Sciences, Hokkaido University

Dr. Aiming Ren
Life Sciences Institute, Zhejiang University, China

Dr. Xiaohua Shen
Tsinghua University, China

Prof. Dr. Thomas Thum
Institute of Molecular and Translational Therapeutic Strategies, Medizinische Hochschule Hannover (MHH), Germany

Dr. Igor Ulitsky
Weizmann Institute of Science, Switzerland

Dr. Yue Wan
Associate Director, Genome Institute of Singapore, Singapore

Prof. Dr. Wensheng Wei
Beijing Advanced Innovation Center for Genomics, Peking University, Beijing, China

Program at a Glance

Program Structure			
	Wednesday 21 July 2021	**Thursday 22 July 2021**	**Friday 23 July 2021**
8:00-11:30 CEST | 14:00-17:30 CST Asia	Welcome from the Chairs Keynote Speaker	Session 2. Part 2.	Session 5. Biotechnology of ncRNA
	Break	**Break**	**Break**
	Session 1. Mechanism of ncRNA Processing and Function	Session 3. ncRNAs in Development and Diseases	Keynote Speaker Closing of the Conference & Award Ceremony
	Break	Break	
From 12:00 CEST | From 18:00 CST Asia	Session 2. Structure and Bioinformatics of ncRNAS	Session 4. ncRNA Therapeutics	
	Break	Break	
	Poster Session	Poster Session	

Conference Program

Wednesday 21 July 2021			
CEST	**CST Asia**	**Speaker**	**Title**
8:00	14:00	Welcome from the Chairs	
8:10	14:10	Narry Kim (Keynote Speaker)	A Quantitative Map of Human Primary microRNA Processing
9:00	15:00	Break	
Session 1: Mechanism of ncRNA Processing and Function			
9:15	15:15	Xiaohua Shen (Invited Speaker)	Phase Separation of RNA-Binding Protein Promotes Polymerase Engagement and Transcription *(sciforum-048051)*
9:45	15:45	Marisa Pereira	Lack of TRMT2A, a tRNA Methyltransferase, Leads to m5U54 tRNA Hypomodification and Generation of tRNA-derived small RNAs *(sciforum-045161)*
10:00	16:00	Rajika Arora	Cytoplasmic L1-RNP Condensate Formation: A Novel Mechanism Regulating LINE-1 Retrotransposition in mESCs *(sciforum-045697)*
10:15	16:15	Break	
10:30	16:30	Eloina Corradi	Pre-miRNAs Locally Processed in Axon to Regulate the Development of Neuronal Circuits *(sciforum-044896)*
10:45	16:45	Joanna Sztuba-Solinska	The Epitranscriptomic Landscape of Viral Long non-coding RNA *(sciforum-044051)*
11:00	17:00	Yue Wan (Invited Speaker)	TBD
11:30	17:30	Break	
Session 2: Structure and Bioinformatics of ncRNAs			
12:00	18:00	Zhao Li	Expression Landscape of Human Long non-coding RNAs Across Diverse Biological Contexts *(sciforum-045018)*
12:15	18:15	Toby Hunt	TAGENE: Integrating Long Read Transcriptomic Data into GENCODE lncRNA Annotation *(sciforum-045817)*
13:30	18:30	Janusz M. Bujnicki (Invited Speaker)	Computational Modeling of ncRNA Structure and Interactions *(sciforum-047446)*
13:00	19:00	Break	
13:15	19:15	Poster Session: (a) Mechanism of ncRNA Processing and Function (b) Structure and Bioinformatics of ncRNAs (c) ncRNA Therapeutics (d) Biotechnology of ncRNA	
14:00	20:00	End of day one!	

Thursday 22 July 2021			
CEST	**CST Asia**	**Speaker**	**Title**
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8:00	14:00	Aiming Ren (Invited Speaker)	Demystifying the Architecture and Mechanism of Some Specific RNA Molecules *(sciforum-047835)*
8:30	14:30	Jinbiao Ma	Cryo-EM Structure of Drosophila Dicer-2 and Loqs-PD in Complex with Long dsRNA Substrate *(sciforum-045165)*
8:45	14:45	Barbara Uszczyńska-Ratajczak	Improving Annotation of Mammalian Long non-coding RNA Orthologues in the Zebrafish Genome *(sciforum-045787)*
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9:15	15:15	Shinichi Nakagawa (Invited Speaker)	Regulation of Mouse Development by Retrotransposon-Related Short RNA
9:45	15:45	Isabelle Plaisance	CARMEN-201 Governs Smooth Muscle Cell Commitment in Human Cardiac Precursor Cells Rate *(sciforum-045158)*
10:00	16:00	Anna Gimbel	Despite a Relatively High Expression, TUG1 Is Not Required for Basal Endothelial Cell Function *(sciforum-044893)*
10:15	16:15	Break	
10:30	16:30	Irene Bozzoni (Invited Speaker)	ncRNAs in Motor Neuron Differentiation and Physiopatology *(sciforum-047988)*
11:00	17:00	Louis Delhaye	The Neuroblastoma-Specific lncRNA NESPR Controls Adrenergic Cell Identity and Neuroblastoma Cell Survival *(sciforum-045145)*
11:15	17:15	Lovorka Stojic	Understanding How Long non-coding RNAs Control Genome Stability in Cancer *(sciforum-045088)*
11:30	17:30	Break	
Session 4: ncRNA Therapeutics			
12:00	18:00	Thomas Thum (Invited Speaker)	Preclinical and Clinical Development of non-coding RNA Based Therapeutics in Heart Failure *(sciforum-048050)*
12:30	18:30	Juliet French	The Role of Long non-coding RNAs in the Genetic Predisposition to Breast Cancer *(sciforum-045194)*
12:45	18:45	Zaneta Zarebska	Function and Clinical Significance of circCLIP2 in Glioblastoma *(sciforum-045572)*
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13:15	19:15	Serena Diazzi	The miR-143/145 Cluster Promotes Mesenchymal Phenotypic Plasticity Associated with Resistance to Targeted Therapies in Melanoma *(sciforum-045080)*

13:30	19:30	Carolina Mathias	Analysis of LncRNA with PUMILIO Binding Sites Differentially Expressed in Cancer and Identification of Co-expressed Genes *(sciforum-045682)*
13:45	19:45	Sven Diederichs (Invited Speaker)	Non-coding RNAs & RNA-Dependent Protein Complexes in Cancer *(sciforum-047448)*
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14:30	20:30	Poster session: ncRNAs in Development and Diseases	
15:15	21:15	End of day two!	

Friday 23 July 2021			
CEST	**CST Asia**	**Speaker**	**Title**
Session: Biotechnology of ncRNA			
8:00	14:00	Wensheng Wei (Invited Speaker)	Non-coding RNA & Editing: From High-Throughput Functional Genomics to Therapy *(sciforum-047938)*
8:30	14:30	Siran Zhu	RNA Pull-Down-Confocal Nanoscanning (RP-CONA) Detects Quercetin as pri-miR-7/HuR Interaction Inhibitor that Decreases α-synuclein Levels *(sciforum-044685)*
8:45	14:45	Liang-Zhong Yang	Dynamic Imaging of RNA in Living Cells by CRISPR-Cas13 Systems *(sciforum-045389)*
9:00	15:00	Break	
9:15	15:15	Igor Ulitsky (Invited Speaker)	Functions and Modes of Action of Long non-coding RNAs *(sciforum-047534)*
9:45	15:45	Taisia Polidori	Drug Discovery Pipeline to Screen for RNA Therapeutics in Lung Cancer *(sciforum-045213)*
10:00	16:00	Erika Pereira Zambalde	Lnc-uc.147 and its Potential as Biomarker in Breast Cancer: A New ncRNA from Ultraconserved Regions *(sciforum-045214)*
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10:30	16:30	Piero Carninci (Keynote Speaker)	Long non-coding RNAs: From Interactome to Function (sciforum-047537)
11:20	17:20	Closing of the Conference	
11:30	17:30	End of Conference!	

Poster Session

Wednesday 21 July 2021 - 13h15-14h00 CEST | 19h15-20h00 CST Asia

Session: Mechanism of ncRNA processing and function

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Fien	Gysens	sciforum-044855	Implementing a high-throughput arrayed CRISPRi screening platform to identify functional lncRNAs
Marta	Podralska	sciforum-045109	Identification of irradiation-induced ATM-dependent lncRNAs
Giulia	Buonaiuto	sciforum-045115	From mouse to human: the nuclear lncRNA Charme as a putative candidate for clinical application?
Juan	Unfried	sciforum-045120	LncRNA NIHCOLE promotes DNA damage repair in hepatocellular carcinoma cells
Guang	Xu	sciforum-045148	lncRNA SLERT controls phase separation of FC/DFCs to facilitate Pol I transcription
Kaliya	Georgieva	sciforum-045168	The role of C1QTNF1-AS1 lncRNA in regulation of cell division
Panagiotis	Chouvardas	sciforum-045169	Systematic discovery and characterization of cis-regulatory long non-coding RNAs
Xu-Kai	Ma	sciforum-045196	Altered processing of lncRNAs in stem cells contributes to non-conserved functions
Giulia	Basile	sciforum-045717	Exploring the molecular mechanism of lncRNAs that promote non-small cell lung cancer
Irem	Avcilar Kucukgoze	sciforum-045789	Investigating molecular mechanism of protein arginylation
Itziar	Gonzalez-Moro	sciforum-045806	A T1D-associated lncRNA modulates the type I interferon signalling and antiviral response in pancreatic & cells
Evgenia	Ntini	sciforum-045816	Distinctive features of long non-coding RNA chromatin (dis-)association
Norbert	Polacek	sciforum-045818	Human vault RNA promotes cell proliferation, tumorigenesis and chemoresistance through the lysosome in hepatocellular carcinoma

Session: Structure and bioinformatics of ncRNAs

First name	Last name	Id	Title
Carolina	Mathias	sciforum-045144	Exploring immune related lncRNAs in breast cancer molecular subtypes

Barry	Digby	sciforum-045675	nf-core/circrna: a nextflow workflow for the quantification, microRNA target prediction and differential expression analysis of circular RNAs in RNA-Seq data
Moritz	Schäfer	sciforum-045702	Integrative analysis allows a global and precise identification of functional miRNA target genes
Hugo A.	Guillen-Ramirez	sciforum-045753	ElementaLdb: a user-friendly webserver for exploring and retrieving lncRNA elements
Alexander	Morozov	sciforum-045766	Single cell RNAseq-based transcriptome profiling of mesenchymal stromal cell (MSC) subpopulations with different responses to profibrotic stimuli
Panagiotis	Chouvardas	sciforum-045779	ConnectOR: Sensitive and practical discovery of long non-coding RNA orthologues
Kengo	Sato	sciforum-045804	RNA secondary structure prediction using deep learning with thermodynamic integration
Sunandini	Ramnarayanan	sciforum-045809	Perturbation-agnostic designs for CRISPR pooled screening libraries targeting lncRNAs
Monika	Kwiatkowska	sciforum-045811	Making zebrafish the dark horse in long non-coding RNA research
Sílvia	Carbonell Sala	sciforum-045821	One lncRNA gene catalog to rule them all: expanding GENCODE with the CapTrap-Capture Long Seq method in human and mouse
Dominik	Meise	sciforum-045823	Integrative target prioritisation pipeline (TPP) of CRISPR-Cas pooled screens for improved detection of cancer lncRNA
Ioannis	Kavakiotis	sciforum-045889	DIANA-miTED: A microRNA Tissue Expression Database

Session: ncRNA therapeutics

First name	Last name	Id	Title
Parisa	Aghagolzadeh	sciforum-045039	Single-cell RNA analysis identifies a novel conserved long non-coding RNAs controlling cardiac fibrosis and remodeling
Francesco Paolo	Ruberto	sciforum-045151	Clipper, a novel long non-coding RNA regulating cardiomyocyte metabolism and proliferation
Beatriz	Llamusi	sciforum-045263	Development of an antimiR-based treatment against Myotonic Dystrophy disease
Simona	Brillante	sciforum-045728	miR-181a/b modulation as potential therapeutic approach for AMD treatment
Shifa	Jebari-Benslaiman	sciforum-045775	Functionalization of reconstituted HDL for theranostic in cardiovascular disease
Megan	O'Malley	sciforum-045802	Identification of lncRNAs as potential novel therapeutic targets in Triple-Negative Breast Cancer

Session: Biotechnology of ncRNA

First name	Last name	Id	Title
Andrew	Hutchins	sciforum-044468	RNA N6-Methlyadenosine manipulating via CRISPR/Cas13-associated RNA Epigenetic Editor
Timofei	Zatsepin	sciforum-045212	A novel strategy of pre-mRNA splicing correction by site-specific pre-miRNA recruitment
Liangzhong	Yang	sciforum-045471	Dynamic imaging of RNA in living cells by CRISPR-Cas13 systems
Saleh	Musleh	sciforum-045833	Long-non-coding LncRNA Identification using novel machine learning-based pipeline purely based on sequence information

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Carlos	Camilleri	sciforum-044578	Regeneration of Drosophila wing imaginal discs is impaired upon the loss of lncRNAs
Álvaro	Andrades	sciforum-044902	Analysis of long non-coding RNA driver mutations in lung adenocarcinoma
Keisuke	Hitachi	sciforum-045013	Identification and characterization of novel long non-coding RNAs associated with multiple skeletal muscle atrophy
Francesca Maria	Stefanizzi	sciforum-045055	Circular RNAs to predict outcome after cardiac arrest
Matilde	Calderoni	sciforum-045068	45A ncRNA expression impairs microtubules dynamics affecting drug response in neuroblastoma
Marios	Lange	sciforum-045096	Investigating the oncogenic role of the lncRNA Gracile2 in gastric cancer
Marta	Kasprzyk	sciforum-045116	Functional dissection of IGH enhancers and eRNAs in B-cell lymphoma
Bowen	Zhang	sciforum-045125	7SK acts as an anti-tumor factor in tongue squamous cell carcinoma
Alina	Naveed	sciforum-045136	Investigating the cellular function of the NEAT1_1 lncRNA in castration-resistant prostate cancer
Erika	Perreira Zambaldi	sciforum-045139	Highlighting the RIDL (repeated insertion of long non-coding RNAs) domains derived from transposable elements in Cancer
Branislav	Kura	sciforum-045155	The impact of molecular hydrogen application during heart transplantation: expression of selected miRNAs

Ifeolutembi	Fashina	sciforum-045162	Exploring the role of MicroRNA variation in Multiple Sclerosis pathology
Rodiola	Begolli	sciforum-045164	Identification of lncRNAs that are regulated by lineage-survival transcription factors in gastric cancer
Rodolfo	Chavez-Dominguez	sciforum-045172	LncRNA expression profile in cisplatin-tolerant lung adenocarcinoma cell lines
Maina	Bitar	sciforum-045191	Assessing the Landscape of Long Non-coding Transcripts in Breast Cell Populations
Roberta	Esposito	sciforum-045215	Cancer cells refine their fitness via function-altering mutations in long non-coding RNAs
Eleni	Birli	sciforum-045216	A lncRNA-NF90 interaction confers chemoresistance in liver cancer
Olga	Burenina	sciforum-045260	Characterization of novel cancer-associated long non-coding RNA and its probable murine homolog
Marthe	Chehade	sciforum-045561	The long non-coding RNA Psoriasis Susceptibility-Related RNA Gene Induced by Stress (PRINS) is a biomarker for Invasive Breast Cancer
Julia	Latowska	sciforum-045570	Expression pattern of circRNA in primary and recurrent glioblastoma
Ariadna	Bargiela	sciforum-045676	Contribution of Musashi-2 to muscle dysfunction in myotonic dystrophy by upregulating autophagy through miR-7 biogenesis repression
Anna	Guisado-Corcoll	sciforum-045708	Small ncRNAs derived from different Huntington's disease patients' brain regions induce diverse neuropathological outcomes in wild-type mice
Adrienne	Vancura	sciforum-045722	Cancer LncRNA Census 2 (CLC2): an enhanced resource reveals clinical features of cancer lncRNAs
Zubaida	Hassan	sciforum-045727	Structural impact and mechanism of Epstein-Barr virus encoded RNA-1-induced proliferation in lymphoid cell line
Nour	Khater	sciforum-045736	MALAT-1 and miR-486-5p: A novel lncRNA-miRNA circuit tuning IGF-1R and its downstream JAK/STAT pathway in Breast Cancer
Samantha	Filipów	sciforum-045759	Long non-coding RNAs in the pathogenesis of focal segmental glomerulosclerosis (FSGS)
Francisco	Enguita	sciforum-045764	Examining host lncRNA expression patterns in response to SARS-CoV-2 infection
Shifa	Jebari-Benslaiman	sciforum-045776	Statin-induced epigenetic deregulation contributes to the development of type 2 diabetes mellitus
Ane	Olazagoitia-Garmendia	sciforum-045819	Describing evolutionary convergent lncRNAs involved in development by k-mer and RNA structural homology analyses
Agnieszka	Fiszer	sciforum-045824	Analysis of competing endogenous RNAs network in neurodegenerative disorders caused by triplet repeat expansions
Rafael	Fort	sciforum-045825	Pan-Cancer chromatin analysis of the human vtRNA genes uncovers their association with cancer biology

Cristina	Sisu	sciforum-045828	Exploration of XIST role in lung cancer reveals a potential gene panel diagnostic biomarker and key cancer regulators
Luisa	Santus	sciforum-045829	In vivo single-cell profiling of long non-coding RNAs during Ebola virus infection

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Abstracts

Session 1. Mechanism of ncRNA Processing and Function

Invited Speaker

sciforum-048051: Phase Separation of RNA-Binding Protein Promotes Polymerase Engagement and Transcription

Xiaohua Shen

[1] School of Medicine, Tsinghua University, China

An RNA-involved phase-separation model has been proposed for transcription control. Yet, the molecular links that connect RNA binding to the transcription machinery remain missing. Here we find RNA-binding proteins (RBPs) constitute half of the chromatin proteome in embryonic stem cells (ESCs), and some are colocalized with RNA polymerase (Pol) II at promoters and enhancers. Biochemical analyses of representative RBPs¾such as PSPC1 and PTBP1¾show that the paraspeckle protein PSPC1 not only prevents the RNA-induced premature release of Pol II, and also makes use of RNA as multivalent molecules to promote Pol II engagement and activity, by enhancing the phase separation and subsequent phosphorylation and release of polymerase condensates. In ESCs, auxin-induced acute degradation of PSPC1 leads to genome-wide defects in Pol II phosphorylation and chromatin-binding and nascent transcription. We propose that the synergistic interplay of RBPs and RNA aids in the rate-limiting step of polymerase condensate formation to promote active transcription.

sciforum-045161: Lack of TRMT2A, a tRNA Methyltransferase, Leads to m5U54 tRNA Hypomodification and Generation of tRNA-derived small RNAs

Marisa Pereira[1], Ana Raquel Soares[1], Diana Ribeiro[1], Miguel Pinheiro[1], Margarida Ferreira[1], Stefanie Kellner[2]

[1] iBiMED - Institute of Biomedicine

[2] Institute of Pharmaceutical Chemistry, Goethe-University

Transfer RNAs (tRNA) are subjected to a wide variety of post-transcriptional modifications to ensure their structural stability, correct folding, and efficient protein decoding. More specifically, modifications in the D- and T-loops of tRNAs are essential for tRNA stability. However, the biological role of the tRNA epitranscriptome in the generation of tRNA-derived small RNA fragments (tsRNA), a class of small non-coding RNAs, is still not totally understood.

The 5-methyluridine (m^5U) modification at position 54 of cytosolic tRNAs is one of the most common and conserved tRNA modifications among species. In mammals, this modification is catalyzed by the tRNA methyltransferase TRMT2A. To study the relevance of m^5U54 for tRNA-derived small RNA formation, we have knockdown TRMT2A in human cells and found that m^5U54 hypomodification resulted in ANG overexpression and tRNA cleavage near the anticodon, with accumulation of 5'tRNA-derived stress-induced RNAs (5'tiRNAs), in particular 5'tiRNA-Gly^{GCC} and 5'tiRNA-Glu^{CTC}. Moreover, we found that exposure to oxidative stress induces TRMT2A downregulation, ANG overexpression and tsRNA generation. Our results establish a direct link between tRNA demethylation and ANG-dependent tRFs formation and propose the m^5U54 as a tRNA cleavage protective mark.

Published: Pereira, M.; Ribeiro, D.R.; Pinheiro, M.M.; Ferreira, M.; Kellner, S.; Soares, A.R. m^5U54 tRNA Hypomodification by Lack of TRMT2A Drives the Generation of tRNA-Derived Small RNAs. *Int. J. Mol. Sci.* **2021**, *22*, 2941. https://doi.org/10.3390/ijms22062941.

Funding: This research was funded by the Portuguese Foundation for Science and Technology (FCT), POCH, FEDER, and COMPETE2020, through the grants SFRH/BD/135655/2018, SFRH/BD/146703/2019, POCI-01-0145-FEDER-016630 and POCI-01-0145-FEDER-029843, and by Centro 2020 program, Portugal 2020 and European Regional Development Fund Centro-01-0145-FEDER-000003, and the European Union thought the Horizon 2020 program: H2020-WIDESPREAD-2020-5 ID-952373.

Mechanism of ncRNA Processing and Function

Selected Speaker

sciforum-045697: Cytoplasmic L1-RNP Condensate Formation: A Novel Mechanism Regulating LINE-1 Retrotransposition in mESCs

Rajika Arora, Maxime Bodak, Jennifer Steiner, Constance Ciaudo

[1] Department of Biology, Institute of Molecular Health Sciences, RNAi and Genome Integrity, ETH Zürich, Switzerland

Mechanisms regulating LINE-1 transposition include DNA methylation in somatic cells and the Piwi-interacting RNA (piRNA) pathway in the germline. During pre-implantation stages of mouse embryonic development, however, both pathways are inactivated, leading to a critical window necessitating alternate means of L1 regulation. We previously demonstrated that DICER is essential for the control of L1 expression and retrotransposition in mESCs. Nevertheless, the fold increase in L1 expression levels did not translate into a similarly increased L1 transposition rate, suggesting an extra layer of regulation. We now report that L1 RNA and proteins accumulate as L1 Ribonucleoprotein (RNP) condensates in the cytoplasm of *Dicer*_KO mESCs, leading us to hypothesize the existence of a novel cytoplasmic sequestration mechanism preventing L1 retrotransposition. Unlike in cancer cells, these L1-RNPs do not appear to be stress granules or autophagosomes. To understand the mechanism of L1-RNPs formation, we investigated the role of RNA helicase MOV10, a known interactor of both ORF1 and ORF2 L1 proteins. In *Dicer*_KO cells, MOV10 is upregulated via a direct regulation by miRNAs and co-localizes with L1-RNP complexes in the cytoplasm. Additionally, we engineered cells overexpressing L1 RNAs and proteins by using a dCas9 fused with VP160, targeting the 5'UTR of L1 elements ($L1^{UP}$). We did not observe any L1-RNPs formation in $L1^{UP}$ cells but observed that the two-fold increase in L1 transcript level led to an increase in retrotransposition compared to control cells. Moreover, overexpressing MOV10 protein along with L1 mRNAs was sufficient to induce de novo condensate formation in $L1^{UP}$ cells. We are currently testing if this forced L1-RNPs formation is able to reduce the rate of retrotransposition in $L1^{UP}$ cells. In a parallel approach, we showed that downregulating *Mov10* in *Dicer_KO cells* enhances the rate of retrotransposition. Our data are suggestive of a novel mechanism of MOV10-mediated L1-RNP sequestration in the regulation of L1 retrotransposition in mESCs.

Selected Speaker

sciforum-044896: Pre-miRNAs Locally Processed in Axons to Regulate the Development of Neuronal Circuits

Eloina Corradi[1], Irene Dalla Costa[1], Antoneta Gavoci[1], Michela Roccuzzo[1], Tegan Otto[1], Eleonora Oliani[1], Stephanie Strohbuecker[1], Donatella Valdembri[2], Guido Serini[2], Cei Abreu-Goodger[3], Marie-Laure Baudet[1]

[1] Department of Cellular, Computational and Integrative Biology - CIBIO, University of Trento

[2] Department of Oncology, University of Torino School of Medicine, Candiolo, Italy

[3] Unidad de Genómica Avanzada (Langebio), Irapuato, Mexico

mRNA localization is a highly conserved mechanism that confers functional autonomy to subcellular compartments in virtually all animal cells. As with mRNAs, non-coding RNAs (ncRNAs) show preferential enrichment in specific subcellular locations. However, the mechanisms of ncRNA transport and the local biological functions of ncRNAs are still elusive. We investigated both aspects using the elongating axon and its tip, the growth cone, as models.

Short ncRNA trafficking is vastly unexplored, mostly due to the lack of appropriate tools to detect endogenous molecules. Therefore, we developed a new approach to study ncRNA transport dynamics. We adapted the use of molecular beacon (MB) technology to specifically track pre-miRNAs through live imaging. Using an MB strategy, we reveal that specific endogenous precursor microRNAs (pre-miRNAs) are actively trafficked along axons by hitchhiking primarily on late endosomes/lysosomes.

Intriguingly, Dicer is located at the leading tip of the axon, raising the possibility that inactive pre-miRNAs trafficked to the growth cone mature locally to exert their function. We found that pre-miRNAs were indeed processed within axons into newly generated miRNAs (NGmiRNAs) upon exposure to a specific environmental signal. Single axon FRAP and puro-PLA imaging revealed that one of these NGmiRNAs, in turn, locally silences the basal translation of tubulin beta 3 class III (TUBB3), but not amyloid-beta precursor protein (APP). This local pre-miRNA processing was critical for axon development in response to external stimulus ex vivo and in vivo.

Overall, our results uncover a novel endosome-based mode of ncRNA transport from one cytosolic compartment to another within eukaryotic cells. They also unravel a novel ncRNA-based signaling pathway whereby newly generated miRNAs (NGmiRNAs) regulate local protein synthesis on demand to promote neuronal circuit formation.

Mechanism of ncRNA Processing and Function

Selected Speaker

sciforum-044051: The Epitranscriptomic Landscape of Viral Long non-coding RNA

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The discovery of processes that invoke covalent RNA modification, referred to as the epitranscriptome, opened up an exciting field of study that is now recognized as the next frontier in RNA biology. We leverage the study of the polyadenylated nuclear (PAN) RNA, a critical modulator of Kaposi's sarcoma-associated herpesvirus replication, to address how the N6-methyladenosine (m^6A) affects PAN RNA biology and function. We have resolved the m^6A landscape on PAN RNA expressed during latent and lytic stages of KSHV replication by applying the second- and third-(nanopore) generation RNA sequencing analyses. We have shown that PAN is the most extensively modified at the late lytic stages of KSHV replication, in contrast to most of its epitranscriptome. Using a newly developed method, termed selenium-modified deoxythymidine triphosphates (SedTTP)-RT and ligation-assisted PCR analysis of m^6A (SLAP), we have dissected the fraction of modification at each site, showing that two residues near the expression and nuclear retention element (ENE) involved in the PAN triple helix formation are modified at the highest frequency. Proteomic approaches, including RNA antisense purification with mass spectrometry and immunoblotting, have allowed the identification of the specific readers, writers, and erasers that facilitate the modification of the PAN and m^6A phenotypic effects. By applying the selective 2'-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP) in KSHV-positive cells with ablated expression of these enzymes, we have shown that m^6A influences not only the local but also global secondary structure of PAN RNA. To our knowledge, this work represents the first comprehensive overview of the dynamic interplay that takes place between the cellular epitranscriptomic machinery and a specific viral RNA in the context of infected cells.

Abstracts

Session 2. Structure and Bioinformatics of ncRNAs

Invited Speaker

sciforum-047446: Computational Modeling of ncRNA Structure and Interactions

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Understanding how RNA molecules carry out their biological functions requires detailed knowledge of RNA structure. Experimental determination of high-resolution RNA structures is both costly and difficult, which is why the majority of known RNAs remain structurally uncharacterized. Given the lack of experimentally determined high-resolution structures of RNA molecules, theoretical models of RNA 3D structure can help to understand and guide the identification of functionally important regions and can provide a starting point for higher-resolution descriptions. However, "purely theoretical" structure predictions usually suffer from limited accuracy, and furthermore, the deviation between the theoretical model and the "true" (unknown) structure is very difficult to assess in the absence of additional data. Fortunately, although complete high-resolution structures of RNA molecules are scarce, a variety of heterogeneous information is often available from biochemical and biophysical data. Computational techniques can be used to integrate the available data and guide structural elucidation for RNA molecules alone and for their complexes, revealing the molecular basis of RNA interactions with other molecules. I will present strategies for computational modeling of the 3D structure and interactions of RNA, with the possibility of using restraints and constraints from different types of experiments.

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Invited Speaker

sciforum-047835: Demystifying the Architecture and Mechanism of Some Specific RNA Molecules

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Structure-based investigation into the working mechanism of some specific RNA molecules provides essential information to demystify the relationship between architecture and function of RNA molecules, therefore help to improve the property of RNA molecules in application. We have been focusing on three types of RNA molecules including riboswitches, ribozymes and fluorescent RNAs. Pepper fluorescent RNAs are recently reported bright, stable and multi-color fluorogenic aptamer tag that enable imaging of diverse RNAs in live cells. To investigate the molecular basis of the superior properties of Pepper, we determined the structures of complexes of Pepper aptamer bound with its cognate HBC or HBC-like fluorophores at high resolution by X-ray crystallography. The Pepper aptamer folds in a monomeric non-G-quadruplex tuning-fork like architecture composed of a helix and one protruded junction region. The near-planar fluorophore molecule intercalates in the middle of the structure and is sandwiched between one non-G-quadruplex base quadruple and one non-canonical G-U wobble base helical pair. Further, structure-based mutational analysis is evaluated by *in vitro* and live-cell fluorogenic detection. Taken together, our research provides a thorough basis for structure-driven improvement and application of Pepper aptamer in RNA visualization.

Selected Speaker

sciforum-045018: Expression Landscape of Human long non-coding RNAs across Diverse Biological Contexts

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The expression profiling and characterization of long non-coding RNAs (lncRNAs), based on large-scale transcriptome data analysis across diverse biological contexts, provide fundamental insights into their molecular signatures and biological significances. However, it remains unexplored to build an expression landscape of human lncRNAs, particularly by considering a wide range of biological contexts. To systematically characterize the expression landscape of the more than 100,000 lncRNA genes in humans, we performed an integrative analysis of 1977 samples across nine biological contexts, including normal tissue/cell line, cancer tissue/cell line, subcellular localization, exosome, cell differentiation, preimplantation embryo, organ development, circadian rhythm, and virus infection. We found that 90.8% of these lncRNAs are reliably expressed and 3318 genes are ubiquitously expressed in all the nine biological contexts. A total of 34% of the reliable lncRNAs could be highly expressed, albeit mostly in only one biological context. To explore lncRNAs' biological roles, we identified a total of 25,191 featured lncRNA genes, which are specifically expressed in a certain cell line/tissue, differentially expressed in the context of cancer or virus infection, enriched in a subcellular compartment, dynamically expressed during cell differentiation or embryo/organ development, or periodically expressed with circadian rhythm. Moreover, we predicted the potential interacting partners for the featured lncRNA genes based on a co-expression network to enable in-depth exploration. Taken together, we, for the first time, provide an expression landscape of human 101,293 lncRNAs across nine biological contexts, which would be of great significance to deepen our understanding of lncRNAs and facilitate the functional studies. Finally, all these expression profiles as well as their associated data are publicly available in LncExpDB at https://bigd.big.ac.cn/lncexpdb.

sciforum-045817: TAGENE: Integrating Long-Read Transcriptomic Data into GENCODE lncRNA Annotation

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The GENCODE consortium produces the reference annotation used in the Ensembl and UCSC genome browsers for all human and mouse protein-coding genes, pseudogenes, long non-coding RNAs, and small RNAs. Accurate gene annotation is of fundamental importance for genome biology and clinical genomics; annotation that is incorrect or incomplete impacts downstream analysis and introduces potentially significant errors. Nonetheless, GENCODE remains a work in progress: the geneset contains only a fraction of the introns that have been identified in large-scale RNAseq reprocessing projects; in particular, these suggest that our annotation is missing many novel lncRNA loci and transcripts. It is hoped that integrating the ever-expanding datasets offered by long-read sequencing technologies such as PacBio ISOseq and ONT will enable us to greatly enhance and improve the lncRNA annotation within GENCODE.

Here, we present TAGENE, an annotation workflow that incorporates long-read sequences into the GENCODE genesets. It starts with alignments of long-read transcriptomic data such as PacBio Isoseq provided by the GENCODE consortium. Alignments are quality filtered and merged, introns confirmed with RNAseq data and the resulting transcripts clustered into models and filtered for novelty. In order to achieve consistency in the quality of the TAGENE annotation and existing

manual GENCODE annotation, a QC assessment of the output is performed to ensure only transcripts that pass manual scrutiny are added to the geneset. The first novel lncRNA loci/transcripts generated by this pipeline were added to GENCODE 31 and new iterations of the pipeline will be run for future GENCODE releases as new sequence data becomes available. A total of 17,641 novel transcripts were added by the first TAGENE run (and a further 3134 existing transcripts extended). Future iterations are expected to add significantly more splicing variation and greatly enhance the GENCODE lncRNA catalogue.

Selected Speaker

sciforum-045165: Cryo-EM Structure of Drosophila Dicer-2 and Loqs-PD in Complex with Long dsRNA Substrate

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Drosophila Dicer-2 (Dcr-2) processes long double-stranded RNAs (dsRNAs) with its cofactor Loqs-PD and generates mature siRNA duplex. The molecular mechanism of recognition and processing of dsRNA by Dcr-2 is not well understood. Here, we report the cryo-electron microscopy structures of Dcr2-Loqs-PD in apo state and in complex with dsRNA substrate at global resolutions of 3.3Å and 3.6Å, respectively, elucidating a significant conformational change of the helicase domain induced by dsRNA binding that makes the whole complex transform from the original self-inhibition state to a pre-cleavage state, in which the Duf283 domain plays a pivotal role by serving as the bridge domain between helicase and RNase III domains. In addition, both RNase III domains of Dcr-2 have an insertion domain in the same region that binds to the PAZ platform region and helicase domain to form a head-to-base homodimer in the absence of ATP. Our study sheds light on the mechanism of RNA-binding-induced conformational change of Dcr2-Loqs-PD complex, which is the first step of RNA processing by Dcr-2, and also provides a possible inhibitory state Dcr-2-Loqs-PD-dsRNA complex while lacking ATP.

sciforum-045787: Improving Annotation of Mammalian Long noncoding RNA Orthologues in the Zebrafish Genome

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Zebrafish (*Danio rerio*) is a powerful vertebrate system for modeling gene function and studying human diseases. The basic body plan for all vertebrates is conserved, and zebrafish have the same major organs and tissues as humans. Interestingly, the transcriptional network controlling organogenesis is also well preserved across vertebrates. Thus, zebrafish holds promise as an excellent model for studying lncRNA-related human disease. It has become even more attractive, after it was established that a number of its long noncoding RNAs (lncRNA) are conserved with those of human. However, zebrafish lncRNA annotations lag far behind those for human or mouse and this discrepancy severely hampers functional characterization of their mammalian counterparts.

We will address this issue by detecting human and mouse lncRNA orthologues in the zebrafish genome using a synteny-based approach. Next, we will improve their annotation by employing capture long seq (CLS), which combines targeted RNA capture with third-generation long-read sequencing. Full-length, manual quality annotation of mammalian orthologues in biomedically relevant adult zebrafish tissues will definitely facilitate their functional characterization. This also has direct relevance to human disease, as many lncRNAs have been linked to pathological processes in our cells.

Abstracts

Session 3. ncRNAs in Development and Diseases

Invited Speaker

sciforum-047988: ncRNAs in Motor Neuron Differentiation and Physiopatology

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Non-coding RNAs (ncRNAs) are emerging as essential players in nervous system physiopathology. In neurodegenerative disorders such as Amyotrophic Lateral Sclerosis (ALS) disease-related mutations point to an important role of abnormal RNA metabolism. Our aim is to study the function of ncRNAs in motor neurons (MN) physiology and to discover their link to FUS-linked ALS pathology.

The transition from dividing progenitors into post-mitotic motor neurons is orchestrated by a series of events mainly studied so far at the transcriptional level by analyzing the activity of specific programming transcription factors. In our work, we identified several ncRNAs which control such transition at the post-transcriptional level.

Taking advantage of *in vitro* MN differentiation systems of HB9::GFP mESCs and hiPSCs, we have been able to identify several lncRNA and circRNA species which are specifically expressed in mature MNs and whose expression is altered in MNs carrying ALS-associated mutations of the FUS protein. RNAi screening has allowed to identify several species which play important functions in MN differentiation and activity.

Through the use of *in vitro and in vivo* CRISPR/Cas9 mutants we have identified several regulatory circuitries through which ncRNAs control MN activity and we have analyzed how the alteration of their functioning affects MN differentiation and survival.

Selected Speaker

sciforum-045158: CARMEN-201 Governs Smooth Muscle Cell Commitment in Human Cardiac Precursor Cells

Isabelle Plaisance[1], Panagiotis Chouvardas[2], Yuliangzi Sun[3], Mohamed Nemir[1], Parisa Aghagolzadeh[1], Farhang Aminfar[1], Sophie Shen[3], Rory Johnson[2], Nathan Palpant[3], Thierry Pedrazzini[1]

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Developmental lncRNAs emerge as key regulators of specification in the cardiovascular system. In this context, alternative transcription start sites and terminations, as well as alternative splicing expand the diversity of lncRNAs. However, the functional importance of spliced variants has not been evaluated. Thus, we take advantage of primary cardiac precursor cells (CPC) isolated from the fetal and adult human heart to investigate the relevance of lncRNA isoforms during cardiovascular lineage commitment. The locus encoding the enhancer-associated lncRNA *CARMN* plays crucial roles in dictating specification into the cardiomyocyte (CM) and the smooth muscle cell (SMC) lineage. We use, therefore, an integrated approach to dissect the molecular mechanisms mediated by specific *CARMN* isoforms. In particular, we identify *CARMN-201* as crucial for SMC specification. Mechanistically, *CARMN-201* associates with the transcriptional repressor REST (RE1 Silencing Transcription Factor aka Neuron Restrictive Silencer Factor), targets the repressor to cardiogenic loci, namely *IRX1*, *IRX5*, *SFRP1,* and *ISL1*, and blocks the emergence of a CM gene program. Binding to REST depends on the inclusion of the second exon in the *CARMN-201* transcript. This exon contains itself a short interspersed nuclear element of the MIR family of transposable element indispensable for the capacity of the isoform to determine the SMC fate. Furthermore, two other *CARMN-201* protein partners have been identified, i.e., the RNA methyltransferase NOP2/Sun RNA Methyltransferase 6 (NSUN6), and the Replication Protein A1 (RPA1), a protein implicated in stabilization of single-stranded DNA, suggesting *CARMN-201* represses loci via forming RNA:DNA triplex at target promoters. In contrast, in CPCs adopting a CM fate, *CARMN-201* is not expressed, allowing cardiac factors to be expressed and enabling cardiogenic specification and differentiation. Altogether, these data support lncRNA isoform-specific actions, and exemplify the complexity of lncRNA-mediated regulatory mechanisms.

Selected Speaker

Selected Speaker

sciforum-044893: Despite a Relatively High Expression, TUG1 Is Not Required for Basal Endothelial Cell Function

Anna Theresa Gimbel[1], Kosta Theodorou[1], Susanne Koziarek[1], Laura Stanicek[2], Stefanie Dimmeler[1], Reinier A. Boon[1, 2]

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Background and purpose

Cardiovascular diseases (CVD) are the leading cause of death worldwide, with ageing as a major risk factor. In the last decade, long non-coding RNAs (lncRNA) have been shown to play a role in the pathophysiology of CVDs. The evolutionary conserved gene *Taurine Upregulated Gene 1* (*TUG1*) is a ubiquitously expressed lncRNA, and is one of the highest expressed lncRNAs in endothelial cells (EC). Interestingly, *TUG1* was recently described to comprise a small open reading frame that is likely translated into protein. However, the role of *TUG1* in the cardiovascular system remains largely unknown. The objective of this study is to unravel the function of *TUG1* in ECs.

Methods and results

TUG1 expression was decreased by ageing in carotid ECs from 18 months old mice (0.29 fold, p 0.05) and by in vitro ageing of human ECs (0.81 fold, p 0.05). Intriguingly, GapmeR-mediated silencing of *TUG1* (0.15 fold, p 0.05) did not affect basal EC proliferation, metabolism, apoptosis, barrier function, or migration. In contrast, *TUG1* knockdown slightly decreased cumulative sprout length upon vascular endothelial growth factor A stimulation in an angiogenic sprouting assay in vitro (0.79 fold, p 0.05), which could be reproduced by siRNA-mediated *TUG1* knockdown in human umbilical Vein ECs (HUVECs; 0.75 fold, p 0.05). Moreover, RNA sequencing of GapmeR-mediated silencing of HUVECs revealed only minor changes in gene expression compared to a control, despite a robust reduction in *TUG1* levels.

Conclusion

In summary, *TUG1* is a highly expressed transcript and downregulated with advanced age in human and mouse ECs. Functionally, *TUG1* silencing in combination with a proangiogenic stimulus slightly attenuates angiogenesis in vitro. In contrast, *TUG1* is not required for basal EC functions including basal cell turnover, metabolism, barrier function, and migration.

ncRNAs in Development and Diseases

Selected Speaker

Selected Speaker

sciforum-045145: The Neuroblastoma-Specific lncRNA NESPR Controls Adrenergic Cell Identity and Neuroblastoma Cell Survival

Louis Delhaye[1, 2, 3, 4], Eric James de Bony[1, 3, 4], Dries Rombaut[1, 3, 4], Lim Fang Qi[5, 6], Bieke Decaesteker[1, 3, 4], Amber Louwagie[1, 3, 4], Eva D'haene[1, 4], Celine Everaert[1, 4], Roberto Ciaccio[7], Nurten Yigit[1, 3, 4], Delphine De Sutter[1, 2], Giorgio Milazzo[7], Yves Heremans[8], Matthias Fischer[9, 10], Thorsten Simon[11], Johan Van Nes[12], Rogier Versteeg[12], Björn Menten[1, 4], Giovanni Perini[7], Jo Vandesompele[1, 3, 4], Steve Lefever[1, 3, 4], Frank Speleman[1, 3, 4], Takaomi Sanda[5, 6], Sven Eyckerman[1, 2, 3], Pieter Mestdagh[1, 3, 4]

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Neuroblastoma is a childhood cancer derived from the sympathoadrenal lineage that is defined by an adrenergic and mesenchymal cell identity. Through a pan-cancer lncRNA expression analysis, we identified the neuroblastoma-specific lncRNA NESPR. *NESPR* is located in the super enhancer of the adrenergic transcription factor PHOX2B. NESPR is abundantly expressed, efficiently spliced, highly conserved in mammals, and correlates with poor patient survival. Notably, NESPR expression is restricted to adrenergic neuroblastoma cells and downregulated upon de-differentiation to the mesenchymal state. Knockdown of the nuclear NESPR fraction significantly decreases cellular

survival and induces an apoptotic program. RNA-sequencing upon NESPR knockdown revealed a significant reduction in the expression of PHOX2B-, as well as other core transcription factors of the adrenergic cell identity. 4C-sequencing demonstrated that the *NESPR* and *PHOX2B* loci form an insulated neighborhood in adrenergic but not mesenchymal cells, suggesting NESPR regulates PHOX2B *in cis*. However, RNA-sequencing data from NESPR and PHOX2B knockdown experiments revealed a PHOX2B-independent function for NESPR. ChIRP-sequencing revealed hundreds of NESPR binding sites throughout the genome and HOMER motif enrichment analysis of these binding sites highlighted possible co-binding with other adrenergic core transcription factors, such as GATA3 and ISL1. Interestingly, a *de novo* motif enrichment analysis revealed several motifs that map to a distinct G-rich region in the NESPR transcript. We hypothesize that NESPR is binding the genome through this region via R-loop formation. This hypothesis is further strengthened by the identification of R-loop-associated proteins in NESPR ChIRP-MS experiments. Among the NESPR target genes, we prioritized ATF4, a master regulator of amino acid metabolism and stress response pathways. NESPR binds the ATF4 promoter region and NESPR knockdown reduces expression levels of ATF4 and its downstream targets. We hypothesize that NESPR maintains ATF4 levels in adrenergic neuroblastoma cells to cope with stress induced by overexpression of the MYCN oncogene. Experiments are ongoing to validate further our hypothesis with respect to the molecular mechanism and function of NESPR in adrenergic neuroblastoma cells.

Selected Speaker

sciforum-045088: Understanding How Long non-coding RNAs Control Genome Stability in Cancer

Lovorka Stojic

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Genome stability is paramount to cellular homeostasis throughout the human lifespan. Cells have developed several surveillance mechanisms to protect the genome from mutations and ensure faithful duplication and transmission of the genetic material. Defects in any of these mechanisms lead to genome instability, which drives cancer evolution and contributes to tumour heterogeneity, drug resistance, and poor prognosis. Protein-mediated mechanisms controlling genome stability are well described; however, the biological and regulatory function of RNA-based mechanisms in this context, and in particular, the contribution of long noncoding RNAs (lncRNAs), is largely unknown. We have recently identified novel lncRNAs linked to chromosome mis-segregation, a process common to different types of cancer. I will focus on two nuclear-localised lncRNAs whose expression is altered in cancer, and highlight mechanisms through which these lncRNAs safeguard genome integrity and their relevance to cancer.

Abstracts

Session 4. ncRNA Therapeutics

sciforum-048050: Preclinical and Clinical Development of non-coding RNA Based Therapeutics in Heart Failure

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Noncoding RNAs hold great promise as next generation targets in a broad range of diseases including cardiovascular disease.

We recently moved several noncoding RNA inhibitors forward to clinical trials. In particular, cardiac microRNA-132-3p (miR-132) levels increased in patients with heart failure (HF) and mechanistically drive cardiac remodelling processes. CDR132L, a specific antisense oligonucleotide, is a first-in-class miR-132 inhibitor that attenuates and even reverses HF in preclinical models. We performed a Phase 1b study to assess safety, pharmacokinetics, target engagement, and exploratory pharmacodynamic effects of CDR132L in patients on standard-of-care therapy for chronic ischemic HF in a randomized, placebo-controlled, double-blind, dose-escalation study (NCT04045405). Twenty-eight patients were randomized to receive CDR132L (0.32, 1, 3, and 10 mg/kg body weight) or placebo (0.9% saline) in two intravenous infusions, 4 weeks apart in four cohorts of seven (five verum and two placebo) patients each. CDR132L was safe and well tolerated, without apparent dose-limiting toxicity. A pharmacokinetic/pharmacodynamic dose modelling approach suggested an effective dose level at ≥1 mg/kg CDR132L. CDR132L treatment resulted in a dose-dependent, sustained miR-132 reduction. Patients given CDR132L ≥1 mg/kg displayed a median 23.3% NT-proBNP reduction, vs. a 0.9% median increase in the control group. CDR132L treatment induced significant QRS narrowing and encouraging positive trends for relevant cardiac fibrosis biomarkers. We performed the word-wide first clinical trial of an antisense drug in HF patients. CDR132L was safe and well tolerated, confirmed linear plasma pharmacokinetics with no signs of accumulation, and suggests cardiac functional improvements. Although this study is limited by the small patient numbers, the indicative efficacy of this drug is very encouraging justifying additional clinical studies to confirm the beneficial CDR132L pharmacodynamic effects for the treatment of HF.

sciforum-047448: Non-coding RNAs & RNA-dependent Protein Complexes in Cancer

Sven Diederichs

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Non-coding RNAs and their interacting proteins play important roles in health and disease. Here, I review our research on the role of long non-coding RNAs (lncRNAs) and RNA-protein complexes in cancer. On the one hand, we discovered important lncRNAs like MALAT1, CASC9 and lincNMR and characterized their role in lung and liver cancer. On the other hand, we proposed the concept of RNA-dependent proteins and developed the R-DeeP assay to identify RNA-dependent proteins proteome-wide.

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sciforum-045194: The Role of Long noncoding RNAs in the Genetic Predisposition to Breast Cancer.

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Genome wide association studies (GWAS) have identified >200 loci associated with breast cancer. Genetic fine-mapping of these regions has shown that the putative causative variants at each GWAS locus fall outside protein-coding regions. Recent analysis has shown that many breast cancer risk variants fall in regulatory elements such as transcriptional enhancers, however the contribution of long non-coding RNAs (lncRNA) is unclear. Using targeted RNA sequencing combined with de novo transcript assembly we systematically annotated multi-exonic lncRNAs transcribed from 1.5Mb intervals surrounding breast cancer GWAS loci and assessed their contribution to breast cancer risk. We identified >4000 lncRNA genes and show their expression distinguishes normal breast tissue from tumors and different breast cancer subtypes. The breast cancer risk variants were significantly enriched in lncRNA exons but not the promoters or introns. Expression quantitative trait loci (eQTL) analyses, conducted in a cohort of breast tumours, identified several lncRNAs whose expression is associated with breast cancer risk variants. Approximately half of these variants fell within lncRNA exons suggesting that the variants may alter the stability of transcripts, the remainder were located more distally. Using Capture Hi-C data, we provide evidence that these risk variants fall in distal enhancers that regulate lncRNA genes through long-range chromatin interactions. For one of these lncRNAs, that we named KLnc, we show that the risk-associated variant reduces Klnc half-life and is associated with reduced KLnc expression. Klnc is an intron-derived lncRNA that is induced with estrogen. Overexpression of Klnc using both CRISPR based activation or lentiviral over expression reduces cell proliferation and promotes apoptosis consistent with a role for Klnc in breast cancer development.

sciforum-045572: Function and Clinical Significance of circCLIP2 in Glioblastoma

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Gliomas have always been one of the greatest challenges for medicine due to their location, significantly impeding the use of conventional methods of diagnosis and treatment. One of the biggest obstacles is intratumoral heterogeneity, characterized by distinct genetic alterations that occur in individual tumors, and allows for the classification of these tumors into various molecular subtypes. Nowadays, much effort has been made to explore the way in which malignancy develops and identify key players and mechanisms. Recent studies indicate a significant role of the new class of regulatory RNAs—circular RNAs (circRNAs)—in many biological processes, including tumorigenesis. In order to establish their role in gliomagenesis and glioblastoma progression, we performed RNA sequencing of primary and recurrent glioblastoma tissue and glioblastoma stem cells (GSC), which revealed the disruption of several circRNAs in tumor and GSC samples. Moreover, with these results, we provide circRNAs expression patterns of specific subtypes of GBM, as a step forward to personalized treatment of GBM patients. One of the differentially expressed circRNAs—

circCLIP2—derived from the *CLIP2* gene, emerges more often in the mesenchymal subtype of GBM, which is considered as the one with the poorest patient prognosis. Our results indicated decreased migration and proliferation potential of glioma cells after circCLIP2 knockdown, which is significantly enhanced in combination with the anti-proliferative agent temozolomide. We also analyzed the potential interaction of circCLIP2 with miRNAs and RBPs, finding a potential RBP that might be involved in circCLIP2 biogenesis. As a future perspective, analysis of circCLIP2 will be performed utilizing patient-derived neurospheres and organoids.

sciforum-045080: The miR-143/145 Cluster Promotes Mesenchymal Phenotypic Plasticity Associated with Resistance to Targeted Therapies in Melanoma

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Treatment of BRAF-mutant metastatic melanoma with MAPK pathway inhibitors (MAPKi) has revolutionized the management of patients with advanced-stage disease, but therapeutic resistance inevitably occurs. Due to its high plasticity, melanoma cells are capable of developing non-genetic resistance towards MAPK-targeted therapy that is frequently associated with dedifferentiation into a mesenchymal invasive phenotype displaying fibrotic markers and extracellular matrix (ECM) remodeling activities. However, the molecular mechanisms that regulate this resistant phenotype remain poorly defined. In this study, an expression screening of a pool of miRNAs defined as "FibromiRs" revealed high expression of the miR-143/145 cluster in dedifferentiated mesenchymal MAPKi-resistant melanoma cells. In addition, inhibition of the $BRAF^{V600}$ pathway in vitro and in the xenograft model triggered cluster expression along with ECM reprogramming. Remarkably, the administration of the anti-fibrotic drug Nintedanib in combination with MAPKi reverted ECM remodeling, prevented miR-143/145 cluster upregulation and delayed tumor relapse. Ectopic expression of the two miRNAs in melanoma cells caused ECM reprogramming, the switch to an invasive dedifferentiated slow-cycling melanoma cell state and resistance to MAPKi. Conversely, miR-143/145 inhibition impaired MAPKi-driven ECM remodeling and increased drug sensitivity. Mechanistically, Fascin actin-bundling protein 1 (FSCN1) was identified as a key target of both miRNAs for the acquisition of phenotypic alterations mediated by the cluster. These findings identify the profibrotic miR-143/145 cluster as a key regulator of phenotypic plasticity and non-genetic resistance to MAPKi in melanoma. They also provide pre-clinical evidence that normalizing the fibrotic stromal reaction driven by MAPK-targeted therapy using antisense oligonucleotides (ASOs) directed against these two miRNAs can be exploited therapeutically to delay relapse and disease progression.

ncRNA Therapeutics

Selected Speakers

sciforum-045682: Analysis of LncRNA with PUMILIO Binding Sites Differentially Expressed in Cancer and Identification of Co-Expressed Genes

Cristiane Sato Mara Muller, Igor Samesima Giner, Carolina Mathias, Jaqueline Oliveira, Enilze Ribeiro, Daniela Gradia

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Long non-coding RNAs (lncRNA) are molecules that have more than 200 nucleotides and are often untranslated. They act in distinct regulatory mechanisms at multiple levels. LncRNAs can associate with RNA-binding proteins (RBP), forming ribonucleoprotein complexes (RNP) in both the cytoplasm and nucleus, regulating gene expression. PUMILIO proteins represent an RBP family that act as negative regulators of gene expression by binding to 3'UTR regions of hundreds of mRNAs and have already been associated with cancer. LncRNAs that present sites for PUMILIO may act sequestering these proteins, interfering in their targets' fate. RNA-seq data of 9 types of cancer from the cancer genome atlas (TCGA) database were analyzed using the GDCRNATools package. We compared the expression levels of lncRNAs with binding sites for PUMILIO to their respective non-tumor tissues using the Limma package (p0.05) in the R environment. We use the RTN package to select the PUMILIO target genes co-expressed with the lncRNAs with increased expression in tumors --- via mutual information inference --- and utilizing a p-value set for each cancer type according to the samples' number. We performed survival analyses using the Survival and Survminer packages for the selected genes with higher co-expression values. The analyses indicate a more significant differential expression of lncRNAs in the breast cancer (BRCA) samples, and the results point out the lncRNA NORAD as the most relevant. Among the PUMILIO target genes with significant expression correlated to NORAD, the RALGAPB gene stands out (p0.00001). Survival analyses indicated a shorter survival for Luminal A BRCA patients with increased expression of RALGAPB (p=0.042). Interestingly, NORAD and RALGAPB were already related to chromosome stability in different contexts. For the first time, this study suggests the potential role of lncRNA NORAD in chromosome instability through the NORAD-PUMILIO-RALGAPB regulatory axis in breast cancer.

Abstracts

Session 5. Biotechnology of ncRNA

sciforum-047938: Non-coding RNA & Editing: From High-Throughput Functional Genomics to Therapy

Wensheng Wei

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We have previously developed two high-throughput screening (HTS) methods based on CRISPR/Cas9 system for the functional identification of long non-coding RNAs. One is to employ genomic deletion strategy to screen for functional long non-coding RNAs (lncRNAs), and another approach is to establish genome-wide screening of lncRNA function using sgRNAs to target splice sites and achieve exon skipping or intron retention. Besides these high-throughput strategies to facilitate the accurate and rapid identification of functional genomic elements in various settings, we have recently developed a novel programmable RNA editing strategy called LEAPER, which enables precise and efficient RNA editing with broad applicability for both therapeutic and biomedical research.

sciforum-047534: Functions and Modes of Action of Long non-coding RNAs

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It is now clear that many intergenic regions in eukaryotic genomes give rise to a range of processed and regulated transcripts that do not appear to code for functional proteins. A subset of these are long (>200 nt), capped, and polyadenylated RNAs transcribed by RNA polymerase II and collectively called long noncoding RNAs (lncRNAs). The recent estimates are that the human genome may have >50,000 distinct lncRNA-producing loci, many of which show tissue-specific activity and dysregulation in human disease, including cancer and neurodegeneration.

Given the growing number of lncRNAs implicated in human disease or required for proper development, fundamental questions that need to be addressed are: Which lncRNAs are functional? How is functional information encoded in the lncRNA sequence? Is this information interpreted in the context of the mature or the nascent RNA? What are the identities and functional roles of specific sequence domains within lncRNA genes? These are challenging questions, primarily because of the substantial heterogeneity in mechanisms utilized by lncRNAs and the current paucity of lncRNAs with well-understood mechanisms. I will describe our work towards tackling these questions by combination of experimental methods with a focus on lncRNA functions in early cell fate decisions and computational methods focused on lncRNA evolution.

sciforum-044685: RNA Pull-Down-Confocal Nanoscanning (RP-CONA) Detects Quercetin as pri-miR-7/HuR Interaction Inhibitor that Decreases α-synuclein Levels

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MicroRNAs (miRs) are important non-coding RNAs that inhibit protein expression by base-pairing their target mRNAs. miR-7 targets the 3'-UTR of α-synuclein mRNA and thus decreases the level of α-synuclein, a protein that contributes to the etiology of Parkinson's disease (PD). Meanwhile, miR-7 is downregulated in PD patients. Previously, we showed that HuR (ELAVL-1) protein inhibits the biogenesis of miR-7 by binding to its precursor (pri-miR-7) at the conserved terminal loop. This suggests that disrupting the pri-miR-7/HuR interaction will rescue the miR-7 level and reduce α-synuclein production, providing a revolutionary strategy for PD therapy.

To identify small-molecule pri-miR-7/HuR inhibitors, we developed a new technique named RNA Pull-down-Confocal Nanoscanning (RP-CONA). Combining the RNA pulldown technique (RP) with the on-bead fluorescence imaging screen system (CONA), we wereable to monitor RNA/protein interaction in eukaryotic cell extracts as quantifiable fluorescent rings. With RP-CONA, we identified a natural product, quercetin, as the pri-miR-7/HuR inhibitor with the most potential. Importantly, quercetin upregulates cellular miR-7 levels and downregulates the expression of α-synuclein in an HuR-dependent manner. Our work opens up new therapeutic avenues towards the treatment of Parkinson's disease and and provides a novel methodology to search for RNA–protein interaction modulators.

sciforum-045389: Dynamic Imaging of RNA in Living Cells by CRISPR-Cas13 Systems

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Visualizing the location and dynamics of RNAs in live cells is key to understanding function. Here, we identify two endonuclease-deficient, single-component programmable RNA guided RNA-targeting Cas13 RNases (dCas13) that allow efficient RNA labeling and tracking in live cells, even when using single guide-RNAs between 20 and 27nt. Compared to the aptamer-based MS2-MCP strategy, an optimized dCas13 system achieves a comparable RNA labeling efficiency and is user-friendly without requiring genetic manipulation. The dCas13 system is capable of labeling NEAT1, SatIII, MUC4, and GCN4 RNAs, as well as studying the paraspeckle- associated NEAT1 dynamics. Applying orthogonal dCas13 proteins or combining dCas13 and MS2 -MCP allows dual-color imaging of RNAs in single cells. Further combination of dCas13 and dCas9 systems allows simultaneous visualization of genomic DNA and RNA transcripts in living cells. Collectively, the CRISPR-dCas13 system enables efficient and robust real-time imaging of RNA in live cells.

sciforum-045213: Drug Discovery Pipeline to Screen for RNA Therapeutics in Lung Cancer

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Non-small cell lung cancer (NSCLC) is the leading cause of cancer death worldwide with a longstanding unmet clinical need for effective therapies. The human genome contains many thousands of uncharacterized long non-coding RNAs (lncRNAs), which represent an untapped resource of therapeutic targets, given their promising patient- and tumor-specific expression patterns and demonstrated roles in tumorigenesis. To address the need for effective and durable therapeutics, we propose a novel end-to-end cancer drug discovery pipeline that is focused on three principal innovations: long non-coding RNA targets, CRISPR-deletion pooled screening, and antisense oligonucleotide (ASOs) therapeutics. This approach revealed novel oncogenic and potentially druggable cancer-promoting lncRNAs whose knockdown inhibits NSCLC growth in a variety of two- and three-dimensional models but displays negligible effects on untransformed cells. To gain insights into their molecular mechanisms, we performed the transcriptome

sequencing upon knock-down of selected lncRNAs with independent ASOs. This revealed concordant transcriptional changes in different NSCLC cell lines and a strong association with known cancer pathways. These findings demonstrate the power of CRISPR for functionally screening non-protein-coding genomic elements, and open an alternative route to personalized therapies for cancer.

sciforum-045214: Lnc-uc.147 and its Potential as Biomarker in Breast Cancer: A New ncRNA from Ultra-Conserved Regions

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Long RNAs are non-coding RNAs (lncRNA) with more than 200 nucleotides in length, with essential regulatory roles in several biological processes, including in breast cancer (BC). An intriguing class of lncRNAs are the transcribed from ultra-conserved regions (T-UCR) with near-perfect evolutionary conservation in many mammalian genomes. Most T-UCRs are differentially expressed in tumors, but few T-UCRs have been functionally characterized and associated with BC. We characterized and demonstrated the association with BC of the lnc-uc.147, a lncRNA transcribed from an ultra-conserved region. Using TCGA data, we found 302 T-UCRs related to clinical features in BC: 43% were associated with molecular subtypes, 36% with estrogen-receptor positivity, 17% with HER2 expression, 12% with stage, and 10% with. We chose 12 T-UCRs to validate differential expression in Brazilian patients (n=51) by RT-qPCR based on T-UCR mapping in intergenic or intronic regions with greater difference among molecular subtypes. Analysis of TCGA data showed that lnc-uc.147 is highly in luminal versus triple-negative patients. These results were confirmed in Brazilian patients. High expression of uc.147 was associated with worse overall survival among luminal A patients. Strand-specific RT-qPCR showed that uc.147 is transcribed in the opposite direction from its host gene. By subcellular fractionation, we demonstrated that uc.147 is nuclear. The lnc-uc.147 is a 2,8 kb mono-exonic transcript characterized by northern blotting and sequencing by RACE. The silencing of lnc-uc.147 increases apoptosis, arrests the cell cycle, and reduces cell viability and

colony formation in luminal BC cell lines. Additionally, we identified 19 proteins that interact with lnc-uc.147 through mass spectrometry. These proteins are mainly involved in cytoskeletal and centrosome organization as well as in epithelial-mesenchymal transition. This study characterized the lnc-uc.147 and demonstrated its oncogenic effect on BC cell lines. Furthermore, uc.147 has the potential as a BC prognostic marker in luminal patients.

sciforum-047537: Long non-coding RNAs: From Interactome to Function

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Long non-coding RNAs (lncRNAs) are widely expressed in the human genome and play key roles in the regulation of gene expressions. To understand genome regulation, we developed the CAGE technology, which identifies transcription start sites (TSSs) generally overlapping promoters, enhancers and also lncRNAs, quantitatively measuring their activity genome-wide. Using CAGE, the FANTOM5 project broadly explored such regulatory elements activity in a large panel of human and mouse primary cells, cell lines and tissues. To further explore the functions of lncRNAs, FANTOM6 established a large knockdown data set of lncRNAs by systematically knocking down 285 lncRNAs in human primary fibroblasts as well as unpublished datasets.

An important subset of lncRNA is comprised of antisense RNAs. We have identified a new class of non-coding antisense RNAs, named SINEUPs, which counterintuitively up-regulate protein translation of the sense RNA that they overlap. Enhancement of protein translation is mediated by SINE elements and the specificity of action is mediated by the region antisense to the 5'UTRs of the target mRNAs. SINEUPs can be designed to target specifically target mRNAs for therapies, including but not limited to haploinsufficiencies. The map of precise TSSs identified by CAGE in FANTOM5 gives us information to flexibly design the antisense region for effective SINEUPs in each cell type, allowing to correct unbalanced gene expression in disease models.

Looking into the future, the field is moving into single cell genomics, including multi-omics. Expanding these activities, we are working with the Human Cell Atlas (HCA) project, aiming at the creation of a comprehensive transcriptional and regulatory map of all human cells at single cell level. For the transcriptome analysis at single cell level, we have developed single cell CAGE and bioinformatics pipeline for the 10X Genomics platform (Moody et al, Biorxiv, https://doi.org/10.1101/2021.04.04.438388). Precise analysis of transcriptional at individual cell level will dramatically increase our understanding of biology.

Abstracts

Poster Session

sciforum-044298: Regulatory RNAs: A Universal Language for Inter-Domain Communication

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In eukaryotes, microRNAs (miRNA) have roles in development, homeostasis, disease, and the immune response. Recent work has shown that plant and mammalian miRNAs also mediate cross-kingdom and cross-domain communications. However, these studies remain controversial and are lacking critical mechanistic explanations. By comparing and contrasting the biogenesis and functions of eukaryotic miRNAs, small interfering RNAs (siRNA) and P-element-induced wimpy testis (PIWI)-interacting RNAs (piRNA), we discovered several conserved features and homologous components in these distinct pathways. These findings enabled us to propose novel mechanisms to explain how eukaryotic miRNAs could function in bacteria. Further understanding in this area is necessary to validate the findings of existing studies and could facilitate the use of miRNAs as novel tools for the directed remodelling of the human microbiota.

sciforum-044855: Implementing a high-throughput arrayed CRISPRi screening platform to identify functional lncRNAs

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Technological advances in RNA sequencing revealed that the human genome is pervasively transcribed, resulting in the production of thousands of long non-coding RNAs (lncRNAs). Several lncRNAs are now recognized as key components of diverse physiological processes. However, molecular genetics lacks a more comprehensive view of lncRNome functionality and the mechanisms through which lncRNAs operate. Current high-throughput approaches to study lncRNA function (i.e., pooled CRISPR library screens) are typically limited to a single cellular phenotype or dedicated molecular reporter based on which functional candidates are selected. Here, we present a scalable platform enabling serial cellular and molecular phenotyping to catalog lncRNA functions in a high-throughput and arrayed approach using CRISPR interference (CRISPRi). We applied PCR, in vitro transcription, and bead-based purification for high-throughput production of single gRNAs in 96-well plate format. Using lipid-based transfection, sgRNAs were delivered to HEK293T cells stably expressing a deficient Cas9 protein fused to repressive complexes (dCas9-KRAB-MeCP2). Cells were monitored in real-time using the Incucyte platform to quantify growth, proliferation, and apoptosis. After 48 hours, cells were lysed and RNA-sequencing libraries were generated directly from crude cell lysates, followed by shallow RNA-sequencing to infer the molecular phenotype associated with each condition. A proof-of-concept screen including 10 sgRNAs for 20 lncRNA targets demonstrated the feasibility of our approach, revealing differentially expressed genes and pathways upon lncRNA knockdown. Subsequently, we initiated the systematic silencing of over 300 lncRNAs through this platform in order to characterize their associated cellular and molecular phenotypes. Additional dCas9-KRAB-MeCP2 models are being generated to probe the functionality of the long non-coding transcriptome in various disease-relevant model systems.

sciforum-045109: Identification of irradiation-induced ATM-dependent lncRNAs

Marta Podralska[1], Marcin Sajek[1], Antonina Bielicka[1], Magdalena Żurawek[1], Iwona Ziółkowska-Suchanek[1], Katarzyna Iżykowska[1], Tomasz Kolenda[2], Marta Kazimierska[1], Marta Kasprzyk[1], Weronika Sura[1], Barbara Pietrucha[3], Bożena Cukrowska[3], Natalia Rozwadowska[1], Agnieszka Dzikiewicz-Krawczyk[1]

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DNA double-strand breaks (DSB) pose a serious threat to the genome. The central player in DSB detection and repair is the ATM kinase. Despite substantial knowledge of DSB repair, still several aspects of DNA damage detection and signaling are not fully understood.

Here, we aimed to verify the hypothesis that ATM-dependent lncRNAs are essential players involved in DNA damage response.

DSBs were induced by ionizing radiation (IR) in immortalized lymphoblastoid cell lines derived from 4 patients with ataxia-telangiectasia (AT) and 4 healthy donors. Cells were collected 1h and 8h after IR to allow identification of lncRNAs involved in the early and late response to DNA damage.

A strand-specific RNAseq approach identified 7 mRNAs and 10 lncRNAs significantly induced 1h after IR in healthy donors, whereas none in AT patients. In total, 447 mRNAs and 149 lncRNAs were induced 8h after IR in the control group, while only 100 mRNAs and 3 lncRNAs in AT patients. Gene set enrichment analysis revealed delayed induction of key DNA damage response pathways in AT patients compared to controls. Based on transcription factor ChIP-seq ENCODE data, we found 71 TFs with binding sites within 1kb from differentially expressed lncRNAs. The majority of TFs are involved in pathways connected with DNA repair. RT-qPCR validation on a larger group and inhibition of ATM with KU-60019 confirmed the ATM-dependent induction of 10 selected lncRNAs. Some of the detected lncRNAs are localized next to protein-coding genes involved in DNA repair. We observed that induction of lncRNAs after IR preceded changes in the expression of adjacent genes. This indicates that IR-induced lncRNAs may regulate the transcription of nearby genes and thus affect cellular response to DNA damage.

In conclusion, we identified lncRNAs induced in response to DNA damage in an ATM-dependent manner.

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sciforum-045115: From mouse to human: the nuclear lncRNA Charme as a putative candidate for clinical application?

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In recent years, several groups have demonstrated that muscle-specific long non-coding RNAs (lncRNA) play essential functions in the regulation of muscle cells growth, differentiation, and physiology. Even though their de-regulation was often associated with skeletal and cardiac diseases, the knowledge concerning their involvement in in vivo myogenesis is still far from complete. By using a high-throughput transcriptome screening, our group identified a novel nuclear lncRNA, *Charme*, expressed in skeletal and cardiac muscle (Ballarino et al., 2015). *Charme* is an abundant and highly conserved transcript that acts in the nucleus as a structural RNA, contributing to the formation of transcriptional foci where coordinated expression of pro-myogenic genes occurs. Its depletion leads to the downregulation of important myogenic genes, including genes linked to cardiomyopathies. *Charme* ablation in vivo resulted in a very distinct cardiac pathological phenotype in which the morphology of the murine heart is deeply remodeled (Ballarino et al., 2018). This defect is already present in new-born mice suggesting a critical role for *Charme* during development. By combining transcriptomic data, whole-mount in situ hybridization approaches and immunohistochemistry assays, we are working to characterized *Charme* role in heart development and the pathological phenotype that derive from its ablation. Intriguingly, the orthologous transcript (*hs-Charme*) has most characteristics in common with its murine counterpart. It regulates the same subset of target genes, suggesting an evolutionarily conserved function and offering many interesting possibilities for its future study. Since the World Health Organization classifies cardiomyopathies among the most frequent and fatal diseases in human, understanding *hs-Charme* involvement in cardiac development and pathologies could be extremely important. Accordingly, we are very much interested in the possible application of *hs-Charme* as a diagnostic tool or as a putative candidate for the treatment of cardiomyopathies, especially those without a clear genetic cause.

sciforum-045120: LncRNA NIHCOLE promotes DNA damage repair in hepatocellular carcinoma cells

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Long noncoding RNAs (lncRNA) are emerging as key players in cancer by enabling poorly understood molecular mechanisms. Here, we identified NIHCOLE, a novel lncRNA induced in hepatocellular carcinoma (HCC) with an oncogenic role in ligation efficiency of DNA double-strand breaks (DSB). NIHCOLE overexpression associates with several clinical parameters of bad prognosis and decreased overall survival of HCC patients. Depletion of NIHCOLE from HCC cells leads to impairment in proliferation, G2/M arrest, apoptosis, accumulation of DNA damage, and decreased non-homologous end-joining (NHEJ) activity. Biochemistry and electron and atomic force microscopy, reveal that NIHCOLE recruits several copies of the Ku70/Ku80 heterodimer into condensate-like clusters. Further, putative structural domains within NIHCOLE support stable multimeric complexes formed by several NHEJ factors, including Ku70/80, APLF, and the ligation complex formed by XRCC4 and DNA Ligase IV. Remarkably, NHEJ reconstitution assays show that

NIHCOLE promotes the ligation efficiency of blunt-ended DSBs. Collectively, we show that NIHCOLE serves as a scaffold and facilitator of the NHEJ machinery, conferring an advantage to hepatocellular carcinoma cells which unmasks a novel cancer vulnerability that could potentially be exploited using antisense drugs and DNA damaging agents as a therapeutic alternative for HCC patients.

sciforum-045148: lncRNA SLERT controls phase separation of FC/DFCs to facilitate Pol I transcription

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RNA polymerase I (Pol I) transcription takes place at the border of Fibrillar Centers (FC) and Dense Fibrillar Centers (DFC) in the nucleolus. Here we report that individual spherical FC/DFC units are coated by the DEAD-box RNA helicase DDX21, whose closed and open conformations allow the formation of hypo (loose)- and hyper (dense)-multimerized clusters, respectively, via their N- and C-terminal disordered domains. The long noncoding RNA SLERT, via a 103 nt loop region, binds to DDX21 RecA domains to promote the DDX21 closed conformation at a sub-stoichiometric ratio via a molecular chaperone-like mechanism. This results in the formation of loose DDX21 clusters coating DFCs required for proper FC/DFC liquidity and Pol I processivity. Without SLERT, DDX21 adopts an open conformation and forms dense clusters surrounding DFCs, dampening the liquidity and space in FC/DFC units, and hijacking rDNAs to suppress Pol I transcription. Together, these results reveal that SLERT is a master RNA regulator to control the biophysical properties of FC/DFCs for ribosomal RNA production.

sciforum-045168: The role of C1QTNF1-AS1 lncRNA in the regulation of cell division

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Long non-coding RNAs (lncRNAs) display a vast functional diversity, which contributes to their involvement in fundamental cellular processes. Different RNA-based mechanisms such as lncRNAs and RNA-binding proteins have been shown to regulate the assembly of the mitotic apparatus, which is essential for the fidelity of cell division. We recently identified *C1QTNF1-AS1* as a nuclear lncRNA involved in the regulation of mitotic progression. *C1QTNF1-AS1* is transcribed in an antisense orientation to *C1QTNF1*, which encodes a secreted glycoprotein and is a member of the C1q and TNF-related protein superfamily that has not been previously associated with mitosis. Depletion of *C1QTNF1-AS1* using different loss-of-function methods causes chromosome congression defects and mitotic delay in several human cell types. To gain insight into how *C1QTNF1-AS1* regulates mitosis, we hypothesised that *C1QTNF1-AS1* regulates the expression of the neighbouring protein-coding gene *C1QTNF1*. Indeed, *C1QTNF1-AS1* depletion led to upregulation of specific *C1QTNF1* isoforms, suggesting that *C1QTNF1-AS1* regulates alternative splicing of *C1QTNF1*. Furthermore, co-depletion of *C1QTNF1-AS1* and *C1QTNF1* was able to rescue the mitotic delay, indicating that *C1QTNF1-AS1* suppresses the expression *of C1QTNF1* isoforms that inhibit mitotic progression. We are currently investigating the mechanisms by which *C1QTNF1-AS1* regulates alternative splicing of *C1QTNF1* and the biological relevance of these isoforms in the control of cell division.

sciforum-045169: Systematic discovery and characterization of cis-regulatory long noncoding RNAs

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Long noncoding RNAs (lncRNAs) regulate target gene expression by two distinct modes: in cis, where target genes are located in genomic proximity to the lncRNA, and in trans, when not. Despite its central importance for understanding the biological roles of lncRNAs, the cis-trans framework lacks a rigorous definition. Furthermore, its mechanistic basis, including its relationship with DNA elements such as enhancers, is unclear. Here, we introduce “TransCistor”, a quantitative framework for classifying regulatory lncRNAs, based on the simple notion that cis-regulatory lncRNAs’ (cis-lncRNAs’) target genes should be statistically enriched for those located nearby in DNA, as identified using standard whole-transcriptome gene perturbation experiments. Applied to 526 perturbation datasets (targeting 293 lncRNAs) from human and mouse models, we identify 45 putative cis-lncRNAs, or 15% of those tested. In addition to several previously known cases, we identify numerous novel and known lncRNAs as cis-regulatory, including those not previously thought to act in cis, and which we can confirm experimentally. Consistent with our predictions, cis-lncRNAs are enriched for expression quantitative trait loci (eQTLs) for their cis-target mRNAs. This dataset enables us to examine the mechanistic basis for cis-lncRNA activity, and test longstanding hypotheses regarding the roles of chromatin modifications and looping. Overall, we provide a widely applicable and quantitative means for identifying cis-lncRNAs, estimate their prevalence, and shed light on their molecular mechanism.

sciforum-045196: Altered processing of lncRNAs in stem cells contributes to non-conserved functions

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A substantial amount of noncoding RNA (ncRNA) is expressed genome-widely. Compared to messenger RNAs (mRNA), the lncRNAs commonly have a relatively low expression, conservation level, and high species-specific, cell-specific expression patterns, which imply the different functions of lncRNAs during evolution. Different processing, localization, and functions of lncRNAs across species remains to be further investigated. For the conserved linear RNAs, we analyzed their subcellular localization, and function among human, monkey and mouse and further explore the function of one lncRNA *FAST* (*FOXD3* antisense transcript) in the embryonic stem cells (ESC). We find that lncRNAs have distinct subcellular localization patterns between human and mouse ESCs. The cytoplasm localized lncRNA h*FAST* can regulate WNT signaling pathway to induce the maintenance of pluripotency of human ESCs. Otherwise, the processing of nuclear retention lncRNA mouse m*Fast* is suppressed by splicing factor PPIE and has no effect on pluripotency maintenance. Taken together, the distinct processing and localization of lncRNAs during evolution is associated with their functions.

sciforum-045717: Exploring the molecular mechanism of lncRNAs that promote non-small-cell lung cancer.

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Increasing evidence suggests that long non-coding RNAs (lncRNAs) play critical roles in human tumorigenesis. Understanding such lncRNAs' molecular mechanism is an important step towards clinical translation. In a recent CRISPR screen, we identified CHiLL 1 and CHiLL 2, two novel oncogenic lncRNAs displaying a pro-survival role in non-small-cell lung cancer (NSCLC). The two lncRNAs are localized in the cytoplasm and nucleus of tumour cells, respectively. This study explores the molecular mechanisms of CHiLL 1 and 2 in terms of their interacting protein partners. We performed in vitro RNA pulldown from purified cellular fractions followed by mass spectrometry, to comprehensively identify their protein interactomes. This approach revealed dozens of potential partners, which are notably enriched for players in oncogenic pathways. This study sheds light on the molecular mechanisms by which oncogenic lncRNAs effect changes in disease hallmarks and marks an important step in clinical translation.

sciforum-045789: Investigating molecular mechanism of protein arginylation

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Posttranslational modifications are fundamental processes that expand the functional diversity of the proteome. Arginylation mediated by arginyltransferase ATE1 is a posttranslational modification of emerging importance that regulates mammalian embryogenesis, cell migration, and normal brain function, and has been recently proposed as a global biological regulator. However, the molecular mechanism of arginylation is elusive. Our previous study showed that arginylation is very specific to Arg-tRNA^{Arg}. Interestingly, tRNA-derived fragments (tRF^{Arg}) conjugated to Arg are capable of mediating arginylation in vitro. This result suggests that generation of physiologically important tRFs can potentially play a critical role in a switching mechanism between protein translation and arginylation, and points to a new role of tRFs in protein modifications. Here, we address this possibility and investigate the intracellular factors that can serve as the limiting step in regulating the yield of arginylation in vivo, including tRNA, Arg, and the associated enzymatic machinery. Our results suggest that the abundance of tRNA^{Arg} has no effect on arginylation efficiency in vivo, however the availability of Arg is a limiting factor for arginylation. Interestingly, ATE1 level does not correlate with higher arginylation efficiency, indicating that activity of ATE1, rather than abundance, determines the arginylation yield. These results show that availability of intracellular Arg may be a determining factor for arginylation in vivo.

sciforum-045806: A T1D-associated lncRNA modulates the type I interferon signaling and antiviral response in pancreatic β cells.

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Type 1 diabetes (T1D) is a complex autoimmune disease characterized by the destruction of insulin-producing pancreatic β cells. This autoimmune assault is triggered by genetic factors along with environmental factors (e.g., viral infections). The majority of T1D-associated SNPs lay into non-coding regions of the human genome, and many are predicted to alter the structure, expression and function of long non-coding RNAs (lncRNA). The molecular mechanisms by which most of these lncRNAs influence T1D development remain poorly unstudied.

Lnc10 is a lncRNA which harbours a T1D-associated SNP that disrupts the secondary structure of the RNA molecule. Intracellular exposure of pancreatic β cells to a synthetic viral dsRNA (poly:IC) upregulated the expression of *Lnc10*. Localization studies revealed that *Lnc10* is preferentially located in the nucleus of β cells, both, in basal and after poly:IC transfection, suggesting a potential role in transcriptional regulation. RNAseq of *Lnc10*-overexpressed pancreatic β cells revealed an upregulation of several genes related to type I IFN signaling and antiviral responses, including *ISG15*, *ISG20 IFI6*, *STAT1,* and *OAS1* among others. Interestingly, the T1D-associated SNP in *Lnc10* was previously annotated as intergenic and defined as an eQTL of an antiviral gene network in immune cells. Our results suggest that *Lnc10* may be a regulator of this pathway in pancreatic b cells and participate in virus-induced pancreatic β cell inflammation.

Further studies are needed to clarify the molecular mechanisms by which *Lnc10* regulates the expression of inflammatory and antiviral genes in pancreatic β cells, and to determine the impact of the T1D-associated SNP in the function of *Lnc10* and in the pathogenesis of T1D at the pancreatic β cell level.

sciforum-045816: Distinctive features of long non-coding RNA chromatin (dis-)association

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Long non-coding RNAs (lncRNAs) constitute a large heterogeneous class, some of which are transcribed from enhancer-like regions and are involved in the regulation of gene expression in cis. Although lncRNAs are generally found to be enriched in the chromatin fraction of the cell, to what degree this defines their broad range of functions remains unclear. In addition, the factors that contribute to lncRNA chromatin tethering, as well as the molecular basis of efficient lncRNA chromatin dissociation and its functional impact on cognate enhancer activity and target gene expression, remain to be resolved. Here, we combined pulse-chase metabolic labeling of nascent RNA with chromatin fractionation and deep sequencing from the chromatin-associated and nucleoplasmic fraction to follow nascent RNA transcripts from their co-transcriptional state to their release into the nucleoplasm. By incorporating physical and functional characteristics in machine learning, we build models to predict chromatin (dis-)association of lncRNAs, and compare these to mRNAs. We find that parameters such as co-transcriptional splicing of the worst processed intron per transcript contribute to lncRNA chromatin dissociation. Intriguingly, lncRNAs transcribed from enhancers display reduced chromatin retention, suggesting that, in addition to splicing, lncRNA chromatin dissociation may contribute to shaping cognate enhancer activity and target gene expression.

sciforum-045818: Human vault RNA promotes cell proliferation, tumorigenesis and chemoresistance through the lysosome in hepatocellular carcinoma

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The human genome encodes four vault RNAs (vtRNA) that were thought to play roles in the vault complex, a gigantic RNP of 13 MDa. Despite decades of dedicated research, the function of the vault complex or the vtRNAs remained enigmatic. We demonstrated that only a minor fraction of vtRNA is actually associated with the vault complex and uncovered a functional role of this ncRNA beyond the vault particle. We have demonstrated previously that vtRNA1-1 confers apoptosis resistance in several malignant cell lines by modulating pro-survival pathways (e.g., MAPK signaling). Furthermore, it has also been suggested that vtRNA1-1 regulating the autophagic flux in hepatocytes, thus highlighting its pro-survival role.

Here we describe a new function of vtRNA1-1 in regulating in vitro and in vivo tumor cell proliferation, tumorigenesis, and chemoresistance. KO of vtRNA1-1 in human hepatocellular carcinoma cells reduced nuclear localization of TFEB (transcription factor EB), leading to a downregulation of the coordinated lysosomal expression and regulation (CLEAR) network genes and lysosomal compartment dysfunction. We demonstrate further that impaired lysosome function due to loss of vtRNA1-1 potentiates the anticancer effect of conventional chemotherapeutic drugs. Finally, loss of *VTRNA1-1* reduced drug lysosomotropism allowing higher intracellular compound availability, and thereby significantly reducing tumor cell proliferation in vitro and in a subcutaneous xenograft mouse model in vivo. These findings reveal a so far unknown role of vtRNA1-1 in the intracellular catabolic compartment and describe its contribution to lysosome-mediated chemotherapy resistance. This novel pro-survival function may identify vtRNA1-1 as a new therapeutic target to overcome chemo-insensitivity caused by passive lysosomal sequestration of antitumor drugs.

sciforum-045144: Exploring immune related lncRNAs in breast cancer molecular subtypes

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Breast cancer (BC) is the most leading cause of cancer worldwide. It is a very heterogeneous disease with at least five molecular subtypes. These molecular subtypes can distinct specific mRNA expression patterns and also consider ncRNAs. Among the ncRNAs class, the long non-coding RNAs (lncRNA) are molecules with more than 200 nucleotides with versatile regulatory roles; and are being evaluated for BC molecular subtypes differentiation. The heterogeneity of BC can also be reflected regarding tumor microenvironment, which can directly impact a patient's prognosis and therapy response. Using BC immunogenomics data from previous studies, we propose a molecular subtype-specific lncRNA signature to characterize these subtypes better. RNA-seq data from the cancer genome atlas (TCGA) BC cohort was analyzed, and signal-to-noise ratio metrics were applied in two steps, first considering molecular subgroups and then the immune subtypes. A list of approximately 10 specific lncRNAs for each molecular subtype was generated and functionally analyzed using GSEA enrichment and survival analysis. We highlighted here some lncRNAs in each molecular subtype. LINC01871 is related to immune response activation and favorable overall survival in basal-like samples; EBLN3P is related to immune response suppression and overall, and progression in luminal B, MEG3, XXYLT1-AS2, and LINC02613 were related with immune response activation in luminal A, Her2, and normal-like subtypes, respectively. In this way, we emphasize the actual need to know better the role of lncRNAs as regulators of immune response to provide new perspectives regarding diagnosis, prognosis and therapeutically targets in BC molecular subtypes.

sciforum-045675: nf-core/circrna: a nextflow workflow for the quantification, microRNA target prediction and differential expression analysis of circular RNAs in RNA-Seq data

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Circular RNAs (circRNAs) are a class of non-coding RNA (ncRNA) that have gained attention due to their unique covalently closed loop structure conferring resistance to RNase degradation, tissue-specific expression, abundant expression in saliva, blood, plasma, and exosomes. These characteristics make circRNAs ideal candidates as both diagnostic and prognostic biomarkers in a clinical setting, facilitating the use of non-invasive liquid biopsies for the detection and monitoring of disease status. Furthermore, highly expressed circRNAs have the ability to titrate microRNAs (miRNAs) via miRNA response elements within their mature spliced sequence, making the study of circRNAs within the competing endogenous RNA (ceRNA) network at the systems level highly pertinent in the context of novel therapeutic drug design. To date, no pipeline exists that seamlessly integrates multiple circRNA quantification tools, performs miRNA target prediction and differential expression analysis of circRNAs in a modular fashion, utilising Docker/Singularity containers requiring minimal installation steps by the user, executable across all POSIX compute environments, enabling high-performance cluster and cloud-based deployment.

To address these shortcomings, we present nf-core/circrna, a multi-functional, automated high-throughput pipeline implemented in Nextflow that allows users to fully characterise circRNAs in RNA-Seq datasets via three analysis modules—(i) circRNA quantification, robust filtering and annotation; (ii) miRNA target prediction of the mature spliced sequence; and (iii) differential expression analysis between pathological conditions complete with statistical results, and diagnostic and expression plots. nf-core/circrna has been developed within the nf-core framework, ensuring robust portability across compute environments via containerisation, deployment on cluster/cloud-based infrastructure, comprehensive documentation, and maintenance support. Source code, documentation, and installation instructions are freely available at https://github.com/nf-core/circrna and at https://nf-co.re/circrna.

sciforum-045702: Integrative analysis allows a global and precise identification of functional miRNA target genes

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Despite almost two decades of research and development for accurate detection methods allowing the identification of direct miRNA target genes, a precise picture of miRNA regulation in a given cell type or context is still missing. We reasoned that instead of focusing on the function of individual RNA interference genes in a single dataset, the global analysis would provide a comprehensive view of important mechanisms, generating new insights and hypotheses. We performed an integrative analysis combining OMICs data from a unique series of miRNA deficient mESC lines generated in the laboratory (*Drosha*, *Dgcr8*, *Dicer*, *Ago2&1*_KO) with other datasets publicly available (Prediction models, AGO-bound miRNAs) to determine global and accurate direct miRNA interactions in mESCs. We further validated our findings by measuring the impact of the deletion of specific miRNA clusters on gene expression and established that only about 6% of expressed genes are subject to direct miRNA-regulation in mESCs, refining previous higher estimations. We are now deepening our original computational analysis by developing a novel miRNA-interaction prediction model in a data-driven manner, which will be useful to generate similar predictions in other biological contexts.

sciforum-045753: ElementaLdb: a user-friendly webserver for exploring and retrieving lncRNA elements

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It is thought that long noncoding RNAs (lncRNA) are composed of modular units called elements, and these discrete sequences act as molecular docking sites for proteins, RNAs, and DNA through sequence motifs or structures in the mature lncRNA molecule. In order to facilitate the exploration and discovery of such elements, we present the ElementaLdb webserver. ElementaLdb contains a curated set of annotations previously implicated in lncRNA function, such as Repeat Insertion Domains of LncRNAs (RIDL), conserved RNA secondary structures (CRS) and RNA binding protein sites (RBP). ElementaLdb is paired with the latest GENCODE annotation and allows the user to produce publication-ready figures of lncRNAs alongside subsets of elements of interest. Furthermore, the user could specify to generate a transcript-relative annotation and plot, which removes all intronic regions and transform the coordinates relatives to the mature lncRNA. ElementaLdb outputs annotations in tabular form (BED and CSV formats), as well as an XML Integrative Genome Viewer (IGV) session for further local inspection. ElementaLdb will be available at http://elementaldb.crg.eu/.

sciforum-045766: Single-cell RNAseq-based transcriptome profiling of mesenchymal stromal cell (MSC) subpopulations with different responses to profibrotic stimuli

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Fibrosis is a complex outcome of the healing process when connective tissue replaces parenchyma, leading to the loss of function and organ failure. It is associated with abnormally high accumulation of myofibroblasts differentiated from cells of mesenchymal origin. They excessively produce extracellular matrix components such as collagen and fibronectin. Elimination of myofibroblasts is essential for fibrosis regression. Multipotent mesenchymal stromal cells (MSCs) could play a dual role in the development of fibrosis: some MSCs replenish a myofibroblast pool promoting the progression of fibrosis, but they could also suppress fibrosis due to the antifibrotic effects of their secretome, including transfer of non-coding RNAs. MicroRNAs (miRNAs) make up the class of non-coding RNAs that control gene expression by gene silencing on the transcriptional level and thus can contribute to MSC regulation of myofibroblast differentiation, which makes them promising targets for fibrosis management.

In our study, we explore if MSCs are heterogeneous in responses to profibrotic stimuli and which miRNAs could contribute to these responses. Human adipose-derived MSCs were cultured for 4 days with a profibrotic factor TGFb on fibrotic-like decellularized extracellular matrix produced by fibroblasts. Single-cell RNA-seq datasets were created from MSCs under profibrotic versus standard conditions (10x Genomics). Loupe Browser was used to cluster cells according to their gene expression pattern. Two clusters were preliminarily defined as “profibrotic” and “antifibrotic” depending on the expression of myofibroblast markers. MiRNAs targeting genes highly expressed in antifibrotic and silenced in profibrotic populations with potential antifibrotic properties were revealed. The top five miRNAs (hsa-let-7b-5p, hsa-let-7a-5p, hsa-miR-22-3p, hsa-miR-27b-3p, hsa-miR-29a-3p) with the greatest number of fibrosis-associated gene targets and overrepresented in MSCs and MSC extracellular vesicles were selected. Their contribution to MSC antifibrotic effects needs to be validated in further experiments.

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sciforum-045779: ConnectOR: Sensitive and practical discovery of long noncoding RNA orthologues

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Advances in sequencing technologies have enabled the discovery of thousands of novel long non-coding RNAs (lncRNAs) across metazoan species, but the biological roles of just a tiny fraction have been elucidated. Orthology relationships between conserved lncRNAs are considered valuable evidence for functionality. Presently available methods for orthology prediction employ computationally intensive sequence homology, and require extensive preprocessing of data. Here, we present ConnectOR, an orthologue discovery pipeline that reduces computational burden by employing precomputed genome chain alignments and eliminates preprocessing by running with widely available gene transfer format annotation files. ConnectOR discovers mouse orthologues for ~8% of human GENCODE lncRNAs, including many not found by other methods. Most interesting, analysis of the largest possible annotations between mammals and fish substantially expands the set of known anciently conserved pan-vertebrate lncRNAs to ~1.5%. Thus, ConnectOR is a practical and powerful means of extending lncRNA orthology maps.

sciforum-045804: RNA secondary structure prediction using deep learning with thermodynamic integration

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Accurate predictions of RNA secondary structures can help uncover the roles of functional non-coding RNAs. Although machine learning-based models have achieved high performance in terms of prediction accuracy, overfitting is a common risk for such highly parameterized models. Here we show that overfitting can be minimized when RNA folding scores learnt using a deep neural network are integrated together with Turner's nearest-neighbor free energy parameters. Training the model with thermodynamic regularization ensures that folding scores and the calculated free energy are as close as possible. In computational experiments designed for newly discovered non-coding RNAs, our algorithm (MXfold2) achieves the most robust and accurate predictions of RNA secondary structures without sacrificing computational efficiency compared to several other algorithms. The results suggest that integrating thermodynamic information could help improve the robustness of deep learning-based predictions of RNA secondary structure. The MXfold2 source code is available at https://github.com/keio-bioinformatics/mxfold2/, and the MXfold2 web server is available for use at http://www.dna.bio.keio.ac.jp/mxfold2/.

sciforum-045809: Perturbation-agnostic designs for CRISPR pooled screening libraries targeting lncRNAs

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CRISPR-Cas9-mediated, large-scale, targeted genome editing is a powerful technique for identifying genes promoting disease states. CRISPR offers a solution for perturbing noncoding RNAs via two principal means: CRISPR-deletion (CRISPR-del) and CRISPR-inhibition (CRISPRi). CRISPR-del requires paired guide RNAs designed for sites flanking the target region. CRISPRi, on the other hand, can be achieved using single or paired guide RNAs delivering dCas9-repressor fusions immediately downstream of the transcription start site. In the past, separate screening libraries had to be created for either application. Here, I describe a strategy for the creation of multi-purpose paired sgRNA libraries compatible with both CRISPR-del and CRISPRi. The resulting libraries provide high coverage for candidate sets of long noncoding RNAs. Multi-purpose CRISPR library designs increase the flexibility of this increasingly used screening approach.

sciforum-045811: Making zebrafish the dark horse in long noncoding RNA research

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Genomes of multicellular eukaryotic organisms produce tens of thousands of long noncoding RNAs (lncRNA). Although many lncRNAs have been linked to fundamental physiological processes in the cell, 99% of them still remain functionally uncharacterized. Application of animal models helps to understand the biological roles of lncRNAs. At the same time, zebrafish (*Danio rerio*) has emerged as a powerful vertebrate model for investigating gene function and holds a great promise for studying lncRNAs. However, current zebrafish lncRNA annotations show evident signs of incompleteness with many gene models being fragmented or uncatalogued. To accelerate lncRNA annotation in zebrafish, we will use nanopore capture long sequencing (nanoCLS), which combines targeted RNA capture with long-read Oxford nanopore RNA sequencing. With this project, we also aim to remove the bias of zebrafish gene annotation towards developmental stages by focusing on transcriptomically complex and biomedically relevant adult tissues. Full-length, highly accurate transcript models produced by nanoCLS will facilitate functional studies of lncRNAs and enhance the detection of human and mouse lncRNA orthologues.

sciforum-045821: One lncRNA gene catalog to rule them all: expanding GENCODE with the CapTrap-Capture Long Seq method in human and mouse

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Long noncoding RNAs (lncRNA) are a heterogeneous group of transcripts that do not encode any identifiable peptide product. Although genomes of multicellular eukaryotic organisms produce tens of thousands of lncRNAs, the overwhelming majority of them (>97%) remains functionally uncharacterized. Understanding the biological roles of lncRNAs requires accurate genome annotations, which describes their precise genomic location, gene boundaries and transcript structures. Present lncRNA annotations are far from being complete, with many gene models being fragmented or uncatalogued. This incompleteness can have a strong impact on downstream analyses. We recently developed the capture long-read sequencing (CLS) method, which has helped to considerably improve the GENCODE lncRNA catalog's accuracy and completeness. Despite its benefits, CLS still produces large proportions of 5'-incomplete transcript models, mainly due to technical limitations of cDNA synthesis methods. To address this issue, we developed CapTrap-CLS, an upgraded version of the CLS method that specifically enriches for 5'-capped, full-length RNAs. CapTrap-CLS was applied to a panel of adult and fetal tissues, embryonic stem cells, as well as immortalized cell lines from human and mouse. CapTrap-CLS experiments were performed using a very broad custom RNA capture design that targets a large number of GENCODE-unannotated

elements from various ncRNA catalogs (Bigtranscriptome, FANTOMCat, miTranscriptome, etc.) and computational predictions. Using CapTrap-CLS, we report the discovery of thousands of novel, 5'-to-3'-complete lincRNA transcripts and loci in both human and mouse. This augmented catalog provides one of the broadest and most accurate views of the long noncoding transcriptome to date, while contributing a robust foundation for future lncRNA functional characterization.

sciforum-045823: Integrative target prioritisation pipeline (TPP) of CRISPR-Cas pooled screens for improved detection of cancer lncRNA

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Long non-coding RNAs (lncRNAs) are of increasing importance in cancer research as therapeutic targets. CRISPR-Cas pooled screening is a powerful and versatile means of discovering novel cancer lncRNAs. To date, most studies have performed screens using a single cell type or functional readout, and no solutions exist for leveraging diverse datasets to improve detection power. Here, we propose to resolve this using an integrative target prioritisation pipeline (TPP) capable of integrating any number of CRISPR screens. TPP balances both effect size and statistical significance, enabling prioritisation of lncRNA drug targets based on their overall performance and identifying hits that would be overlooked by traditional methods. Using protein-coding controls, TPP yields improved performance compared to conventional, single-screen analyses, in terms of sensitivity (TPP: 0.37; average single screens: 0.17) and accuracy (TPP: 0.51; average single screens: 0.36). TPP also displays improved sensitivity in detecting known cancer lncRNAs. In summary, integrative analysis is a useful tool for improving the detection of target lncRNAs for precision oncology.

sciforum-045889: DIANA-miTED: A microRNA Tissue Expression Database

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DIANA-miTED is a web database offering the expression of microRNAs (miRNA) obtained via consistent analysis from scratch of thousands of miRNA-Seq datasets. miRNA expression information is catered for cell-lines and tissues alike. DIANA-miTED holds information for >240 cell-lines from sequence read archive (SRA) and for >190 healthy/disease tissues or organs derived and efficiently catalogued from 32 projects of the cancer genome atlas project (TCGA), as well as SRA. In total, users can retrieve expression values of 2656 miRNAs and relevant meta-information from 15,183 analyzed experiments from the two repositories (27.3% of samples from NCBI-SRA and 72.7% from TCGA). Pre-processing and analysis of the datasets was performed following a well-defined workflow for small RNA-Seq analysis, “DIANA-mAP” (Alexiou et al., Genes, 2021), that consists of the following steps: data acquisition, quality checking utilizing FastQC, contaminant detection using DNApi and Minion, alignment and quantification using miRDeep2. miRNA expression units offered are read counts, reads per million (RPM) and Log2RPM. miTED offers rich visualizations (grouped boxplots, pie charts) depicting the expression, as well as distribution of requested data across variables, such as health status and gender. Sankey diagrams and network graphs are provided, enabling exploratory analyses of the relationships between variables (tissue-anatomical locations, tissue-diagnosis, etc.). miTED’s interconnection with other DIANA-Tools enables users to easily retrieve relevant targets and conduct functional analyses. The miRNA tissue-expression database was developed utilizing the latest technologies in web development, namely MongoDB (noSQL database), PHP and Laravel8 for data access layer development and Typescript and Angular 9 for the presentation layer. We aspire that miTED will prove a valuable utility to the non-coding RNA community, facilitating research in miRNA tissue expression patterns.

sciforum-045039: Single-cell RNA analysis identifies a novel conserved long noncoding RNAs controlling cardiac fibrosis and remodeling

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Coronary artery disease is a leading cause of death worldwide, typically leading to myocardial infarction (MI) and heart failure. The aim of this project was to characterize the heterogeneity in the cardiac non-myocyte cell populations after MI via single-cell analysis of the long noncoding (lnc) RNA transcriptome. The non-myocyte fraction of a mouse heart was isolated using a Langendorff approach before and three days after MI. Following isolation, cardiomyocytes were eliminated by gentle centrifugation, and FACS was used to remove leukocytes ($CD45^+$) and endothelial cells ($CD31^+$) from metabolically active ($Calcein^+$) and viable ($DAPI^-$) cells. The single-cell suspension was analyzed using a 10X Genomics platform. An average number of 200,000 reads per cell allowed mapping coding and noncoding genes in 8413 cells from sham-operated and infarcted hearts. Dimensionality reduction approaches were used to visualize cells in low-dimensional space-based exclusively on the expression of the individual lncRNA transcriptomes. The major cardiac cell types were readily identified. Focusing our analysis on fibroblasts and myofibroblasts, we were able to identify seven different subpopulations, three of them being present only in the infarct heart. In the end, we selected the novel enhancer-associated lncRNA CF31245 for further validation. CF31245 was found to be induced in the early response phase after MI and associated with the myofibroblast population. Its expression correlated with the expression of relevant matrix proteins and the development of fibrosis. Silencing CF31245 using specific ASOs in vitro blunted the fibrotic gene program in differentiating myofibroblasts. Furthermore, CF31245 knockdown in adult mice after MI led to a significant decrease in infarct size, a drastic reduction of fibrosis, a large diminution of remodeling, and improved heart function. Importantly, CF31245 is conserved in humans and therefore represents a novel therapeutic target for heart disease.

sciforum-045151: Clipper, a novel long noncoding RNA regulating cardiomyocyte metabolism and proliferation

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Adult heart regeneration following infarction is limited by the inability of cardiomyocytes (CM) to proliferate in response to stress. Therefore, enhancing CM proliferation represents a promising approach for promoting regeneration in the damaged heart. In this context, long noncoding RNAs (lncRNA) are regulatory molecules controlling cell identity and behavior. We exploited a high throughput-screening assay and neonatal mouse CMs to identify anti-proliferative lncRNAs. Specifically, we used antisense oligonucleotides (GapmeRs) to silence individually 350 novel heart-enriched lncRNAs in CMs and evaluated the impact on proliferation. We identified *Clipper*, a nuclear enhancer-associated lncRNA located in the *Lipin1* locus. GapmeR-mediated *Clipper* knockdown reduced also *Lipin1* expression, indicating that *Clipper* could work via regulating *Lipin1*. Interestingly, *Lipin1* is a phosphatidic acid phosphatase, controlling a lipid cascade that eventually regulates mitochondrial fission. Two different types of fission events have been previously observed, namely midzone and peripheral fissions, depending on the position of the event along the mitochondrion. Midzone fission is MFF-dependent and associated with proliferation whereas peripheral fission is FIS1-dependent and associated with stress. Upon *Clipper* knockdown, midzone fissions were increased while peripheral fissions were not affected. Inhibition of midzone fission following Mff knockdown blunted CM proliferation following *Clipper* silencing, implicating mitochondrial energy metabolism in the process. *Clipper* knockdown induced a shift from oxidative phosphorylation to glycolysis in proliferating CMs. This was associated with a reduction of ROS production and ROS-induced DNA damage, an important determinant of CM cell cycle arrest. Finally, we evaluated the effects of *Clipper* knockdown on CM proliferation and heart function in a mouse model of myocardial infraction. CM proliferation, decreased infarct size and improvement of heart function were observed after anti-*Clipper* GapmeR administration in vivo. Altogether, this

study suggests the existence of a coordinated regulation of CM metabolism and proliferation, in part through a *Clipper*/*Lipin1* axis controlling mitochondrial dynamics.

sciforum-045263: Development of an antimiR-based treatment against Myotonic Dystrophy disease

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Myotonic dystrophy type 1 (DM1) is a chronically debilitating, rare genetic disease that originates from an expansion of a non-coding CTG repeat in the dystrophia myotonica protein kinase (DMPK) gene. The expansion becomes pathogenic when DMPK transcripts contain 50 or more repetitions due to the sequestration of the muscleblind-like (MBNL) family of proteins. Depletion of MBNLs causes alterations in splicing patterns in transcripts that contribute to clinical symptoms, such as myotonia and muscle weakness and wasting. We previously found that microRNA (miR)-23b directly regulates MBNL1 in DM1 myoblasts and mice and that antisense technology (“antagomiRs”) blocking this microRNA (miRNA) boosts MBNL1 protein levels. Here, we show the therapeutic effect over time in response to administration of antagomiR-23b as a treatment in human skeletal actin long repeat (HSALR) mice. Subcutaneous administration of antagomiR-23b upregulated the expression of MBNL1 protein and rescued splicing alterations, grip strength, and myotonia in a dose-dependent manner with long-lasting effects. The pharmacokinetic data obtained provide further evidence that miR-23b could be a valid therapeutic target for DM1.

sciforum-045728: miR-181a/b modulation as a potential therapeutic approach for AMD treatment

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MicroRNAs (miRNAs) are small noncoding RNAs actively involved in the regulation of gene expression and represent promising therapeutic tools due to their capability to simultaneously fine-tune modulating multiple molecular pathways involved in human pathogenesis and progression. The miR-181 family is highly expressed in different regions of the CNS, including the retina, where they regulate neurotrophic signaling, axon guidance, immunity, and mitochondrial-related pathways. miR-181a/b regulates genes involved in mitochondrial biogenesis and function and ROS detoxification, thus pointing to a critical role of these microRNAs in the regulation of mitochondrial turnover. The reduction of miR-181a/b levels preserves retinal cells from death and ameliorates visual function in several disease models of mitochondrial dysfunction, indicating these microRNAs as attractive therapeutic targets for these conditions. Age-related macular degeneration (AMD) is a complex multifactorial degenerative form of vision impairment due to photoreceptor (PR) damage secondary to retinal pigment epithelium (RPE) degeneration. Increasing evidence indicates that mitochondrial dysfunction in both RPE and PRs exacerbate the production of reactive oxygen species (ROS) and contribute to the pathogenesis of AMD.

Preliminary results suggest that the genetic inactivation of miR-181a/b exerts a protective role in an AMD-like mouse model, the Abca4$^{-/-}$ mice. Weare also evaluating the putative therapeutic effect of miR-181a/b downregulation in Abca4$^{-/-}$ mice by subretinal injection with AAV-encoding miR-181a/b ”sponges”, which will allow long-term loss-of-function of miR-181a/b in vivo. The miRNA sponge-based strategy will be helpful in defining the protective effect of miR-181a/b silencing and the

underlying molecular mechanisms and will potentially lead to the design of a novel non-coding RNA-based treatment for AMD and other forms of multifactorial retinal degeneration.

sciforum-045775: Functionalization of reconstituted HDL for theranostic in cardiovascular disease

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Aim: Cardiovascular disease (CVD), the leading cause of mortality in industrially developed countries, is primarily caused by atherosclerosis, characterized by an abnormal lipid and inflammatory cell accumulation in the intima, which is associated with atheroma plaque formation. The interaction of HDLs with membrane cholesterol transporters is especially relevant in reducing the intracellular cholesterol content of macrophages that interact with the atheroma plaque. Therefore, HDL mimetics have focused attention both as a potential therapeutic tool and as an inspirational source for biomedical engineering. The goal of the present work was to deliver microRNA to macrophages to stimulate ABCA1 expression in order to improve cholesterol efflux.

Methods: In vitro production of rHDL was studied by mixing apoA-1 with different lipids. microRNAs were delivered to macrophages using nanoparticles to overexpress ABCA1 receptor and cholesterol efflux assays on macrophages were carried out.

Results: We have obtained stable rHDLs that have been functionalized with microRNA to favour RCT from macrophages.

Conclusions: Functionalized rHDL are suitable molecule nanocarriers for microRNAs that efficiently enhance cholesterol efflux from macrophages.

sciforum-045802: Identification of lncRNAs as Potential Novel Therapeutic Targets in Triple-Negative Breast Cancer

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Triple-negative breast cancer (TNBC) lacks the three common receptors associated with breast cancer. Consequently, there are a limited number of targeted therapies available for TNBC patients. Recently, long non-coding RNAs (lncRNA) have emerged as potential therapeutic targets in cancer treatment. LncRNAs are a subtype of non-coding RNA classified by their length (> 200 nt). By definition, they lack a significant open reading frame which means they are not translated into proteins. Despite not coding for proteins, lncRNAs are transcribed by RNA polymerase II and can be spliced, alternatively spliced, 5'-capped, and polyadenylated. Hundreds of lncRNAs have recently been discovered as key players in cancer. We aim to assess the biological importance of lncRNAs in TNBC progression and metastasis.

We performed loss-of-function (LOF) viability screens to assess the impact of lncRNAs on TNBC cell growth. Illumina sequencing was used to measure sgRNA enrichment following 20 cell doublings (T20). Candidate genes were selected based on average depletion of sgRNAs at T20 compared to the day of infection (T0) and prioritised based on therapeutic relevance in TNBC. loss-of-function (LOF) models were created for select candidate genes using antisense oligonucleotides (ASO) and CRISPRi in TNBC cells. The LOF models were compared to control cell lines in regard to key aspects of cancer progression such as cell proliferation, migration, and invasion.

The overall aim of this research is to identify a specific lncRNA gene as a key driver in TNBC cell growth to allow for the future development of a novel targeted therapy.

sciforum-044468: RNA N6-Methlyadenosine manipulating via CRISPR/Cas13-associated RNA Epigenetic Editor

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RNA N^6-Methyladenosine(m^6A) is the most abundant mRNA modification and forms part of an epitranscriptomes system analogous to the epigenetic system based on the chemical modification of DNA and histones. RNA modification can be reversibly catalyzed by several specific enzymes, and those modifications can be recognized by RNA binding proteins that are, in turn, related to many biological processes. Although there are many reports demonstrating m^6A participation in the critical parts for those biological functions, this exploration has mainly been conducted through the global knockout or knockdown of the m^6A writers, erasers, or readers. Consequently, the lack of information about the role of m^6A on the single transcript in biological processes still a challenge for us to understand m^6A's biological function precisely. --Here, we demonstrate CRISPR/dCas13a associated RNA epigenetic-editor (termed 'CARE') which can single or multiple targets specific mRNAs or lincRNAs for methylation and demethylation of m^6A, systematically assay its abilities can enabling the targeted rewriting of m^6A dynamics. Especially RNA stability dependent gene expression level will be changed during RNA's m^6A be rewrites by CAREER and XIST repression of the X chromosome can be rescued in human cells by editing XIST's m^6A modification level. Based on our technique, the function of m^6A modifications on single transcripts can be explored and will help us understand how m^6A functions in specific biological processes much deeply.

sciforum-045212: A novel strategy of pre-mRNA splicing correction by site-specific pre-miRNA recruitment

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Alternative splicing is an important cellular process, during which different RNA transcripts are generated from pre-mRNA due to varying alternations in the skipping or inclusion of exons. This leads to different protein isoforms that differ in their structure and function. We suggest a new model for miRNA regulation of the splicing mechanism, in which pre-miRNAs change the affinity (or strength) of the U1-5'SS binding. This new type of regulation has therapeutic potential in combination with short oligonucleotide adaptors that attract pre-miRNA to the targeted splicing site and enhance the inclusion of the target exon to the mature mRNA. The use of the natural mechanism and tissue-specific miRNAs without changing the level of miRNA in the cell allows to achieve high specificity and selectivity, as well as potentially reducing side effects. We compare the efficiency of this approach with standard methods of alternative splicing regulation. We have also performed data analysis of genes involved in mono-genetic diseases, mis-spliced exons, and miRNA expression data, and defined the exons and diseases that can be potentially regulated using the proposed mechanism. This work was supported by the Russian Foundation for Basic Research, grant № 19-54-06009.

sciforum-045471: Dynamic imaging of RNA in living cells by CRISPR-Cas13 systems

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Visualizing the location and dynamics of RNAs in live cells is key to understanding their function. Here, we identify two endonuclease-deficient, single-component programmable RNA-guided RNA-targeting Cas13 RNases (dCas13) that allow efficient RNA labeling and tracking in live cells, even when using single guide-RNAs between 20 and 27 nt. Compared to the aptamer-based MS2-MCP strategy, an optimized dCas13 system achieves a comparable RNA labeling efficiency and is user-friendly without requiring genetic manipulation. The dCas13 system is capable of labeling NEAT1, SatIII, MUC4, and GCN4 RNAs as well as studying the paraspeckle-associated NEAT1 dynamics. Applying orthogonal dCas13 proteins or combining dCas13 and MS2 -MCP allows dual-color imaging of RNAs in single cells. Further combination of dCas13 and dCas9 systems allows the simultaneous visualization of genomic DNA and RNA transcripts in living cells. Collectively, the CRISPR-dCas13 system enables efficient and robust real-time imaging of RNA in live cells.

sciforum-045833: Long-noncoding LncRNA Identification using novel machine learning-based pipeline purely based on sequence information

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The rapid development of sequencing technology has leveraged the opportunity to discover many long non-coding RNAs (lncRNAs) transcripts. An array of sequencing techniques (e.g., microarray, RNA-seq, northern blot, etc.) have provided us a good number of lncRNA transcript catalogs from different labs across the globe. As a result, there is a significant variation in the lncRNA catalog generated from multiple labs, and the cost and time-consuming nature of sequencing techniques barred the research community from focusing on the identification of novel lncRNA transcripts. To overcome the inherent limitation of sequencing technologies, computational methods have been widely used in the literature for the identification of lncRNA. In this study, we propose a novel machine learning-based pipeline for the identification of lncRNA purely based on sequence information. We formulated this as a classification framework for distinguishing lncRNA from mRNA. As a feature set, we used k-mer, information of the open reading frame, the Fickett score, UTR regions, GC content, and HMMer score to distinguish the lncRNA transcripts from the mRNA transcripts. On the recent version (Release 37) of the GENCODE catalog for Human (GRCh38), the proposed random forest model outperformed all other existing models with an AUC accuracy of 99%. For the very first time, we also tested the proposed model for the FANTOM CAT lncRNA catalog, having high accuracy. We believe our tool will support the research community to identify the lncRNA transcripts in an easy and inexpensive manner.

sciforum-039200: microRNAs link obesity and cancer

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Obesity is a risk factor for several cancer types, suggesting shared molecular mechanisms. We identified cancer-relevant microRNAs that respond to metabolic hormone signaling in cultured cells and/or to metabolic changes in human subjects.

In collaboration with Lorna Harries (Exeter University, UK), we reported that miR-10b is more strongly downregulated in the primary breast tumors of obese patients, demonstrating that the metabolic state of the organism can lead to a significant difference in the molecular pathology of cancer (Meerson et al, BMC Cancer 2019). In ductal but not lobular tumors, significant inverse correlations were observed between the tumor levels of miR-10b and miR-30c and the mRNA levels of cancer-relevant target genes SRSF1, PIEZO1, MAPRE1, CDKN2A, TP-53, and TRA2B, as well as the tumor grade. Suppression of miR-10b levels in BT-549 primary BC-derived cells increased cell proliferation and invasive capacity, while exogenous miR-10b mimic decreased invasion. Manipulation of miR-10b levels also inversely affected the mRNA levels of miR-10b targets BCL2L11, PIEZO1, and NCOR2.

Previously, we reported that in cultured colon cancer cells, miR-4443 was upregulated by leptin and insulin in a MEK1/2-dependent manner. MiR-4443 overexpression decreased invasion and proliferation and directly downregulated NCOA1 and TRAF4, genes involved in metastasis. Insulin and/or leptin resistance (e.g., in obesity) may suppress this tumor-suppressive pathway and increase cancer risk (Meerson et al. BMC Cancer 2016). Supporting this notion, the miR-4443 locus is frequently deleted in cancers. Our recent publication showed that miR-4443 is a non-canonical microRNA with a yet-unknown biogenesis pathway (Meerson, Biomolecules 2020).

Our current research explores the mechanisms that regulate these cancer-relevant microRNAs under conditions of obesity. Understanding the roles of metabolically controlled microRNAs may help to improve the risk assessment, diagnosis, and treatment of metabolism-dependent cancers.

sciforum-044578: Regeneration of Drosophila wing imaginal discs is impaired upon the loss of lncRNAs

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Long non-coding RNAs (lncRNAs) have emerged as potential regulators of many different processes, from chromatin remodeling to post-transcriptional regulation. Although the expression of lncRNAs is generally lower than coding genes, their expression pattern is highly specific, and clear differences are usually observed between different tissues and developmental stages. LncRNAs generally participate in highly regulated processes, which may not be compromised upon the loss of the lncRNA expression. Using genome-wide approaches to interrogate chromatin dynamics, we recently identified the regulatory elements governing tissue recovery in *Drosophila* imaginal discs, which show a high regenerative capacity after genetically induced cell death [1]. Here, we propose a robust set of non-coding genes differentially expressed during the early stages of regeneration. Among them, the lncRNA genes *CR40469* and *MRE16* are present in this gene set and show an antagonistic expression pattern in regeneration, being upregulated and downregulated, respectively, during the early recovery process. Mutant flies for either *CR40469* or *MRE16* are able to develop normal wings under standard conditions; however, their regeneration capacity is clearly impaired upon damage, suggesting a putative role of these lncRNAs during the early stages of regeneration.

sciforum-044902: Analysis of long non-coding RNA driver mutations in lung adenocarcinoma

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Background. Lung adenocarcinoma (LUAD) is one of the major subtypes of lung cancer, the leading cause of cancer death worldwide. An average LUAD tumor accumulates ~1,000--10,000 point mutations, but only ~4-5 mutations per tumor are directly responsible for tumorigenesis (“driver” mutations). Currently, up to ~40--50% LUAD tumors have no identified drivers. Long non-coding RNAs (lncRNA) play critical roles in all hallmarks of cancer, but their mutational landscape is largely unexplored. Here, we aimed to identify novel LUAD drivers among lncRNAs.

Methods. We performed targeted next-generation sequencing on genomic DNA from 70 LUAD primary tumors, 27 paired normal samples, and 38 LUAD cell lines. We developed a novel pipeline to identify high-confidence somatic point mutations by combining three state-of-the-art tools. In addition, we corroborated our findings in a whole-genome sequencing dataset from The Cancer Genome Atlas (TCGA-LUAD). Then, to identify candidate driver lncRNAs, we searched for signals of

positive selection in our mutational data by combining OncoDriveFML and OncoDriveCLUSTL. Finally, we manually curated the candidates based on various genomic, regulatory, and population features of the affected lncRNAs and their mutated nucleotides.

Results. In unpaired analyses, we identified 3435 mutations in lncRNAs in our LUAD primary tumors and 3379 in cell lines. In paired analyses, we found 806 mutations in our LUAD primary tumors and 736 in TCGA-LUAD. We identified 57 candidate driver lncRNAs based on exonic or promoter mutations. After manual curation, we obtained three high-confidence candidate driver lncRNAs based on mutations in exons and two based on mutations in promoters. Experimental validations are underway to confirm whether two of these candidates are true LUAD drivers.

Conclusion. A small but relevant set of lncRNAs may accumulate driver point mutations in LUAD. Experimental validations are crucial to confirm the driver role of the identified mutations in lncRNAs.

sciforum-045013: Identification and characterization of novel long non-coding RNAs associated with multiple skeletal muscle atrophy

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Skeletal muscle is a mechanically pivotal organ, and its mass decreases through aging, immobilization, malnutrition, and diseases, including cancer, cardiovascular diseases, and neurodegenerative disorders. The loss of skeletal muscle mass, also known as muscle atrophy, decreases the quality of life, survival rates, and healthy life expectancy in humans. We previously revealed the molecular function of long non-coding RNA (lncRNA) *Myoparr* as a novel inducer of skeletal muscle atrophy (Hitachi et al., *EMBO Rep*, 2019; *Noncoding RNA*, 2019). Using multiple mouse models of muscle atrophy, we also found that several lncRNAs, including *linc-MD1* and *LncMyoD,* showed altered expression dependent on the models of muscle atrophies (Hitachi et al., *IJMS*, 2020). However, the molecular function of lncRNAs in muscle atrophy is not fully revealed. In this study, we tried to identify novel lncRNAs associated with skeletal muscle atrophy by RNA-sequencing using four muscle atrophy conditions (denervation, casting, fasting, and cancer cachexia) in mice. As a result, we successfully identified 33 annotated lncRNAs and 18 novel/unannotated lncRNAs with common expression changes in all four muscle atrophy conditions. Further analysis focusing on lncRNA–mRNA expression-correlation identified four lncRNAs that affected small-molecule biosynthetic processes during muscle atrophy (Hitachi et al., *IJMS*, 2021). Thus, our results provide novel insights into the molecular function of lncRNAs in muscle atrophy and may be useful for the identification of promising therapeutic targets against human muscle atrophy.

sciforum-045055: Circular RNAs to predict outcome after cardiac arrest

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Introduction: Out-of-hospital cardiac arrest (CA) is one of the most devastating disease worldwide. An average 400,000 people are affected each year in Europe. Among the patients admitted to the intensive care unit (ICU), the mortality rate is approximately of 50%. The current multimodal approach and biomarkers recommended to predict clinical outcome after cardiac arrest lack accuracy. Hence, the discovery of novel and specific prognostic biomarkers is necessary. Among the non-coding RNA family, circular RNAs (circRNA) seem to have some biomarker potential.

The goal of this project is to identify circulating circRNAs able to predict outcome after CA.

Methods: Whole blood samples were collected from patients of the target temperature management trial (TTM-trial; NCT01020916) 48h after CA. A subgroup of 46 patients were enrolled in a discovery phase by RNA sequencing (RNA-seq). In total, 23 patients survived with no major neurological sequelae (cerebral performance category score 1 – CPC1) and 23 patients died within 6 months (CPC5). The expression levels of candidate circRNAs identified in RNA-seq experiments were measured by quantitative RT-PCR in the entire cohort of the TTM-trial (n=542).

Results*:* A total of 27 circRNAs were differentially expressed between the CPC1 and CPC5 groups (p-value 0.05; fold-change ≥1.5). Among these, a circRNA named here circ01 predicted 6-month neurological outcome with an odds ratio (OR) +/- 95% confidence interval (CI) of 1.4 (1.15-1.63). This prediction was preserved after adjustment with demographic and clinical parameters (OR +/- 95% CI 1.36 (1.06-1.76)). Kaplan-Meier curves and Cox proportional hazards analysis (adjusted hazards ratio for the prediction of survival: 1.3 (1.1-1.5)) confirmed the ability of circ01 to predict survival at the end of the trial. Patients with high levels of circ01 were at higher risk of poor outcome after CA.

Conclusion: Circulating levels of circ01 measured 48h after CA predict neurological outcome and survival.

sciforum-045068: 45A ncRNA expression impairs microtubules dynamics affecting drug response in neuroblastoma

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45A non-coding (nc) RNA overexpression induces deep modifications of neuroblastoma (NB) cell cytoskeleton leading to a cascade of reactions which interferes with proliferation control, cell migration, tumorigenic potential, and cell adhesion properties.
In this work, we investigate the association between 45A ncRNA expression and the tuned regulation of several proteins involved in microtubules dynamics with relevant effects on cancer development. Indeed, the proper function of these proteins is fundamental for the cytoskeleton organization and its impairment leads to the particularly dangerous condition known as chromosomal instability (CIN).
We performed real-time qPCR, western blot, and immunocytochemical analysis to evaluate the different expression and localization of G2 And S-Phase Expressed 1 (GTSE1), Mitotic Centromere-Associated Kinesin (MCAK), Aurora B, p53, and the altered organisation of tubulin in different NB cell (SKNBE2) models stably overexpressing or downregulating 45A ncRNA.
We demonstrate that 45A ncRNA not only directly regulates the expression of aforementioned proteins but can even affect the subcellular localization of GTSE1: in 45A-overexpressing cells the protein is accumulated in nuclei, while 45A-downregulation leads to a significant GTSE1 cytoplasm relocation (nuclear GTSE1 being 86% vs 14%, respectively, p0.01). The simultaneous cytoplasmic sequestration of p53, driven by GTSE1, affects apoptosis leading to the detected resistance of 45A-downregulating cells to spindle poisons (e.g., paclitaxel, vincristine, vinblastine). Furthermore, 45A-overexpression leads to an increased number of abnormal spindles, probably driven by the observed dysregulation of Aurora B and MCAK during mitosis phases, thus promoting CIN and possibly explaining the increased tumorigenic potential exhibited by 45A-overexpressing cells. These data highlight the role of 45A ncRNA in the regulation of the expression of several proteins involved in microtubules dynamics, causing variations in drug response, and suggesting its possible relevance in NB prognosis and therapy.

sciforum-045096: Investigating the oncogenic role of the lncRNA Gracile2 in gastric cancer

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Gastric cancer ranks fifth in prevalence and second in mortality on a global scale. The ranking difference between frequency and mortality is due to delayed diagnosis since early stages of the disease are usually asymptomatic. Early diagnosis of gastric cancer relies mainly on gastroscopy, which is unsuited for routine monitoring of the population on a large-scale. Therefore, development of novel and specialized markers for early and non-invasive diagnosis and therapy is imperative. LncRNAs refer to regulatory non-coding transcripts that are involved in multiple aspects of physiology and pathology, often ina tissue- or cancer-specific manner, and, therefore, are regarded as valuable diagnostic and therapeutic agents. During our study, we focus on the characterization of GrACILe2, a lncRNA that is over-expressed in all stages of gastric cancer, with its expression levels correlating with the survival probability of the patient. We evaluated the half-life time point for the transcript, as well as its subcellular localization in gastric cancer cells. Additionally, we performed a lenti-viral mediated knock-down of GrACILe2, and after observation of the phenotype, we proceeded to an RNA-seq experiment to detect possible downstream target genes that are directly or indirectly targeted by GrACILe2 function in the background of gastric cancer cells.

sciforum-045116: Functional dissection of IGH enhancers and eRNAs in B-cell lymphoma

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Characteristic feature of B-cell non-Hodgkin lymphomas are recurrent translocations juxtaposing an oncogene (e.g., *MYC, BCL2*) with immunoglobulin heavy chain (IGH) enhancers: Eμ and 3'regulatory regions (3'RR1, 3'RR2). Survival of many B-cell lymphomas depends on the expression of the translocated oncogene. The function of IGH enhancers in B-cell maturation is well established, while the precise mechanisms of their involvement in oncogene expression and lymphomagenesis are yet to be determined. Enhancer RNA (eRNA) transcription is a feature of active enhancers. This class of non-coding RNAs plays a role in transcriptional regulation by recruitment and trapping of transcription factors, chromatin remodelers or RNA Pol II. Our goal is to identify functional elements in the IGH enhancers and eRNAs transcribed from them, essential for oncogene expression, and B-cell lymphoma cell growth.

We performed a tiling CRISPR interference screen in two Burkitt lymphoma (BL41, DG75) cell lines with an sgRNA library densely covering the IGH enhancers. Cells were harvested after transduction (T0) and further cultured for 25 population doublings (T1). Changes in abundance of sgRNAs in the cell pool at T1 vs T0 were determined by NGS. We identified 680 sgRNAs at least two-fold depleted in DG75 and 279 in BL41. Sliding window analysis revealed three 500-700 bp regions, one in the Eμ and two in the 3RR enhancers, whose targeting profoundly inhibited BL cell growth. In parallel, we performed chromatin-associated RNA-Seq in lymphoma and normal B cells, which confirmed ongoing transcription at each IGH enhancer and identified several differentially expressed eRNAs and lncRNAs. Subsequent validation of sgRNAs targeting CRISPRi-essential regions confirmed their inhibitory effect on eRNA and c-MYC expression in BL cells. The precise role of these regions will be further examined in IGH translocation positive B-cell lymphomas.

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sciforum-045125: 7SK acts as an anti-tumor factor in tongue squamous cell carcinoma

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Mounting evidence has provided mechanistic insights about non-coding RNA 7SK in controlling transcription. However, the biological function and mechanism of 7SK in cancer are largely unclear. Here, we showed that 7SK was downregulated in human tongue squamous carcinoma (TSCC) and acted as a TSCC suppressor through multiple cell-based assays including migration assay and a xenograft mouse model. The expression level of 7SK was negatively correlated with the size of tumor in the 73 in-house collected TSCC patients. Through combined analysis of 7SK knockdown RNA-seq and the available published 7SK ChIRP-seq data, we identified 27 of 7SK regulated genes that were involved in tumor regulation and whose upstream regulatory regions were bound by 7SK. Motif analysis showed that the regulatory sequences of these genes were enriched for transcriptional factors FOXJ3 and THRA, suggesting a potential involvement of FOXJ3 and THRA in 7SK regulated genes. Interestingly, the augmented level of FOXJ3 in TSCC patients and previous reports on THRA in other cancers suggested that these two factors may promote TSCC progression. Supportively, we found that 21 out of 27 aforementioned 7SK-associated genes were regulated by FOXJ3 and THRA, and 12 of them were oppositely regulated by 7SK and FOXJ3/THRA. We also found that FOXJ3 and THRA dramatically promoted migration in SCC15 cells. Collectively, we identified 7SK as an anti-tumor factor and suggested a potential involvement of FOXJ3 and THRA in 7SK-mediated TSCC progression.

sciforum-045136: Investigating the cellular function of the NEAT1_1 lncRNA in castration-resistant prostate cancer

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Long non-coding RNAs (lncRNA) are highly dysregulated in cancer and are emerging as therapeutic targets. The lncRNA NEAT1 consists of two overlapping isoforms, with the well-studied longer NEAT1_2 (23 kb) responsible for scaffolding gene-regulatory nuclear bodies termed paraspeckles. In contrast, the lesser-studied NEAT1_1 (3.7 kb) is not sufficient for paraspeckle formation and has paraspeckle-independent roles. Paraspeckle-independent nuclear foci containing NEAT1_1 have been dubbed microspeckles, and NEAT1_1 is emerging as an oncogenic lncRNA in different cancers.

The NEAT1 isoform ratio is dependent on the efficient cleavage and polyadenylation of NEAT1_1 at the expense of NEAT1_2. In neuroblastoma, a targeted antisense oligonucleotide (ASO) approach to sterically block NEAT1_1 processing was achieved, resulting in upregulation of NEAT1_2 at the expense of NEAT1_1 expression. NEAT1_1 downregulation reduced neuroblastoma cell viability. These NEAT1 ASO RNA therapeutics can transiently decrease NEAT1_1 levels, yet a more persistent change in NEAT1_1 expression is required for further characterisation. Here, we aim to identify the role of NEAT1_1/microspeckles in prostate cancer, where NEAT1_1 has already been reported to bind to active chromatin sites and positively upregulate oncogene expression. We aim to investigate the biophysical properties of microspeckles, including NEAT1_1-binding proteins, genomic regions and RNA transcripts to uncover the structure of microspeckles. We aim to utilise CRISPR-Cas9 genome editing to create NEAT1_1-expressing stable cell lines, as well as incorporate patient-derived prostate cancer organoids, to decipher the functional role of NEAT1_1. An in-depth investigation of NEAT1_1 may lead to the development of further NEAT1-RNA therapeutics.

sciforum-045139: Highlighting the RIDL (repeated insertion of long non-coding RNAs) domains derived from transposable elements in cancer

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Cancer features include genomic instability and epigenetic modifications that may be associated with the activation of transposable elements (TEs). The RIDL (repeated insertion of long non-coding RNAs) domains are insertions of repetitive sequences that influence the long non-coding (lncRNAs) function, including regulatory roles in cell processes. We investigated lncRNAs differentially expressed in eight types of cancer compared to their non-tumoral counterparts using data from The Cancer Genome Atlas; among them, we selected the lncRNAs containing sequences of RIDLs (lncRNA-RIDLs) and found 944 lncRNA-RIDLs genes differentially expressed in cancer. Three lncRNA-RIDLs—SNHG3, MAGI2-AS3, and PVT1—stood out for their presence in at least seven types of cancer; their RIDLs were identified as FRAM, L1MC4, and MIRb, respectively. In the top-20 most-differential lncRNA-RDILs, 81% of genes were associated with only one tumor type and have been represented in a heatmap; furthermore, 44% were associated with survival time in at least one tumor type, CARMN, LINC00261, LINC00511, and MIR99AHG being the lncRNA-RIDLs associated with survival in more cancer types. In the list of the top-20 lncRNA-RIDLs in the eight types of cancer, the TE class/order that was more abundant was SINE, and the TE superfamily was MIR. Additionally, among a compilation of 122 lncRNAs with causal roles in cancer phenotypes, 19 of these genes have RIDLs. The lncRNA-RIDLs FENDRR, ADAMTS9-AS2, MIR99AHG, LINC00511, PVT1, LINC00982, and LINC00261 were highlighted based on their high differential expression and association with survival time in patients, recognized as having causal roles in cancer. Our study has brought additional knowledge about lncRNA-RIDLs genes and new possibilities for the identification of biomarkers in cancer, reinforcing the importance of additional studies in the TEs domain in lncRNA functions.

sciforum-045155: The impact of molecular hydrogen application during heart transplantation: expression of selected miRNAs

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Heart transplantation is usually the last option for patients with acute and chronic heart failure. This procedure offers a significantly better chance for patients to prolong their life. However, despite the progress in heart preservation and improved surgery techniques, there are still unavoidable negative consequences after surgery due to the presence of ischemia/reperfusion and the production of reactive oxygen species. Molecular hydrogen is a novel substance with significant antioxidant and anti-inflammation capacity used in many experimental models and clinical trials on various diseases.

This study aimed to examine the effect of molecular hydrogen on the heart allograft of pigs (female Přeštice black-mottled pig, 4 months old). The simulation of heart transplantation consisted of occluding *venae cavae* and pulmonary veins, cross-clamping of ascending aorta, and connection to extracorporeal circulation (ECC). Cold crystalloid cardioplegia (Custadiol) was administered for 3 hours. After the time of cold arrest, the coronary arteries were flushed with Plasmalyte solution and the aortic clamp was released. This was followed by rewarming the heart. After 60 minutes of reperfusion, the pig was detached from extracorporeal circulation and the experiment was terminated. Molecular hydrogen was administered in two forms: saturated in Custadiol (at least 1 ppm) and in gas form during oxygenation of blood (50% O_2, 3% H_2). In this experiment, levels of lactate dehydrogenase, thiobarbituric acid reactive substances, and tumor necrosis alpha expression were reduced in pigs with hydrogen treatment. In addition, the expression levels of miRNA-1, miRNA-21, and miRNA-107 were normalized in hearts where hydrogen was administered compared with non-treated hearts.

In conclusion, based on the results of this study, we demonstrated that the addition of molecular hydrogen during heart transplantation could be a new potential therapeutic strategy for

minimalizing the negative effect of ischemia/reperfusion injury, leading to better recovery of patients.

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sciforum-045162: Exploring the role of MicroRNA variation in Multiple Sclerosis pathology

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Genome-wide association studies (GWAS) have provided insights into the genetic architecture of complex conditions including Multiple Sclerosis (MS). While over 200 autosomal variants have been associated with MS through GWAS studies, variants in non-coding sequences have not been explored adequately despite strong evidence of miRNA dysregulation in MS model organisms.

This research hopes to bridge that gap by using bioinformatics tools to generate a miRNA prioritization pipeline in order to interrogate MS GWAS studies. To achieve this, open-access catalogues including dbSNP and miRBase have been utilised to identify all existing single nucleotide polymorphisms (SNP) in miRNA genes. So far, 2,434 common variants in miRNA genes have been identified within seed, non-seed, mature, and precursor regions. In addition, miRNA target genes have been collated by their presence in prediction tools such as RNA22, TargetScan, miRDB, miRWalk, DIANA-microT, experimental validation datasets such as CLASH, as well as within the literature. This collation step primarily focuses on target gene variants within 3'UTR-associated miRNA binding sites. Additionally, by integrating variant frequency data from GNOMAD and the 1000genomes project, we will explore the population structure of these miRNA-associated variants.

Linkage disequilirium (LD) information from the 1000genomes reference panel will, therefore, be leveraged for imputation of these miRNA and target gene SNPs into the summary statistics used in the most recent MS GWAS (2019). Next, association analysis will be carried out on those imputed summary statistics. Specifically, variants in miRNA genes will be imputed and tested separately from variants in nominated target genes. The validation status of the resulting target gene susceptibility variants will be examined, alongside the location and putative effects of the miRNA gene risk variants. Results from these analyses will then be enhanced by delving further into cell and tissue-specific association studies. The ultimate aim is to integrate this insight with clinical data to create a predictive model for causation and progression of the condition.

sciforum-045164: Identification of lncRNAs that are regulated by lineage-survival transcription factors in gastric cancer

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Gastric cancer is globally characterized by high incidence and mortality rates compared to other types of cancer. This malignancy is categorized into many subtypes based on histological and molecular criteria, highlighting the strong heterogeneity that is typically observed among patients. The high mortality rates relate to the delayed diagnosis of the disease, which is asymptomatic in its early stages. Therefore, it is imperative to develop highly specialized and non-invasive markers for molecular diagnosis. Biomedical research that focuses on the molecular and genetic background of gastric cancer has discovered oncogenes that predispose patients to gastric cancer due to the genetic amplification of their loci. Such loci include lineage-survival transcription factors that are normally involved in the maintenance of specific cell types during the developmental stages of the stomach. Such factors physically interact, forming oncogenic regulatory complexes with crucial roles in gastrointestinal cancer progression. However, their pivotal regulatory function also extends to normal tissues, preventing their direct therapeutic targeting and drawing attention to their tissue-specific targets or interactors. Such targets are lncRNAs that frequently specialize in the function of transcription factors through their tissue-specific and often cancer-specific expression during the progression of the disease. The goal of this study is to identify lncRNAs that are directly regulated by lineage-survival transcription factors in the context of gastric cancer. To this end, bioinformatics analysis was performed using a combination of ChIP and RNA-seq data from patient biopsies and gastric cancer cell lines. Using an optimized lentiviral transduction system, transient silencing of a lineage survival transcription factor was achieved, the cellular phenotype of its knock-down was characterized, and experimental validation of three candidate lncRNAs that are directly controlled by this transcription factor was performed in the background of gastric cancer cells.

sciforum-045172: LncRNA expression profile in cisplatin-tolerant lung adenocarcinoma cell lines

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Background: Platinum-based chemotherapy is the cornerstone of treatment for advanced lung tumors. However, not all patients benefit from cisplatin therapy as some show intrinsic resistance. Cisplatin-tolerant tumor cells are those that remain viable after one or more exposures to treatment. These drug-tolerant cells may induce cancer relapse. Long non-coding RNA (lncRNA) are molecules exerting transcriptional and epigenetic regulatory functions. Aberrant expression of lncRNA has been found in cancer to play an important role in the development of chemotherapy resistance. However, no reports indicate the participation of lncRNA in the intrinsic resistance to cisplatin in lung cancer. A comparative analysis of the transcriptomes of tolerant cells from lung adenocarcinoma cell lines with different degrees of sensitivity to cisplatin identified a profile of lncRNAs associated with resistance.

Materials and methods: Lung adenocarcinoma cell lines A549, H1299 and H1975 were obtained from the ATCC; 3B1A cells were derived from a patient. Cisplatin cytotoxic effect was measured using the MTT assay at 24 h of treatment. We evaluated cell death by Annexin-V/PI assay, activation of caspase-3/7 and release of lactate dehydrogenase. We sequenced total RNA from drug-tolerant cells, and differential expression analysis was executed preserving lncRNA showing a log2-fold change > |1| and p adjusted value 0.05.

Results: Cisplatin induced a cytotoxic effect mediated by apoptosis in the studied cell lines. Interestingly, cell lines showed distinct levels of sensitivity to cisplatin. PCA showed that cisplatin-

tolerant cells clustered together and separated from untreated cells. Drug-tolerant cells exhibited differential expression of 195 lncRNA (99 over-expressed and 96 sub-expressed). Using the differentially expressed lncRNA, cell lines clustered according to their degree of sensitivity to cisplatin.

Conclusions: The lncRNAs identified and was associated with the mechanisms of intrinsic resistance to chemotherapy can be used as response markers to treatment in patients with lung adenocarcinoma.

sciforum-045191: Assessing the Landscape of Long Noncoding Transcripts in Breast Cell Populations

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Breast cancer is a heterogeneous disease characterised by multiple subtypes, each with distinct molecular features and clinical outcomes. Notably, one of the major factors that define these molecular features is the cell of origin. Thus, delineating the different cell types in the normal breast is critical for understanding the aetiology of breast cancer. Single-cell RNA sequencing (scRNAseq) is allowing us to exploit differential gene expression as a means to define cell types. Recent single-cell studies on normal breast tissue have discovered new cell types and mapped the trajectory of mammary epithelial lineages. However, the expression of unannotated genes is lost in these studies, as only sequencing reads that map to known genes are quantified. Importantly, the majority of the unannotated genes are long noncoding RNAs (lncRNA), often undetected by current methods. In the present study, we implemented a state-of-the-art computational pipeline to annotate thousands of new lncRNAs and applied to deep RNAseq of sorted cells from normal breast samples. We then used available scRNAseq data to comprehensively map the transcriptional landscape of these new lncRNAs across subpopulations of cells within the tissue. We found that clustering cells according to the expression levels of lncRNAs alone, we were able to recapitulate well-defined subpopulations of breast cells. Using this strategy we were also able to identify new lncRNA markers for breast cell populations. Mapping the transcriptional landscape of lncRNAs across the normal breast can, therefore, better characterise these subpopulations and identify lncRNAs implicated in breast cancer and linked to specific subtypes. As products of these genes may represent subtype-specific drug targets, our results can support subsequent studies in the field of biomarker discovery and pave the way for novel treatment strategies targeting lncRNAs, thus contributing to future breast cancer prevention and therapy.

sciforum-045215: Cancer cells refine their fitness via function-altering mutations in long noncoding RNAs

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Tumours evolve through fitness-altering somatic mutations that enable cells to replicate uncontrollably and invade new sites. Genes carrying these “driver” mutations are prime therapeutic targets, and hundreds of protein-coding driver genes have been identified through targeted exome sequencing. However, the lack of entire tumour genomes has made it impossible to assess the existence of driver elements in the non-protein coding genome, including the thousands of long noncoding RNAs (lncRNAs) that are known to play roles in tumorigenesis.

This hurdle has recently been overcome thanks to the Pan Cancer Analysis of Whole Genomes (“PCAWG”) project. By comprehensively mapping mutations across >2500 tumour genomes, PCAWG has enabled comprehensive screens for hypothetical driver lncRNAs. Such screens make use of the fact that, due to their effect on cell fitness, driver mutations display signatures of positive evolutionary selection.

We have developed a bioinformatic pipeline, ExInAtor2, which identifies candidate driver lncRNAs using integrated signatures of positive evolutionary selection from mutational burden (MB) and functional impact (FI). Applying ExInAtor2 to thousands of primary and metastatic tumour genomes, we present a panorama of putative driver lncRNAs across multiple cancer types. The dozens of driver lncRNAs display genomic and clinical features of genuine cancer genes. Furthermore, we have validated their contributions to cell fitness by knockdown and over-expression in a variety of cancer types.

To further test the notion that lncRNAs can act as driver genes, we employed CRISPR-Cas9 to model the mutagenic processes that take place in tumour cells. Taking the example of a well-known cancer-associated lncRNA, we find that small nucleotide variants (SNV) at several locations reproducibly give rise to alterations in nuclear architecture and accelerated cell growth.

In conclusion, we present a range of evidence that transformed cells can hone their fitness via function-altering mutations in lncRNAs, and lay the foundation for new therapeutics targeting these genes.

sciforum-045216: A lncRNA-NF90 interaction confers chemoresistance in liver cancer

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Background: Long non-coding RNAs (lncRNA) regulate DNA damage response (DDR), a major obstacle to effective chemotherapy, but their effect on hepatocellular carcinoma chemoresistance remains largely unknown.

Methods: Chemotherapy-resistant liver cancer cell lines were established and whole-transcriptome analysis was performed. A series of functional assays were performed following lncRNA transient knockdown using LNA GapmeRs, stable knockdown through three shRNAs or stable overexpression through lentiviral transduction. Cell growth was interrogated through luminescence viability, live cell analysis (IncuCyte) and colony formation assays. LncRNA expression and cellular localization was assessed in liver human tissues by RT-qPCR and in situ hybridization, respectively. LncRNA-interacting protein partner(s) were identified by RNA pull-down experiments followed by mass spectrometry (MS) and verified by protein-based RNA immunoprecipitation. DNA damage and apoptosis-associated genes were assessed through western blot analysis or RT-qPCR following treatment with different DNA damage-inducing drugs. DNA damage and apoptosis were evaluated through Comet and IncuCyte caspase-3/7 assays, respectively.

Results: Transcriptome analysis revealed 15,857 up-regulated and 22,332 down-regulated lncRNAs in chemoresistant liver cancer cell lines. Among 31 lncRNAs, a novel lncRNA was identified to exhibit the strongest effect on cell growth upon GapmeR silencing. LncRNA depletion inhibits, whereas its overexpression induces liver cancer cell growth and invasion. In patients, the nuclear-dominant lncRNA was significantly overexpressed in liver cancer tissues when compared to normal. Importantly, sensitivity of cell growth to chemotherapy is enhanced upon lncRNA silencing. DDR is an intrinsic biological factor affecting treatment response and tolerance, and NF90, a DDR regulator, was identified as lncRNA-interactor. LncRNA overexpression inhibits, whereas its silencing induces DNA damage, as evidenced by phospho-H2AX and the comet assay. Interestingly, this effect is lost upon disruption of the lncRNA-NF90 interaction.

Conclusion: LncRNA-NF90 interaction regulates liver cancer chemoresistance, through the DNA damage response pathway, and its disruption may be exploited as novel therapeutic approach.

sciforum-045260: Characterization of novel cancer-associated long non-coding RNA and its probable murine homolog

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Cancer development and progression are often followed by changes in expression levels of different long non-coding RNAs (lncRNA). These cancer-associated lncRNAs are widely considered as perspective tools in early diagnostics of carcinogenesis, probable targets for cancer therapy or prognostic biomarkers. However, thorough investigation of lncRNAs and their functions is often limited due to the lack of appropriate animal models. LncRNAs usually share poor sequence homology between species, which implies difficulties in the identification of murine candidates. Moreover, analogous lncRNAs from human and mice often participate in different cellular pathways. In the present work we characterized a novel human lncRNA CHOL, which ismiserably expressed in different human tissues, but is up-regulated in cholangiocarcinoma (CCA) and pancreatic duct adenocarcinoma cancer (PDAC) tissues (in comparison to adjacent liver or pancreas samples, respectively). This lncRNA has extremely short length about 200 nt, but is comprised of several exons. We detected various CHOL isoforms with different expression patterns both in cancer tissues and in hepatic and pancreatic cancer cell lines. We also found a probable murine analogue of CHOL RNA, a ~800 nt transcript with an identical sequence of 27 nt. Further analysis revealed this region to be a complement for specific miRNA expressed both in human and mice. We also showed active expression of murine CHOL RNA exclusively in testis. Thus, we suggest these two lncRNAs to serve as sponges for miRNA, that might have function in cell proliferation. This work is supported by Russian Science Foundation grant No. 20-74-00141.

sciforum-045561: The long non-coding RNA Psoriasis Susceptibility-Related RNA Gene Induced by Stress (PRINS) is a biomarker for Invasive Breast Cancer

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Purpose:

Breast cancer accounts for approximately 25% of cancers in women worldwide. Although patients have benefited from advances in hormone therapy, no cure exists for the 8%--10% who have distant metastases at diagnosis. Advances in transcriptomics have uncovered the unique RNA signature of breast cancer, and long non-coding RNAs (lncRNA) have emerged as key players with the potential to be exploited for therapeutic purposes. *Psoriasis Susceptibility-Related RNA Gene Induced by Stress* (*PRINS*) is a novel lncRNA which is underexpressed in breast cancer in the cancer genome atlas (TCGA). The purpose of this study was to verify *PRINS* underexpression in breast cancer clinical samples and determine its clinical correlates, ultimately providing evidence for *PRINS* as a biomarker in breast cancer.

Methodology:

In total, 75 normal breast tissues, breast tumours and metastatic breast cancer tissues were obtained from The Kolling Institute Tumour Bank with de-identified clinical data. Total RNA was extracted using the RNeasy mini kit (Qiagen) and relative *PRINS* expression was determined using RT-qPCR. Clinical correlates of *PRINS* expression were determined.

Results:

Breast cancer primary tumours under-express *PRINS* relative to normal breast tissues (mean difference in log(2) *PRINS* expression 1.213; CI 95%, 0.389-2.038; p = 0.002), and *PRINS* relative expression is an excellent discriminator between them (AUC, 0.851; CI 95%, 0.748-0.954; p 0.001; sensitivity, 80%; specificity, 72%). Breast cancer metastases under-express *PRINS* relative to normal breast tissues (mean difference in log(2) *PRINS* expression 1.614; CI 95%, 0.790-2.438; p 0.001), but no significant difference in *PRINS* relative expression was detected between primary tumours and metastases. Subgroup univariate analysis revealed a significant effect of advanced primary tumour histological grade on *PRINS* relative expression ($F_{2,41}$ = 4.308; p = 0.021). In the normal breast tissues of patients with grade 1 tumours, mean log(2) *PRINS* expression was 1.909, compared with -1.384 in grade 2 and -0.165 in grade 3 tumours. In primary tumour tissue, mean log(2) *PRINS* expression was -0.556 for grade 1 tumours, -1.315 for grade 2 tumours and -1.701 for grade 3 tumours. These differences remained significant for grades 1 and 3 only on post-hoc Tukey analysis (p = 0.048).

Conclusion:

PRINS underexpression is a biomarker for invasive breast cancer. Further studies to investigate its molecular and intracellular actions are warranted to establish its functional significance in this disease.

sciforum-045570: Expression pattern of circRNA in primary and recurrent glioblastoma

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Glioblastoma (GBM) is one of the most lethal and the most aggressive malignant brain tumors. Recently, the world of non-coding RNAs has become a field of intensive research since the discovery of their essential impact on carcinogenesis. The role of circular RNAs (circRNAs) has been extensively studied in various diseases, and a growing body of evidence shows that their disruption may play an important role in tumor development. Circular RNA can affect cellular processes associated with cancer at many levels. They can sponge miRNAs and proteins and regulate transcription and parental gene translation, and eventually, they can also be translated into proteins. The aim of our study was to identify circRNAs differentially expressed in GBM and glioma stem cells that are potentially involved in GBM aggressiveness, invasiveness, and tumor recurrence. We performed RNA sequencing in which we analyzed 29 freshly obtained GBM samples, 3 glioma

stem cells-enriched samples, and 4 blood samples from GBM patients. We conducted differential analysis, distinguishing dysregulated circRNAs among primary and recurrent GBM samples, and we also managed to establish circular RNAs expression patterns among different GBM molecular subtypes, namely classical, mesenchymal, proneural, and neural. In our analysis, we identified over 29,598 circRNAs (4663 exclusive for primary GBM and 1255 for recurrent GBM), and further analysis resulted in distinguishing 978 downregulated circRNAs and 129 upregulated circRNAs in GBM samples. Moreover, we discovered circulating circRNAs with biomarker potential in blood samples. The accomplished large-scaled analysis now gives the good opportunity to select circRNA candidates for further structural and functional investigations, which will have importance for GBM development and progression.

sciforum-045676: Contribution of Musashi-2 to muscle dysfunction in myotonic dystrophy by upregulating autophagy through miR-7 biogenesis repression

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Skeletal muscle symptoms strongly contribute to mortality of myotonic dystrophy type 1 (DM1) patients. DM1 is a neuromuscular genetic disease caused by CTG repeat expansions that, upon transcription, sequester the Muscleblind-like family of proteins and dysregulate alternative splicing of hundreds of genes. However, mis-splicing does not satisfactorily explain muscle atrophy and wasting, and several other contributing factors have been suggested, including hyperactivated autophagy leading to excessive catabolism. *miR-7* has been demonstrated to be necessary and sufficient to repress the autophagy pathway in cell models of the disease, but the origin of its low levels in DM1 was unknown. We have found that the RNA-binding protein Musashi-2 (MSI2) is upregulated in patient-derived myoblasts and biopsy samples. Because it has been previously reported that MSI2 controls *miR-7* biogenesis, we tested the hypothesis that excessive MSI2 was repressing *miR-7* maturation. Using gene silencing strategies (siRNAs and gapmers), and the small molecule MSI2-inhibitor Ro 08-2750, we demonstrate that reducing MSI2 levels or activity boosts *miR-7* expression, represses excessive autophagy, and downregulates atrophy-related genes of the UPS system. We also detect a significant upregulation of MBNL1 upon MSI2 silencing. Taken together, we propose MSI2 as a new therapeutic target to treat muscle dysfunction in DM1.

sciforum-045708: Small ncRNAs derived from different Huntington's disease patients' brain regions induce diverse neuropathological outcomes in wild-type mice

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Progressive motor alterations and selective death of medium-sized spiny neurons in the caudate and putamen are key pathological hallmarks of Huntington's disease (HD), a neurodegenerative disorder caused by a CAG trinucleotide repeat expansion in the coding region of the huntingtin (*HTT*) gene. Most research has focused on the pathogenic effects of the resultant protein product(s); however, growing evidence indicates that expanded CAG repeats within mutant *HTT* mRNA and derived small CAG repeat RNAs (sCAG) participate in HD pathophysiology. The individual

contribution of protein versus RNA toxicity to HD pathophysiology remains largely uncharacterized and the role of other classes of small RNAs (sRNA) that are strongly perturbed in HD is uncertain.

Here, we show that sRNA produced in the putamen of HD patients (HD-sRNA-PT) are sufficient to induce HD pathology in vivo. Moreover, sRNA obtained from the motor cortex (as an affected region) or from the cerebellum (as a less-affected region) are able to differently compromise motor function in wild-type mice. This observation prompted us to identify which sRNA species are enriched in HD putamen and present neurotoxic potential. We detected high levels of tRNA fragments (tRF) in HD putamen, and we validated the neurotoxic potential of an Alanine derived tRF in vitro. These results highlight that HD-sRNA-PT are neurotoxic, and suggest that multiple sRNA species contribute to striatal neuropathology, favouring therapeutic strategies based on the blockage of sRNA-mediated toxicity.

sciforum-045722: Cancer LncRNA Census 2 (CLC2): an enhanced resource reveals clinical features of cancer lncRNAs

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Long non-coding RNAs (lncRNAs) play key roles in cancer and are at the vanguard of precision therapeutic development. These efforts depend on large and high-confidence collections of cancer lncRNAs. Here, we present the Cancer LncRNA Census 2 (CLC2). With 492 cancer lncRNAs, CLC2 is fourfold greater in size than its predecessor, without compromising on strict criteria of confident functional/genetic roles and inclusion in the GENCODE annotation scheme. This increase was enabled by leveraging high-throughput transposon insertional mutagenesis screening data, yielding 92 novel cancer lncRNAs. CLC2 makes a valuable addition to existing collections: it is amongst the largest, contains numerous unique genes (not found in other databases), and carries functional labels (oncogene/tumour suppressor). Analysis of this dataset reveals that cancer lncRNAs are impacted by germline variants, somatic mutations, and changes in expression consistent with inferred disease functions. Furthermore, we show how clinical/genomic features can be used to vet prospective gene sets from high-throughput sources. The combination of size and quality makes CLC2 a foundation for precision medicine, demonstrating cancer lncRNAs' evolutionary and clinical significance.

sciforum-045727: Structural impact and mechanism of Epstein-Barr virus encoded RNA-1-induced proliferation in lymphoid cell line

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Epstein-Barr virus (EBV) is a γ-herpesvirus associated with human malignancies. It infects and establishes latency in more than 90% of the human population worldwide. EBV-encoded RNAs (EBER) are two small capped, non-polyadenylated, non-coding, structurally conserved transcripts that are expressed in millions of copies per EBV-infected cell. EBERs have been proposed to promote the rate of cell growth. However, the mechanisms by which EBERs drive cell proliferation is poorly understood. Unravelling the functions associated with EBERs conserved structure could shed light on how this common virus causes malignancies. The objective of this study was to investigate the impact of EBER1 structure in relation to its proliferative advantage in a lymphoid cell line. We disrupted EBER1 structure by deleting individually, three of its stem-loops (SL) to create three mutants (DSL1, 3, and 4). These mutants were cloned into Hebo plasmid and expressed in Jurkat cell line. Wildtype EBER1 and Hebo plasmid transfected cells were used as positive and negative control cells, respectively. A total of $5x10^4$ cells were seeded, and cell growth was monitored daily by trypan blue dye. 7AAD viability dye was used to determine cell viability. Cell cycle progression and regulation were studied using PCR, western blot, and flow cytometry analyses. All statistical analyses were performed relative to EBER1 wildtype and $p \leq 0.05$ were considered significant. We found a significantly higher cell count and upregulation of the G2/M-phase genes in EBER1 compared to the Hebo, DSL1, and DSL3, but not DSL4 mutants. Similarly, EBER1 has more DNA content in G2/M-phase than Hebo or the mutants. There was differential expression of cell cycle regulatory markers; CDT1 protein was downregulated in Hebo and DSL1 transfectants. In conclusion, EBER1 conserved structure may be crucial in driving proliferative advantage by disrupting cell cycle regulations and prolonging S-phase.

sciforum-045736: MALAT-1 and miR-486-5p: A novel lncRNA-miRNA circuit tuning IGF-1R and its downstream JAK/STAT pathway in Breast Cancer

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Background

The Insulin-like Growth Factor (IGF) system has been recognized as one of the culprits responsible for the development and progression of breast cancer (BC). The activation of IGF-1 receptor (IGF-1R) is responsible for the activation of several oncogenic signalling pathways such as the JAK/STAT pathway. The deregulation of IGF-1R in several oncological contexts has prompted our research group to investigate the role of non-coding RNAs (ncRNAs) in its regulation. Our previous results have shown that miR-486-5p, miR-615-5p and let-7a are validated regulators of IGF-1R. However, the role of long ncRNAs in regulating IGF-1R still needs further investigation. MALAT-1 is an oncogenic lncRNA that demonstrated its sponging effects on several miRNAs affecting their respective targets. Therefore, the aim of this study is to investigate the role of MALAT-1 and miR-486-5p in regulating IGF/IGF-1R and its downstream JAK/STAT signalling pathways in BC.

Methods

Breast tumour biopsies and their normal counterparts were collected from 20 BC patients. MDA-MB-231 cells were cultured and transiently transfected using MALAT-1 siRNAs and miR-486-5p oligonucleotides using the lipofection method. Total RNA was extracted, reverse-transcribed then quantified using qRT-PCR.

Results

Screening revealed that MALAT-1, IGF-1R and STAT3 were upregulated in BC tissues. In contrast, miR-486-5p was downregulated. In MDA-MB-231 cells, ectopic expression of miR-486-5p and knockdown of MALAT-1 similarly resulted in significant repression of IGF-1R expression, thus highlighting IGF-1R as a common target dually regulated by MALAT-1 and miR-486-5p. However, knocking down of MALAT-1 repressed STAT3 levels, while miR-486-5p showed no effect on STAT3 in

MDA-MB-231 cells. Interestingly, a positive feedback loop was observed between MALAT-1 and miR-486-5p. This was evident in the increased expression levels of MALAT-1 upon induction of miR-486-5p and vice versa.

Conclusions

This study categorizes IGF-1R as a common target dually acted upon by MALAT-1 and miR-486-5p. Moreover, it underscores a novel lncRNA/miRNA crosstalk, MALAT-1/miR-486-5p, as an upstream ceRNA circuit regulating IGF-1R and its downstream JAK/STAT pathway. Therefore, this study crystallises the promising therapeutic role of ncRNAs in trimming the IGF-1R signalling pathway in aggressive BC phenotypes.

sciforum-045759: Long non-coding RNAs in the pathogenesis of focal segmental glomerulosclerosis (FSGS)

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Most kidney diseases are caused by a dysfunction of the renal filtration unit --- the glomerulus. Focal segmental glomerulosclerosis (FSGS) --- i.e., partial scarring of renal glomeruli --- is a histopathological pattern of injury common to many glomerular diseases. However, the pathogenesis of FSGS and the particular molecular mechanisms regarding different underlying etiologies remains incompletely understood. Specifically, the contribution of non-coding RNAs to FSGS is unclear. In this project, we focus on the role of long non-coding RNAs in primary FSGS to unravel novel pathogenetic mechanisms, as well as potential diagnostic and therapeutic strategies. Long non-coding RNAs (lncRNA) are defined by a length of above 200 base pairs and cannot be translated into proteins. These molecules were extensively reported to be involved in epigenetic, transcriptional, and post-transcriptional regulation, which points towards an important role in the

pathogenesis of many disease states. We use a combination of established FSGS mouse models to determine disease-associated lncRNA expression patterns coupled with cell culture experiments to elucidate their molecular function. Additionally, lncRNA knockout mouse models will be combined with analyses of human biopsy samples to test the potential of candidate lncRNAs identified by this approach as future therapeutic targets.

sciforum-045764: Examining host lncRNA expression patterns in response to SARS-CoV-2 infection

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Pathogenic human viruses, such as SARS-CoV-2, are able to interact with the host cells, hijacking their metabolic pathways to facilitate their own replication. Accumulated evidence shows that human viruses can accomplish this by selectively modifying the host's genomic output, using direct or indirect mechanisms. Indirect mechanisms often involve the interaction with the host cell's genomic regulators, such as non-coding RNA molecules (ncRNA). Among the different families of ncRNAs, long non-coding RNAs (lncRNA) have been involved in the regulation of the immune response in some viral infections such as HIV and HCV. LncRNAs are long-regulatory RNA transcripts generated from specific genomic loci, and can act at different cellular levels to regulate the genomic output by interacting either with proteins or with other nucleic acids. Using a cohort of patients tested for SARS-CoV-2 infection, we have studied the expression of lncRNAs in response to viral infection and postulated their involvement in regulatory events mediated by specific proteins.

The project involved transcriptomic analysis, by next generation sequencing, of clinical samples from patients tested for SARS-CoV-2 infection. The patient cohort includes nasopharyngeal swabs from 260 SARS-CoV-2-positive patients with different viral loads, 82 patients infected with other respiratory viruses and 429 non-infected controls. Expression analysis of lncRNAs from raw sequencing data of each patient sample on the Pleiades supercomputer at the NASA Advanced Supercomputing facility. Selected differentially expressed lncRNAs were functionally analyzed by combining gene expression information with already published host proteomic data in SARS-CoV-2 infection.

Our results showed that SARS-CoV-2 infection induces a transcriptional shift in the host cells, mainly characterized by an up-regulation of RNA transcripts that includes a specific group of lncRNAs. We determined a molecular lncRNA expression signature that is correlated not only with the SARS-CoV-2 infection but also with the viral load quantified by qPCR. Some of the differentially expressed lncRNAs, such as NRIR and BISPR, have been previously shown to be involved in the host response

against specific viral infections as mediators of the cellular immunity. We postulate that the overexpressed lncRNAs in response to SARS-CoV-2 infection will establish interaction networks with other cellular molecules such as RNA-binding proteins (RBP). To infer functional relationships of this lncRNA expression signature and RBPs, we took advantage of the already available proteomic data in SARS-CoV-2 infection (Bojkova et al., 2020, Nature, 583, 469). The observed lncRNA response to SARS-CoV-2 infection includes a group of up-regulated ncRNA transcripts that could interact with RBPs that are also up-regulated during infection. These RBPs are functionally related to RNA splicing and the interferon response. Further characterization of these interactions opens a new window for the development of therapeutic strategies that could target lncRNA function in SARS-CoV-2 and other viral infections.

sciforum-045776: Statin-induced epigenetic deregulation contributes to the development of type 2 diabetes mellitus

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Statin therapy is the mainstay of treatment for patients with familial hypercholesterolemia and despite its high effectiveness and safety, increasing evidence shows a greater incidence of type 2 diabetes mellitus (T2DM) among treated patients. Meta-analysis of randomized clinical trials shows a 10–12 % increased risk of new-onset T2DM associated with statin treatment. Although the mechanisms by which statin treatment induces T2DM remain not fully understood, some studies support the hypothesis that statins may disrupt glucose homeostasis through both impaired insulin secretion and diminished insulin sensitivity on target tissues. In this study, we sought to unravel the molecular mechanism by which statin treatment induces T2DM through modulating the expression of some miRNAs implicated in insulin signaling and glucose homeostasis.

We performed a gene expression analysis on genes previously associated with statin treatment, an in silico analysis of human genes encoding nuclear receptors and transcription factors known to affect glucose homeostasis, and gene expression analysis using miRNA mimics or antagomirs to ascertain the role of the upregulated microRNAs in the observed effect.

Our results show that statin treatment induces a downregulation of *INSR, INS, VDR, MAPK14,* and *P2Y2* mRNA levels that is mediated by the upregulation of miR-33 and miR-27b.

Here, we demonstrate that deregulation of microRNA expression induced by statin treatment contributes to the development of T2DM.

sciforum-045819: Describing evolutionary convergent lncRNAs involved in development by k-mer and RNA structural homology analyses

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In the last decades, thousands of long noncoding RNAs (lncRNAs) have been identified in almost all vertebrate species, but the functional characterization of these RNA molecules is being challenged. The lack of linear sequence homology between species is one of the main difficulties when studying lncRNA functionality. In this line, k-mer content and secondary structure-based analyses are emerging as powerful methods to identify functionally related lncRNAs among different species. In the present work, we have used these approaches to find evolutionary convergent lncRNAs involved in development.

EVX1AS is a lncRNA divergent to the *EVX1* coding gene, which is developmentally regulated and has been described to promote *EVX1* transcription in cis. Starting from transcriptomic data of the Madagascar gecko, we found an lncRNA with a similar k-mer content which was structurally concordant with the human *EVX1AS*. Using in vitro and in ovo locus-specific targeting of human and gecko *EVX1AS* lncRNA (i.e., CRISPR Display) in human neuroepithelial cells and chicken mesencephalon, we observed that the gecko *Evx1as-like* lncRNA mimics human *EVX1AS* lncRNA function and induces *EVX1* expression independently of the target species. Additionally, our results suggest that lncRNA-mediated induction of *EVX1* contributes to the alteration of other development-related genes such as *EVX2.*

Our results suggest that k-mer content and secondary structure-based analyses can help identify evolutionary convergent lncRNAs, contributing to the understanding of lncRNA involvement in different developmental pathologies. Indeed, the identification of convergent lncRNAs will allow the analysis and manipulation of their function in vertebrate development model species, such as zebrafish, chickens, or geckos, in which the genetic engineering at embryonic levels is accessible.

sciforum-045824: Analysis of competing endogenous RNAs network in neurodegenerative disorders caused by triplet repeat expansions

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Neurodegenerative polyglutamine (polyQ) diseases are caused by a CAG repeat expansion located in ORF of specific genes and include Huntington's disease (HD) and several spinocerebellar ataxias (SCAs). One example from the latter is SCA3, which is caused by mutation in the *ATXN3* gene. SCA3 is so far incurable and the initial pathogenic pathways are not well understood, especially those related to potential disruptions in a competing endogenous RNAs (ceRNAs) network.

We obtained a new SCA3 cellular model, stably expressing translated or non-translated cDNA of human *ATXN3*, generated in SH-SY5Y cells using the Flp-In T-REx system. Using RNA sequencing, we analyzed a set of samples obtained from cells after three days of ataxin-3 transgene expression induction. The differential expression of microRNAs (miRNAs) and potential ceRNAs such as mRNAs, long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs) were analyzed. Initially identified enriched processes include signal transduction and nervous system development pathways.

Actually, CAG repeat tracts are present in many protein-coding and -non-coding RNAs (ncRNAs). Using bioinformatic tools, we identified a relatively high number of CAG repeat-containing RNAs (possessing at least five units of CAG) in the human transcriptome: about 200 mRNAs and about 150 circRNAs as well as several dozen lncRNAs and pseudogenes (Witkos et al. RNA Biol). We also found a similar number of CUG repeat-containing RNAs, which are complementary to RNAs with CAG repeats. This opens the additional possibility of ceRNAs network functioning based on repeated tracts.

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sciforum-045825: Pan-Cancer chromatin analysis of the human vtRNA genes uncovers their association with cancer biology

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Background: The vault RNAs (vtRNAs) are a class of 84-141-nt eukaryotic non-coding RNAs transcribed by RNA polymerase III, associated with the ribonucleoprotein complex known as vault particle. Of the four human vtRNA genes, vtRNA1-1, vtRNA1-2, and vtRNA1-3, clustered at locus 1, are integral components of the vault particle, while vtRNA2-1 is a more divergent homologue located in a second locus. Gene expression studies of vtRNAs in large cohorts have been hindered by their unsuccessful sequencing using conventional transcriptomic approaches.

Methods: VtRNA expression in The Cancer Genome Atlas (TCGA) Pan-Cancer cohort was estimated using the genome-wide DNA methylation and chromatin accessibility data (ATAC-seq) of their genes as surrogate variables. The association between vtRNA expression and patient clinical outcome, immune subtypes, and transcriptionally co-regulated gene programs was analyzed in the dataset.

Results: VtRNA1-1 has the most accessible chromatin, followed by vtRNA1-2, vtRNA2-1, and vtRNA1-3. Although the vtRNAs are co-regulated by transcription factors related to viral infection, vtRNA2-1 is the most independently regulated homologue. The VtRNA1-1 and vtRNA1-3 chromatin status does not significantly change in cancer tissues. Meanwhile, vtRNA2-1 and vtRNA1-2 chromatin accessibility is widely deregulated in neoplastic tissues and its alteration is compatible with a broad oncogenic role for vtRNA1-2, and both tumor suppressor and oncogenic functions for vtRNA2-1. However, vtRNA1-1, vtRNA1-2, and vtRNA2-1 promoter DNA methylation predict a shorter overall cancer-wide patient survival rate. Gene ontology analyses of vtRNA co-regulated genes identify a chromosome regulatory domain, epithelial differentiation, and immune and thyroid cancer gene sets for specific vtRNAs. Furthermore, vtRNA chromatin accessibility patterns are associated with cancer immune subtypes and vtRNA1-2 expression is positively associated with cell proliferation and wound healing.

Conclusions: Our study presents the landscape of vtRNA chromatin accessibility cancer-wide, identifying co-regulated gene networks and ontological pathways associated with the different vtRNA genes that may account for their diverse roles in cancer.

sciforum-045828: Exploration of XIST role in lung cancer reveals a potential gene panel diagnostic biomarker and key cancer regulators

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Long non-coding RNAs (lncRNAs) perform a wide functional repertoire of roles in cell biology, ranging from RNA editing to gene regulation, as well as tumour genesis and tumour progression. The lncRNA X-inactive specific transcript (XIST) is involved in the aetiopathogenesis of non-small-cell lung cancer (NSCLC). However, its role at the molecular level is not fully elucidated. In this work, we investigated the expression landscape of XIST and co-regulated genes TSIX, hnRNPu, Bcl-2, and BRCA1 analyses in lung cancer (LC) and control samples. Differentially expressed genes (DEGs) were determined by analysing the RNA-seq data from H1975 and A549 NSCLC cell lines following siRNA for XIST. XIST exhibited sexual dimorphism, being up-regulated in females compared to males in both control and LC patient cohorts. RNA-seq revealed 944 and 751 DEGs for A549 and H1975 cell lines, respectively. These DEGs are involved in signal transduction, cell communication, energy pathways, and nucleic acid metabolism. XIST expression associated with TSIX, hnRNPu, Bcl-2, and BRCA1 provided a strong collective feature to discriminate between control and LC samples, suggesting a diagnostic biomarker potential. The results indicate that XIST plays a much more complex role in lung cancer and that further studies should concentrate on sex-specific changes to investigate the signalling pathways of the DEGs following silencing of this lncRNA.

sciforum-045829: In vivo single-cell profiling of long non-coding RNAs during Ebola virus infection

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Ebola virus disease (EVD) represents a major public health threat due to its high mortality rates and limited treatment options in humans. However, the precise pathways altered during infection and the host immune response have been poorly characterized. One emerging class of immune response regulators are long non-coding RNAs (lncRNA). LncRNAs have been shown to play a pivotal modulatory role in a variety of anti-viral response mechanisms, such as cytokine production, interferon-stimulated gene (ISG) transcription, and transcription factor activation. Nonetheless, their role in response to viral infections in vivo has not been thoroughly studied. Indeed, the study of highly infectious pathogens requires strict safety measures which have considerably limited in vivo transcriptome studies. To date, no single-cell study has been carried out describing the non-coding transcriptome variation upon infection of any biosafety level 4 (BSL-4) pathogen, like Ebola virus (EBOV).

Here, we perform an in-depth characterization of the expression changes of long non-coding RNAs at single-cell resolution throughout the course of EBOV infection. As a model system, we use Chinese rhesus macaque (Macaca mulatta). First, we build a full de novo annotation leading to the discovery of nearly 3,000 novel lncRNAs significantly improving the present macaque annotation. Then, we characterize lncRNA expression patterns at the single-cell resolution in peripheral blood

mononuclear cells (PBMC) during the course of EBOV infection. We show that lncRNAs are generally expressed in fewer cells than protein-coding genes but they can reach expression levels similar to those of protein-coding genes when expressed in a comparable number of cells. Our results also suggest that the long-assumed higher specificity of lncRNAs is overestimated due to their expression in fewer cells.

Upon infection, we find that both protein-coding genes and lncRNAs are dysregulated in a cell-type and disease stage-specific manner. We detected the strongest lncRNAs' dysregulation in monocytes, which is the only cell-type actively infected by EBOV. In total, we report 93 dysregulated lncRNAs, some of which have crucial roles in immune response, such as NEAT1, GAS5, and LUCAT1. Additionally, we identify many novel lncRNAs that might participate in the elicited immune response. Remarkably, we find that the expression of a set of lncRNAs correlates with the downregulation of MHCII genes in monocytes. Presentation of viral antigens through major histocompatibility complex (MHC) class II induces adaptive immune response upon infection. This result suggests that lncRNAs may be part of a co-regulatory mechanism involved in MHC class II genes repression and subsequent failure to initiate adaptive immune response. Our observations may be generalizable to other viral infections since MHC class II gene expression is known to become markedly downregulated in other infectious diseases, such as COVID-19. Finally, we identify a set of lncRNAs, such as MIR22HG, that are dysregulated between infected and bystander monocytes. Overall, lncRNAs may be involved in cellular pathways that the virus actively modulates upon entry into the cell and likely elicit the cell immune response.

Our work expands the repertoire of annotated lncRNA in macaque and offers insight on lncRNA expression compared to protein-coding genes at single-cell resolution in the context of a fatal viral infection. We find that the dysregulation of lncRNAs is coupled with the host immune response elicited upon EBOV infection, such as antigen presentation through the MHCII complex. Altogether, our work contributes to better understand the role of lncRNAs in the host response to EBOV infection at single-cell resolution.


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