ISFMS 2019

3rd International Symposium on Frontiers in Molecular Science – RNA Regulatory Networks

LISBON | PORTUGAL | 26 –28 JUNE 2019

Program and Abstract Book

The 3rd International Symposium on Frontiers in Molecular Science—RNA Regulatory Networks

The 3rd International Symposium on Frontiers in Molecular Science—RNA Regulatory Networks

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Ciência Viva Center—Parque das Nações
Lisbon, Portugal
26–28 June 2019

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John Mattick

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Symposium Programme

Day 1: Wednesday 26th June 2019

08:30–09:10	Check-in
09:15–09:20	Opening Ceremony
09:20–10:00	**Keynote Opening Lecture** John Mattick: The New World of RNA Biology
	Session 1—RNA Regulatory Networks in Cancer and Other Disorders (*Part 1*)
10:00–10:30	Coffee Break
10:30–11:00	**George Calin—Invited Lecture** About Chomsky, Non-codingRNAs and Cancer Patients
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12:00–12:20	**Anne-Claire Godet** Role of the Paraspeckle and of the Long Non-Coding RNA NEAT1 in Translational Control during Hypoxia
12:20–12:40	**Catarina Morais** Long Non-Protein Coding RNA MVIH Silencing as a Therapeutic Approach for Glioblastoma
12:40–13:00	**Vanja Todorovski** Long Non-Coding RNA NEAT1—A Promising Mechanosensor in Cancer Metastasis
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15:30–16:00	**Leonard Lipovich** Primate-Specific Long Non-Coding RNA Genes: Causes of Human Disease, Targets for Post-Genomic Therapeutics
16:00–16:20	**Florian Karreth** Identification of Co-Amplified ceRNA Genes and Their Role in Melanoma Progression
16:20–16:40	**Teresa Colombo** TP53 Regulates miRNA Association with AGO2 to Remodel the miRNA-mRNA Interaction Network
16:40–17:00	Coffee Break
	Session 1: RNA Regulatory Networks in Cancer and Other Disorders (*Part III*)
17:00–17:30	**Tao Liu** The Novel Long Noncoding RNA Lncnb1 Promotes Tumorigenesis By Interacting With Ribosomal Protein
17:30–17:50	**Pankaj Trivedi** RNA Aided Immunotherapy for Epstein-Barr Virus Associated Cancers

Day 2: Thursday 27th June 2019

Session 1: RNA Regulatory Networks in Cancer and Other Disorders (*Part IV*)

08:45–09:00	**Organization Remarks**
09:00–09:30	**Miguel Mano—Invited Lecture** Exploring High-Throughput miRNA Screening as a Functional Genomics Tool in Biomedicine
09:30–09:50	**Rosemary Kiernan** NF90-Dependent Regulation of miRNA Expression in Cancer
09:50–10:10	**Afshin Beheshti** Circulating miRNA Signature Predicts Health Risks Associated with Radiation and Microgravity
10:10–10:30	**Pavel Sumazin** Targeting Noncoding RNAs Can Improve the Effectiveness of Radiation Therapy for Cancer Patients
10:30–11:00	Coffee-Break

Session 2: RNA Structure-Function Relationships and Signaling Pathways

11:00–11:30	**Marco Marcia—Invited Lecture** The Core Tertiary Structure of a Long Non-Coding RNA Is Essential for Stimulation of the p53 Pathway
11:30–11:50	**Mihaela Rita Mihailescu** Fragile X Mental Retardation Protein: A Potential Switch Controlling the miR-125a-Mediated Translation Regulation of the G Quadruplex Forming PSD-95 mRNA
11:50–12:10	**Jeffrey Evanseck** Molecular Dynamics Simulations of the $(G_4C_2)_n$ Repeat Expansion within c9orf72 RNA and of Its Interactions with Fragile-X Mental Retardation Protein
12:10–12:30	**Sara Napoli** GECPAR, a Noncoding RNA Typical of Germinal Center B Cell, Impacts on Wnt Pathway
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Session 3: RNA Regulatory Networks in Cell, Developmental and Neural Biology (*Part I*)

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Session 3: RNA Regulatory Networks in Cell, Developmental and Neural Biology (*Part II*)

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16:30–16:50	**Maina Bitar** The Transcriptional Landscape of Human Progenitor Cell Populations
17:10–17:30	**Daria Aleshkina** ncRNA in Mammalian Oocyte and Early Embryo Development

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Day 3: Friday 28th June 2019

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	Giuseppina Pisignano
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45	Daniela Gradia	LncRNA and mRNA network reconstruction highlights novel key regulators in breast cancer subtypes
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47	Peter Hamar	Micro-RNA-17 has a minor role in kidney ischemia-reperfusion injury in mice
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53	Peter Hamar	The role of aging on renal ischemia induced lncRNA profile
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Welcome from the Chairs

Dear Colleagues,

The centrality of RNA in the flow of information from the genome is the basis of the classical dogma of cell biology. However, the rules and roles governing RNA functions have been dramatically expanded during the last two decades with the discovery of the pervasive transcription of eukaryotic genomes and the growing appreciation of non-coding RNA as a plastic and versatile molecule that carries out a myriad of functions ranging from enzymatic catalysis to scaffolding of protein complexes, nucleation of subcellular domains, and the dynamic organization of chromatin.

The fact that noncoding RNAs (ncRNAs) are prevalent in the transcriptomes of humans and other complex organisms suggests that a second tier of genetic output has evolved in these organisms, to enable the integration and coordination of sophisticated suites of gene expression required for differentiation and development, and that may be perturbed in cancer and neurological disorders, among others. Moreover, the expansion of the complement of ncRNAs in the higher organisms suggests that the evolution of complexity may not have been simply dependent on an expanded repertoire of proteins and protein isoforms but on a (much) larger set of genomic design instructions embedded in trans-acting RNAs, which drive the epigenetic trajectories of development and can respond to internal and external cues through RNA editing and modification.

The 3rd International Symposium on Frontiers in Molecular Science—RNA Regulatory Networks (ISFMS 2019), organized by Universidade de Lisboa, will analyze the centrality of RNA regulation in biological processes and human disease, and will constitute an excellent opportunity for the interchange of ideas and the presentation of new scientific developments in the field. It will consider the many dimensions of RNA regulation in development and disease, RNA structure-function relationships, the mechanisms by which plasticity is introduced, and the role of RNA in transgenerational communication.

It is a great pleasure to welcome you in Lisbon for this IJMS congress!

Francisco J. Enguita
John Mattick

Invited Speakers

Dr. Maite Huarte
CIMA, University of Navarra, Pamplona, Spain

Dr. John Mattick
Genomics England, London, UK

Dr. Musa Mlhanga
University of Cape Town, South Africa

Dr. Alena Shkumatava
Institut Curie, Paris, France

Dr. Miguel Mano
CNC, Coimbra, Portugal

Dr. Rory Johnson
University of Bern, Bern, Switzerland

Dr. Tariq Rana
University of California, San Diego, CA, USA

Dr. Anders Lund
Universty of Copenhagen, Denmark

Dr. Marcos Malumbres
Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Dr. Frank Slack
Harvard Medical School, Boston MA, USA

Dr. Marco Marcia
EMBL, Grenoble, France

Dr. George Calin
The University of Texas MD Anderson Cancer Center, TX, USA

Conference Venue

The conference will take place in the **"Pavilion of Knowledge", Pavilhão do Conhecimento—Ciência Viva Center, Largo José Mariano Gago, nº1, Parque das Nações, 1990-073 Lisbon, Portugal.**

Lisbon is the capital and the largest city of Portugal, with an estimated population of 505,526 within its administrative limits in an area of 100.05 km^2.

Its urban area extends beyond the city's administrative limits with a population of around 2.8 million people, being the 11th-most populous urban area in the European Union. About 3 million people live in the Lisbon Metropolitan Area (which represents approximately 27% of the country's population). It is mainland Europe's westernmost capital city and the only one along the Atlantic coast. Lisbon lies in the western Iberian Peninsula on the Atlantic Ocean and the River Tagus. The westernmost areas of its metro area form the westernmost point of Continental Europe, which is known as Cabo da Roca, located in the Sintra Mountains.

Lisbon is recognized as an alpha-level global city by the Globalization and World Cities (GaWC) Study Group because of its importance in finance, commerce, media, entertainment, arts, international trade, education, and tourism. Lisbon is the only Portuguese city besides Porto to be recognized as a global city. It is one of the major economic centers on the continent, with a growing financial sector and one of the largest container ports on Europe's Atlantic coast. Additionally, Humberto Delgado Airport served 26.7 million passengers in 2017, being the busiest airport in Portugal, the 3rd busiest in the Iberian Peninsula, and the 20th busiest in Europe, and the motorway network and the high-speed rail system of Alfa Pendular links the main cities of Portugal (such as Braga, Porto, and Coimbra) to Lisbon. The city is the 9th-most-visited city in Southern Europe, after Rome, Istanbul, Barcelona, Milan, Venice, Madrid, Florence, and Athens, with 3,320,300 tourists in 2017.

Lisbon is one of the oldest cities in the world, and one of the oldest in Western Europe, predating other modern European capitals such as London, Paris, and Rome by centuries.

Conference Gala Dinner

We invite you to have the Gala Dinner at "Espelho de agua" at 20:00 on 27 June. There will be a bus to pick up all the attendees from the conference center at 19:30.

Time: 27 June 2019 at 20:00
Location: "Espelho de agua" (water mirror in English)
Address: Av. Brasília, Edifício Espelho d'Água (next to Padrão dos Descobrimentos)

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Abstract

1. About Chomsky, Non-Coding RNAs and Cancer Patients

George A. Calin

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The newly discovered differential expression in numerous tissues, key cellular processes and multiple diseases for several families of long and short non-codingRNAs (ncRNAs, RNAs that do not codify for proteins but for RNAs with regulatory functions), including the already famous class of microRNAs (miRNAs) strongly suggest that the scientific and medical communities have significantly underestimated the spectrum of ncRNAs whose altered expression has significant consequences in diseases. MicroRNA and other short or long non-codingRNAs alterations are involved in the initiation, progression and metastases of human cancer. The main molecular alterations are represented by variations in gene expression, usually mild and with consequences for a vast number of target protein coding genes. The causes of the widespread differential expression of non-codingRNAs in malignant compared with normal cells can be explained by the location of these genes in cancer-associated genomic regions, by epigenetic mechanisms and by alterations in the processing machinery. MicroRNA and other short or long non-codingRNAs expression profiling of human tumors has identified signatures associated with diagnosis, staging, progression, prognosis and response to treatment. In addition, profiling has been exploited to identify non-codingRNAs that may represent downstream targets of activated oncogenic pathways or that are targeting protein coding genes involved in cancer. Recent studies proved that miRNAs and non-coding ultraconserved genes are main candidates for the elusive class of cancer predisposing genes and that other types of non-codingRNAs participate in the genetic puzzle giving rise to the malignant phenotype. Last, but not least, the shown expression correlations of these new ncRNAs with cancer metastatic potential and overall survival rates suggest that at least some member of these novel classes of molecules could potentially find use as biomarkers or novel therapeutics in cancers and other diseases.

Abstract

2. The Potential for Personalized MicroRNA Therapeutics

Frank J. Slack

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MicroRNAs are small non-coding RNAs that regulate gene expression to control important aspects of development and metabolism such as cell differentiation, apoptosis and lifespan.miR-21, miR-155, *let-7* and miR-34 are microRNAs implicated in human cancer. Specifically, human *let-7* and miR-34 are poorly expressed or deleted in lung cancer, and over-expression of *let-7* or miR-34 in lung cancer cells inhibits their growth, demonstrating a role for these miRNAs as tumor suppressors in lung tissue. *let-7* and miR-34 regulate the expression of important oncogenes implicated in lung cancer, suggesting a mechanism for their involvement in cancer. We are focused on the role of these genes in regulating proto-oncogene expression during development and cancer, and on using miRNAs to suppress tumorigenesis.

In contrast, miR-21 and miR-155 are oncomiRs and up-regulated in many cancer types. We are also developing effective strategies to target these miRNAs as a novel anti-cancer approach.

Lastly we are examining the non-coding portions of the genome for mutations and variants that are likely to impact the cancer phenotype. We have successfully resequenced the 3'UTRome and microRNAome from cancer patients with a family history of cancer.

Abstract

3. The "Weakest Linc": Mutated Long Noncoding RNAs in Tumour Genomes

Andrés Lanzós [1,2], Adrienne Vancura [1,2], Roberta Esposito [1,2], Bernard Merlin [1,2] and Rory Johnson [1,2,*]

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Tumours evolve through somatic mutations that enable cells to replicate uncontrollably and invade new sites. Protein-coding genes carrying these "driver" mutations are considered to be prime therapeutic targets. However interpretation of tumour mutation data is confounded by complex and poorly-understood mutational processes, which can lead to false-positive and -negative predictions. It is debated whether long noncoding RNAs can act as driver genes, and few have been identified to date. I will present our efforts to address this through analysis of recently-sequenced tumour genomes from the International Cancer Genome Consortium's "PCAWG" (Pan Cancer Analysis of Whole Genomes) project. We have developed a new version of, ExInAtor, our driver-lncRNA identification tool. ExInAtor2 uses an improved statistical model for mutational-burden analysis, while now also incorporating functional evidence. The result is updated driver-lncRNA predictions across multiple cancer types, and additional evidence that novel and well-known lncRNAs do indeed have characteristics of cancer drivers. We have also curated additional datasets based on functional studies, clinical data and mutagenesis screens, as means for benchmarking driver predictions. In summary, identification of driver genes using somatic mutations is a promising new approach for identifying lncRNA targets in cancer.

Abstract

4. Role of the Paraspeckle and of the Long Non-Coding RNA NEAT1 in Translational Control during Hypoxia

Anne-Claire Godet [1,*], Florian David [1], Fransky Hantelys [1], Françoise Pujol [1], Isabelle Ader [2], Edouard Bertrand [3], Anthony Henras [4], Laetitia Ligat [5], Marion Peter [6], Patrice Vitali [4], Eric Lacazette [1], Florence Tatin [1], Barbara Garmy-Susini [1] and Anne-Catherine Prats [1]

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Cell stress such as hypoxia triggers major changes of gene expression, including a strong blockade of mRNA translation. However, internal ribosome entry sites (IRESs) allow translation initiation during stress, resulting in translation of specific mRNAs. In particular, mRNAs of (lymph)angiogenic growth factors contain IRESs that are activated by hypoxia. These growth factors have a crucial role in tissue revascularization required in ischemic pathologies, such as heart failure. Several IRES trans-acting factors, including p54^{nrb}, are also components of the paraspeckle, a nuclear body that forms in response to stress. Our present hypothesis is that the paraspeckle may be involved in formation of the IRESome (IRES/ITAF complex), responsible for translation activation via IRESs. We have searched for ITAF role of other main paraspeckle components and analyzed the IRESome composition during hypoxia in cardiomyocytes. We show that p54^{nrb}, the main paraspeckle marker, is required to activate the IRESs of several (lymph)angiogenic factors. Furthermore we show that IRES activities are controlled by two other paraspeckle components, PSCP1 and the long non-coding RNA NEAT1 known to be the structural backbone of paraspeckles. We looked for other IRESome components in hypoxia by BIA-MS using cardiomyocytes extracts and identified vasohibin-1 (VASH1), a protein involved in angiogenesis and stress tolerance. We show the ability of recombinant VASH1 to bind IRES RNA by surface plasmon resonance analysis, and its ITAF function by knock-down experiments. Our data indicate that IRES-dependent translation in hypoxic cardiomyocytes is controlled by at least four ITAFs, p54^{nrb}, PSPC1, VASH1 and the lncRNA NEAT1. Three of them are main components of the paraspeckle, suggesting that the paraspeckle might be an IRESome assembly platform. IRES-containing mRNA recruitment to paraspeckle is presently investigated by smiFISH. This would reveal a novel role of this nuclear body in the control of translation during stress.

Abstract

5. Long Non-Protein Coding RNA MVIH Silencing as a Therapeutic Approach for Glioblastoma

Ana M. Cardoso [1], Catarina M. Morais [2,*], Maria C. Pedroso de Lima [1] and Amália S. Jurado [2]

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Glioblastoma (GBM) is the most frequent and malignant type of brain tumor, for which no effective therapy exists. The high proliferative and invasive nature of GBM, as well as its acquired resistance to chemotherapy, make this type of cancer extremely lethal shortly after diagnosis. Due to its location on delicate areas, complete surgical tumor removal is impossible, which facilitates the regrowth of glioblastoma from the malignant cells infiltrated in the healthy brain parenchyma. A suitable GBM therapy should be able to reduce tumor invasiveness while sensitizing it to chemotherapeutic agents. Long non-protein coding RNAs (lncRNA) are a class of regulatory RNAs whose levels can be dysregulated in the context of diseases, unbalancing several physiological processes. The lncRNA associated with microvascular invasion in hepatocellular carcinoma (MVIH), which is overexpressed in hepatocellular carcinoma, breast and non-small cell lung cancer, co-precipitates with phosphoglycerate kinase (PGK1), preventing its secretion to the extracellular environment. Besides its canonical role in glycolysis, PGK1 is a moonlighting enzyme, catalyzing reactions in other cellular pathways, including cell migration and invasion. In the extracellular environment, PGK1 generates the vascular inhibitor angiostatin from plasminogen, decreasing angiogenesis. We hypothesized that, by silencing the expression of lncRNA-MVIH, the secretion of PGK1 would increase, reducing GBM migration and invasion capabilities. We observed that lncRNA-MVIH silencing in human GBM cell lines resulted in no alterations in cell viability, growth rates or cell cycle progression, but significantly decreased glycolysis, cell migration, and invasion. In addition, lncRNA-MVIH silencing sensitized GBM cells to the chemotherapeutic drug cediranib (a tyrosine kinase inhibitor). This work suggests that lncRNA-MVIH knockdown may be a promising strategy to address GBM invasiveness and chemoresistance.

Abstract

6. Long Non-Coding RNA NEAT1—A Promising Mechanosensor in Cancer Metastasis

Vanja Todorovski *, Yu Suk Choi and Archa Fox

The University of Western Australia
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Over 90% of cancer related deaths are caused by metastasis, which is a complex process whereby tumour cells spread from a primary location to a secondary site. Long non-coding RNAs (lncRNAs) are emerging as promising therapeutic targets due to their role in cancer progression. In particular, the aberrant expression of lncRNA Nuclear Paraspeckle Assembly Transcript 1 (NEAT1), which forms subnuclear bodies known as 'paraspeckles,' has been correlated with tumour progression and metastasis. During metastasis, the tumour microenvironment is subject to hypoxia, which has been shown to induce paraspeckle formation.

Further changes that occur to the tumour microenvironment during metastasis involve the remodelling of the extracellular matrix (ECM), therefore a phenomenon known as mechanotransduction is said to occur. In breast tissue, tissues stiffness has been reported to increase when healthy breast tissue becomes cancerous. Further advances in the field have found that signals from the mechanical environment to the nucleus can induce changes at the transcriptional level.

Therefore, we aim to investigate whether the lncRNA NEAT1/Paraspeckles are able to mechanosense in cancer metastasis. After growing cells on polyacrylamide hydrogels with extreme stiffness properties, we utilized FISH (Fluorescence in situ hybridisation) to quantify paraspeckles, and immunofluorescence to detect mechanotransduction markers YAP/TAZ and Lamin A. Results in MCF10A and MDA-MB-231 cell lines suggested that paraspeckles may respond to stiffness in metastatic cells, with a greater number of paraspeckles observed in cells grown on soft hydrogels. Further studies revealed YAP/TAZ as key molecules involved in the upregulation of NEAT1/paraspeckles. Collectively, our study has suggested that NEAT1/paraspeckles may respond to stiffness via YAP/TAZ which could potentially lead to novel therapeutic approaches directly targeting NEAT1/Paraspeckles and mechanotransduction in cancer metastasis.

Abstract

7. Immune Genes Are Primed for Robust Transcription by Proximal lncRNAs Located in Nuclear Compartments

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Accumulation of H3K4me3 epigenetic marks on multiple immune gene promoters underlie robust transcriptional responses during trained immune responses. However, the molecular basis for this remains unknown. Here we show 3D chromatin topology enables H3K4me3-primed immune genes to engage in chromosomal contacts with a newly identified class of lncRNAs (IP-LncRNAs). We mechanistically characterise UMLILO, a prototypical IPL that regulates the robust transcription of the CXCL chemokines. Pre-formed 3D chromatin topology brings UMLILO proximal to the chemokine genes, prior to their transcriptional activation. We show, that acting in cis, UMLILO directs the WDR5/MLL1 complex across the chemokine promoters facilitating their H3K4me3 epigenetic priming. This mechanism appears shared amongst other trained immune genes. We show that β-glucan, a classic inducer of trained immunity, epigenetically reprograms immune genes by upregulating IPLs in an NFAT-dependent manner. This provides strong evidence that IPL-mediated changes to the epigenetic status of immune gene promoters permit the enhanced pro-inflammatory response observed in trained monocytes. This creates a new paradigm for how nuclear architecture and lncRNA regulation may orchestrate H3K4me3 promoter priming and the robust transcription of innate immune genes.

Abstract

8. Primate-Specific Long Non-Coding RNA Genes: Causes of Human Disease, Targets for Post-Genomic Therapeutics

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The Gencode gene catalog of the ENCODE Consortium determined that two-thirds of human genes do not encode proteins. Long non-coding RNA (lncRNA) genes comprise an abundant, but still poorly understood, class of non-coding RNA genes. In the FANTOM Consortium, we characterized lncRNAs in human and mouse, highlighting the lack of their interspecies conservation (PubMed ID: 16683030). Unlike protein-coding genes, most human lncRNA genes lack conservation beyond primates (PMIDs: 24463510, 24429298). We interrogated the functional potential of non-conserved lncRNAs, a controversial topic (in view of the assumption that functional non-coding sequences should be conserved), in cancer and diabetes. We discovered and functionally validated primate-specific estrogen-activated oncogenic (and estrogen-repressed tumor-suppressor-like) lncRNAs in human estrogen receptor positive breast cancer cells. These spliced, polyadenylated, cytoplasmic, non-conserved lncRNAs regulate cell growth and death (PMID: 28003470).

Over 95% of significant disease-associated variants from Genome-Wide Association Studies are non-coding, but the GWAS field continues to focus on protein-coding candidates. Overlapping all public significant GWAS variants with all Gencode lncRNA exons, we identified, as a top hit, LOC157273, a primate-specific cytoplasmic lncRNA, expressed solely in hepatocytes and found independently by over 20 GWAS of type 2 diabetes, BMI, CVD, and obesity, that we validated as a negative regulator of liver glycogen storage (and that, hence, may contribute to abnormally high fasting glucose levels). We developed oligonucleotide-based drugs targeting this lncRNA. In view of the precedents targeting liver ncRNAs with sequence-based drugs (PMID: 23534542), we posit that siRNA derivatives targeting our lncRNA can be delivered to the liver *in vivo* for type 2 diabetes treatment.

Summarily, we show primate-specific lncRNAs, in cancer and normal cells, with genetic epidemiology and cellular phenotypes jointly pointing to specific roles in disease networks.

Abstract

9. Identification of Co-Amplified ceRNA Genes and Their Role in Melanoma Progression

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Messenger RNAs can engage in coding-independent posttranscriptional regulatory networks by competing with other RNA transcripts for shared microRNAs (miRNAs). Such natural miRNA sponges were termed competitive endogenous RNAs (ceRNAs) and have been causally linked to cancer development in several contexts. To assess if genomic abnormalities promote cancer development via amplification of ceRNA genes we interrogated a cohort of 366 melanoma cases. Intriguingly, we identified over 90 recurrently amplified, highly expressed genes that were predicted to regulate complex and interconnected ceRNA networks. 14 of 15 ceRNA gene 3'UTRs (the predominant sites of miRNA interaction) promoted melanoma cell proliferation, validating that our bioinformatics prediction identified putative ceRNAs with oncogenic potential. Remarkably, most of these putative ceRNAs localized to only a few genomic regions. Computational models predict that the number of sequestered miRNAs directly correlates with the potency of ceRNA regulation, which suggests that co-amplification of ceRNA genes can have synergistic oncogenic effects. One particularly interconnected ceRNA sub-network contains genes located on chromosome 1q, which is amplified in up to 25% of melanoma cases and associated with melanoma progression. Three ceRNA genes on chromosome 1q, CEP170, NUCKS1, and ZC3H11A, are indeed co-amplified and co-expressed in melanoma, and are associated with a melanoma metastasis expression signature. Ectopic overexpression of the 3'UTRs of CEP170, NUCKS1, and ZC3H11A in melanoma cell lines enhanced the transformed cell state and promoted migration and invasion. Importantly, the CEP170, NUCKS1, and ZC3H11A 3'UTRs are able to sequester miRNAs and their oncogenic potential is dependent on miRNA binding. Our work revealed amplified genes that promote melanoma progression via their ceRNA function and we provide a framework for the identification of genomically altered ceRNA genes in other cancers.

Abstract

10. TP53 Regulates miRNA Association with AGO2 to Remodel the miRNA-mRNA Interaction Network

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DNA damage activates TP53-regulated surveillance mechanisms that are crucial in suppressing tumorigenesis. TP53 orchestrates these responses directly by transcriptionally modulating genes, including microRNAs (miRNAs), and by regulating miRNA biogenesis through interacting with the DROSHA complex. However, whether the association between miRNAs and AGO2 is regulated following DNA damage is not yet known. Here, we show that, following DNA damage, TP53 interacts with AGO2 to induce or reduce AGO2's association of a subset of miRNAs, including multiple let-7 family members. Furthermore, we show that specific mutations in TP53 decrease rather than increase the association of let-7 family miRNAs, reducing their activity without preventing TP53 from interacting with AGO2. This is consistent with the oncogenic properties of these mutants. Using AGO2 RIP-seq and PAR-CLIP-seq, we show that the DNA damage-induced increase in binding of let-7 family members to the RISC complex is functional. We unambiguously determine the global miRNA-mRNA interaction networks involved in the DNA damage response, validating them through the identification of miRNA-target chimeras formed by endogenous ligation reactions. We find that the target complementary region of the let-7 seed tends to have highly fixed positions and more variable ones. Additionally, we observe that miRNAs, whose cellular abundance or differential association with AGO2 is regulated by TP53, are involved in an intricate network of regulatory feedback and feedforward circuits. TP53-mediated regulation of AGO2-miRNA interaction represents a new mechanism of miRNA regulation in carcinogenesis.

Abstract

11. The Novel Long Noncoding RNA lncNB1 Promotes Tumorigenesis by Interacting with Ribosomal Protein

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The majority of patients with neuroblastoma due to N-Myc oncogene amplification and N-Myc oncoprotein over-expression die of the disease. Here our analyses of RNA sequencing data identified the novel long noncoding RNA lncNB1 as one of the transcripts most over-expressed in MYCN-amplified, compared with MYCN-non-amplified, human neuroblastoma cell lines and most over-expressed in neuroblastoma, compared with >10,000 cancer tissues from all other organ origins. lncNB1 bound to ribosomal protein to enhance E2F protein synthesis, leading to DEPDC gene transcription. The GTPase-activating protein DEPDC induced ERK protein phosphorylation and N-Myc protein stabilization. Importantly, lncNB1 knockdown abolishes neuroblastoma cell clonogenic capacity in vitro and leads to neuroblastoma tumor regression in mice, and high levels of lncNB1, ribosomal protein and DEPDC in human neuroblastoma tissues predicted poor patient prognosis. This study therefore identifies lncNB1, its binding ribosomal protein and its effect DEPDC as key factors in N-Myc-driven oncogenesis and as novel therapeutic targets.

Abstract

12. RNA Aided Immunotherapy for Epstein-Barr Virus Associated Cancers

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Cancer cells subvert host immune surveillance by altering immune checkpoint (IC) proteins. Indeed, EBV associated tumors have higher PD-L1 expression. However, it is not known how the virus alters ICs in lymphomas and other solid tumors and if cellular miRNAs are involved in this process. Over the years, we have extensively investigated how EBV through its latent growth transformation proteins alters cellular miRNAs involved in tumorigenesis. In a recent study, we found that latency III expressing Burkitt lymphoma (BL), diffuse large B cell lymphomas (DLBCL) or their EBNA2 transfected derivatives express high levels of PD-L1. EBV latency III expressing clinical samples from DLBCL patients showed high levels of PD-L1. The PD-L1 targeting oncosuppressor miR-34a was downregulated in EBNA2 transfected lymphoma cells. We have identified EBF-1 as the main player employed by EBV in miR34a suppression. MiR-34a reconstitution in EBNA2 transfected DLBCL reduced PD-L1 expression and increased its immunogenicity in mixed lymphocyte reactions (MLR) and in three dimensional biomimetic microfluidic chips. Interestingly, ICOSL expression was downregulated in EBNA2 expressing DLBCLs. Presently, we are validating ICOSL targeting miRNAs in EBNA2 transfectants. Given the importance of PD-L1 inhibition in immunotherapy, miR-34a dysregulation in cancers and generally poor efficacy of IC inhibiting antibodies alone in patients, I will discuss how RNA aided immunotherapy, which includes a combination of IC inhibiting antibodies and miR-34a, might improve clinical outcome of EBV associated cancers.

Abstract

13. Exploring High-Throughput miRNA Screening as a Functional Genomics Tool in Biomedicine

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MicroRNAs (miRNAs) are a class of genome-encoded small RNAs that post-transcriptionally regulate gene expression by repressing target transcripts containing partially complementary binding sites. Despite their relatively low number (ca. 2500 miRNAs are currently annotated in the human genome), miRNAs have been shown to directly regulate a significant proportion of the transcriptome, controlling virtually all biological and pathological processes.

The application of high-throughput functional screening technologies to systematically analyze miRNA function using genome-wide libraries of miRNAs in cell-based assays offers, thus, a unique opportunity to probe the transcriptome and gain new insights into the intricate molecular networks underlying complex biological and disease-relevant processes. By using these large-scale screening approaches, we have been identifying and characterizing miRNAs and miRNA targets implicated in different biological contexts and pathologies, including cardiovascular disorders, infection and cancer signaling. Overall, these studies have uncovered novel and unexpected molecular players and pathways, which can reveal novel opportunities for therapeutic intervention.

Abstract

14. NF90-Dependent Regulation of miRNA Expression in Cancer

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MicroRNAs (miRNAs) are predicted to regulate the expression of more than 60% of mammalian genes and play fundamental roles in most biological processes. Dysregulation of miRNA expression is a hallmark of most cancers, which contributes to uncontrolled cell growth. While a number of mechanisms have been described to account for this, further investigation of mechanisms controlling miRNA abundance in cancer is needed. We recently showed that the dsRNA-binding protein, NF90, prevents processing of miR3173 that is embedded in the first intron of DICER thereby facilitating DICER expression. In a nude mouse model, NF90 overexpression reduced proliferation of ovarian cancer cells and significantly reduced tumor size and metastasis while overexpression of miR3173 dramatically increased metastasis in a NF90- and DICER-dependent manner. Clinically, low NF90 expression and high miR-3173-3p expression were found to be independent prognostic markers of poor survival in a cohort of ovarian carcinoma patients. These findings suggest that NF90, by controlling the processing of miR3173 and Dicer expression, can act as a suppressor of ovarian carcinoma.

Using a combination of eClip, miRNA profiling and transcriptomic data, our recent findings indicate that NF90 may modulate a number of human miRNAs in a similar fashion. These findings open up new possibilities for therapeutic intervention by modulating intron-embedded miRNA processing.

Abstract

15. Circulating miRNA Signature Predicts Health Risks Associated with Radiation and Microgravity

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There are many known health risks currently associated with space travel with the majority of the risk from stemming from two components: microgravity and radiation. From our earlier work (which will also be presented here), we predicted that there is a systemic component of the host that causes general increased health risks due to spaceflight driven by a circulating microRNA (miRNA) signature consisting of 13 miRNAs that directly regulates both p53 and TGFβ1 and we have also identified a specific 9 miRNA signature that detects accurately the presence of lymphoma. MiRNAs are small non-coding RNAs that are known to circulate stably throughout the body and have been found in the majority of the bodily fluids. We hypothesize that using our technique there should be an identifiable unique miRNA signature for each specific disease. Here, we start to dissect the actual impact of this miRNA signature on both the radiation and microgravity components and prove that this miRNA signature actually exists in the circulation of a host for cancer and other diseases. To achieve this, we obtained multiple tissues (serum, liver, and spleen) from C57BL/6 male mice that were hindlimb unloaded (HU) to simulated microgravity, irradiated with 2Gy gamma (IR), HU plus IR, and control mice. Utilizing droplet digital PCR (ddPCR), we show that HU vs Controls show modest increase in miRNAs, IR vs control mice showed significant increases (p-value 0.05) for approximately 70% of the miRNAs, and HU + IR vs controls showed significant increases for the majority of the miRNA signature which indicate HU acts as a radiosensitizer for IR and drives the increased presence of the circulating miRNAs. This work demonstrate for the first time the potential of a minimally invasive novel biomarker and countermeasure/therapeutic that can be used to mitigate both radiation and microgravity effects.

Abstract

16. Targeting Noncoding RNAs Can Improve the Effectiveness of Radiation Therapy for Cancer Patients

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Profiling of many tumor types revealed dramatic upregulation of long-noncoding RNAs (lncRNAs). These lncRNAs are promising therapeutic targets because inhibitors that specifically target them may be able to target cancer cells while having minimal toxicity in non-tumor cells. We developed software—including longhorn (Chiu et al., 2018) and bighorn (Chiu et al., 2019)—to infer disease-relevant functions for lncRNAs. Using these tools, in conjunction with data from hundreds of cell lines and thousands of patients, we identified lncRNAs that target DNA-repair pathways and are predicted to improve the effectiveness of x-ray and proton therapies. Expression and genomic alterations at the loci of these lncRNAs predict cell and patient response to radiation therapies. Focusing on pan-cancer lncRNAs, which have high expression in many cancer types, we showed that silencing these lncRNAs in vitro increases the susceptibility of cell lines to radiation and reduces radiation dosages required for killing cancer cells. We argue that tumor-suppressor lncRNAs that regulate the DNA repair pathway can indicate candidates for lower-dosage radiation therapy and that pan-cancer and cancer-specific lncRNAs that are upregulated in tumor cells and are predicted to disrupt DNA repair pathways are excellent candidate therapeutic biomarkers.

Abstract

17. The Core Tertiary Structure of a Long Non-Coding RNA Is Essential for Stimulation of the p53 Pathway

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Long non-coding RNAs (lncRNAs) are key regulatory molecules for cellular processes, ranging from genome structure modulation to epigenetic chromatin remodelling, DNA repair and translation. A subgroup of lncRNAs emerged as particularly suited for mechanistic studies based on their evolutionary conservation, specific cellular distribution, specific tissue localization, and clinical relevance. The secondary structures of a handful of such lncRNAs have thus been experimentally mapped. However, because the size and complexity of these molecules present unprecedented challenges for biophysical studies, it has not yet been possible to systematically connect 3D structural information with biological function for any lncRNA, so far. I will report the results of a structural and functional study on an lncRNA that prevents tumorigenesis by regulating the p53 pathway. This lncRNA adopts a well-defined structural core, in which two distal motifs interact by base complementarity to form alternative, mutually exclusive kissing loop structures. Mutations that disrupt these kissing loop interactions impair stimulation of the p53 pathway. These findings directly connect the 3D structure of an lncRNA to its biological function.

Abstract

18. Fragile X Mental Retardation Protein: A Potential Switch Controlling the miR-125a-Mediated Translation Regulation of the G Quadruplex Forming PSD-95 mRNA

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Fragile X syndrome (FXS), the most common inherited form of intellectual impairment, is caused by the loss of expression of the fragile X mental retardation protein (FMRP) due to a CGG trinucleotide expansion in the 5′-untranslated region of the *Fmr1* gene on the X chromosome. FMRP is an RNA binding protein that has been shown to bind to G quadruplex forming mRNAs. It has been shown that FMRP together with the microRNA miR-125a regulate the translation of PSD-95 mRNA, which encodes for the postsynaptic density protein 95. The miR-125a binding site within the PSD-95 mRNA 3′-untranslated region (UTR) is embedded in a G rich region bound by FMRP, which folds into two parallel G quadruplex structures. The FMRP regulation of PSD-95 mRNA translation is complex, being mediated by its phosphorylation. In this study, we have shown that both unphosphorylated FMRP and its phosphomimic FMRP S500D bind to the PSD-95 mRNA G quadruplexes with high affinity, whereas only FMRP S500D binds to miR-125a. These results point towards a mechanism by which, depending on its phosphorylation status, FMRP acts as a switch that potentially controls the stability of the complex formed by the miR-125a-guided RNA induced silencing complex (RISC) and PSD-95 mRNA.

Abstract

19. Molecular Dynamics Simulations of the (G4C2)n Repeat Expansion Within C9ORF72 RNA and of its Interactions With Fragile-X Mental Retardation Protein

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Since the discovery of the C9orf72(G_4C_2) repeat expansion, accounting for the largest number of familial amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) cases to date, research has been focused on elucidating the mechanisms by which the expansion leads to disease. One such mechanism involves RNA gain of function through the formation of the non-canonical secondary structure involving the G-quadruplex. The G-quadruplex secondary structure provides unwanted targets for the binding of specific RNA binding proteins, potentially disrupting their cellular function. Arginine-glycine-glycine (RGG) box containing RNA binding proteins have been shown to interact with G-quadruplexes. One specific RGG box containing protein, fragile-x mental retardation protein (FMRP), has also been shown to colocalize with C9orf72repeat RNA foci in the nucleus and cytoplasm, making this protein a possible player in the development of ALS and FTD. In this study, we used the technique of multiple trajectory molecular dynamics to sample the configurational space of the C9orf72(G_4C_2)$_n$ repeat expansion of RNA G-quadruplexes and hairpin structures, where n = 4, 8, 16, and 32. A total of 0.5 microseconds of simulation time was achieved from roughly fifty 10-nanosecond simulations. Principal component analysis (PCA) was used to identify unique RNA conformations including both G-quadruplexes and hairpin secondary structures for molecular docking simulations with FMRP. FMRP was docked to (G_4C_2)$_4$,(G_4C_2)$_8$,(G_4C_2)$_{16}$and (G_4C_2)$_{32}$ quadruplex and hairpin models using ZDOCK with Amber03 forcefield parameters. Docking results were further refined using molecular dynamics to equilibrate the structures and find a lower energy conformation of the protein:RNA complex, solvated and ionized under periodic boundary conditions for 100 ns of simulation time using the same multiple trajectory strategy. The RMSD change in RNA structure and complexes were determined.

Abstract

20. GECPAR, a Noncoding RNA Typical of Germinal Center B Cell, Impacts on Wnt Pathway Activation

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Enhancers are cell-type specific regulatory DNA regions which play a key role in differentiation and development [1]. Most active enhancers are transcribed in enhancer RNAs which can regulate transcription of relevant target genes by means of in cis but also in trans action [2]. They stabilize contact between distal genomic regions and mediate interaction between DNA and master transcription factors. Here, we describe an enhancer RNA, GECPAR, (GErminal Center Proliferative Adapter RNA), specifically transcribed in germinal center B cells, the normal counterpart of diffuse large B cell lymphoma (DLBCL). Transcribed in the super-enhancer of POU2AF1, a key gene for the regulation of germinal center reaction [3], GECPAR is able to act *in trans* as we proved by CHART seq experiment. In two DLBCL cell lines, we identified a list of genes, direct target of GECPAR, which we confirmed to be correlated to GECPAR expression also in a RNA seq dataset of 32 DLBCL patients. Gene set enrichment analysis performed on GECPAR direct targets highlighted its function as transcriptional modulator of genes involved in proliferation and differentiation, in particular some negative regulator of Wnt pathway. It exerts this regulatory role through the antagonism with FOXJ2 [4], a transcription factor we proved to shear the same DNA binding sites with GECPAR. GECPAR and FOXJ2 seem to counteract in favoring transcription transactivation by LEF1, the main mediator of Wnt pathway activation. Loss of GECPAR expression is a pro-proliferative event which can contribute to DLBCL aggressiveness.

Abstract

21. Dissecting the In Vivo Functions and Mechanisms of Action of Vertebrate lncRNAs

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Long noncoding RNAs (lncRNAs) have emerged as key regulators of important biological processes including human neurological disorders and cancers. Our research program, driven by our interest in RNA pathobiology aims at understanding the *in vivo* functions of lncRNAs and dissecting their molecular mechanisms of action. To identify the *in vivo* functions of lncRNAs, we focus on lncRNAs that are conserved from basal vertebrates to mammals. Because lncRNA sequences show fast evolutionarily turnover, we reasoned that lncRNAs under considerable evolutionary pressure represent a promising set of biologically relevant lncRNAs. Applying this logic, we have recently found that one of the conserved lncRNAs controls normal zebrafish and mouse behaviour by directing the sequence-specific degradation of a microRNA in the brain. Moreover, aiming to determine the molecular functions of lncRNAs, we developed a novel high-throughput technology that enables the systematic identification of RNA–protein interactions in living cells revealed by quantifiable luminescence.Our technology succeeded in resolving two main obstacles when aiming to identify RNA-bound proteomes: (1) it enabled the identification of proteins associated with RNAs expressed at low endogenous levels, and (2) determined RNA region-specific proteomes allowing the assignment of protein binding to defined regions of a long full-length transcript.In summary, we have developed new tools and have established genetic *in vivo* systems to dissect the functions and mechanisms of action of lncRNAs. Our studies have demonstrated that sequence conservation can guide our understanding of lncRNA functions.

Abstract

22. Re-Wiring Pluripotency with a miRNA

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Full differentiation potential along with self-renewal capacity is a major property of pluripotent stem cells (PSCs). However, cultured PSCs rarely conserve the developmental potency of early embryonic cells. We show here that transient exposure to a single microRNA, expressed in the 2-cell (2C) stage during normal development, promotes the naive condition in murine and human pluripotent stem cells (PSCs). Short exposure to this sequence in PSCs induces an early and transient expression of 2C-markers that later results in sustained naive pluripotency which expands their differentiation potential as well as their efficiency in germline contribution, tetraploid complementation and in human-mouse interspecies chimera assays. Mechanistically, these effects are mediated by direct repression of *de novo* DNA methyltransferases Dnmt3a and Dnmt3b, leading to transient resetting of epigenetic memory. Exposure to the microRNA improves differentiation and maturation of PSCs into cardiomyocytes as well as cardiac regeneration after injury. These data support the use of miRNA strategies to promote naive pluripotency in PSCs and to improve their use in regenerative medicine.

Abstract

23. Epitranscriptomic and Epigenetic Regulation of Immunity

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Two interconnected themes will be discussed in this lecture. (1) N(6)-methyladenosine (m6A) is the most prevalent internal modification of eukaryotic mRNA. Very little is known of the function of m(6)A in the immune system or its role in host-pathogen interactions. We found that HIV infection affects viral and human RNAs by altering the topology and function of m6A, a modification affecting RNA structure and function. Our findings identify a new mechanism for the control of HIV-1 replication and their interactions with the host immune system. This discovery pioneered the new field of epitranscriptomics in virology and immunology and propelled advances in science and careers by pursuing the epitranscriptomic regulation in many other viruses including Influenza, ZIKA, Influenza, HCV, KSHV, and HBV. (2) Long noncoding RNAs (lncRNAs) can regulate target gene expression by acting in *cis* (locally) or in *trans* (non-locally). Here, we performed genome-wide expression analysis of Toll-like receptor (TLR)-stimulated human macrophages to identify pairs of *cis*-acting lncRNAs and protein-coding genes involved in innate immunity. A total of 229 gene pairs were identified, many of which were commonly regulated by signaling through multiple TLRs. We focused on elucidating the function of one lncRNA, named *lnc-MARCKS* or *ROCKI* (Regulator of Cytokines and Inflammation), which was induced by multiple TLR stimuli and acted as a master regulator of inflammatory responses. *ROCKI* interacted with APEX1 at the *MARCKS* promoter. In turn, *ROCKI*–APEX1 recruited the histone deacetylase HDAC1, which removed the H3K27ac modification from the promoter. Finally, genetic variants affecting *ROCKI* expression were linked to a reduced risk of certain inflammatory and infectious disease in humans, including inflammatory bowel disease and tuberculosis. Collectively, these data highlight the importance of *cis*-acting lncRNAs inTLR signaling, innate immunity, and pathophysiological inflammation.

Abstract

24. The Transcriptional Landscape of Human Progenitor Cell Populations

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Although their capacity to self renew and differentiate is limited and variable, somatic stem cell descendants (progenitor cells) are crucial for tissue development and maintenance. These unspecialized often tissue-specific cells have been studied for many years and used for organ repair and regeneration in disease conditions. As unique cell populations, their molecular composition is of great interest and more thorough and profound knowledge of their transcriptional landscape may support further developments in the field. Here we investigate similarities and differences between various types of progenitor cells based on transcript expression profile revealed by RNA sequencing. To ensure the biological relevance of our findings, we first compare results with gene expression data acquired in-house with the NanoString technology. Our analyses suggest high overall similarity among the progenitor cell populations investigated and great conservation at the transcriptome level. In addition, most currently annotated transcripts and genes have very low to null expression, thus suggesting a somewhat specialized transcriptome. Although these cell populations are mostly similar in gene expression, we focus on the differences between them, revealing interesting sources of uniqueness and cell type-specific transcripts, both protein-coding and functional RNAs.

Abstract

25. ncRNA in Mammalian Oocyte and Early Embryo Development

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Fully-grown mammalian oocyte is transcriptionally silent and relies on specific mechanisms involved in mRNA stabilization and translation. Crucial role in regulation of translation belongs to various non-coding RNAs in the cell. We aimed to study the involvement of long and short non-coding RNAs in protein synthesis and consequent influence on the oocyte and early embryo physiology.

We found several non-coding RNAs which exhibit specific expression and localization during mouse oocyte and early embryo development. Particularly, analysis of nuclear/cytoplasmic and monosomal/polyribosomal fractions defined possible candidates involved in the maintaining and modulating of maternal mRNAs. Overexpression of a short ncRNA followed by Dual Luciferase assay detected repression of the cap-dependent translation initiation mechanism. Furthermore, we induced clustering of the FMRP protein in the cytoplasm by overexpression of specific ncRNA. In conclusion, our results indicate significant contribution of the ncRNAs to the regulation of translation of the subset of maternal mRNAs in the mammalian oocyte and embryo.

Abstract

26. MicroRNA Determinants of the Balance between Effector and Regulatory CD4+ T Cells *In Vivo*

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MicroRNAs (miRNAs) are highly conserved small non-coding RNAs that control gene expression at the post-transcriptional level. As in many physiological processes, they have been implicated in the regulation of the immune system, including the differentiation and function of CD4+ T cell subsets. CD4+ T cells are key players in host defense against pathogens, but an incorrect balance between different CD4+ T cell subsets, namely pro-inflammatory effector T cells, including the IFN-γ-producers T helper 1 (Th)1 and the IL-17-producers Th17 cells, and anti-inflammatory regulatory T cells (Treg; FoxP3+ subset), can lead to immune-mediated diseases. We believe that a holistic approach based on *in vivo* models is required to understand how miRNA networks may control the balance between effector and regulatory subsets under physiological conditions. To address this, we have established a triple reporter mouse for *Ifng* (Th1 subset), *Il17* (Th17 subset), and *Foxp3* (Treg subset), and subject it to experimental autoimmune encephalomyelitis (EAE). We have then performed miRNA-seq in Th1, Th17 and Treg cells isolated from the spleen and lymph nodes of these mice at peak-plateau stage. Our data indicate that 110 miRNAs are differentially expressed between effector and regulatory subsets *in vivo*. From these, we selected 10 miRNA candidates that were specifically upregulated in one of the T cell subsets when compared with the others. To understand the functional impact of miRNA candidates in T cell differentiation *in vitro*, we first assessed the miRNA expression profile of naïve CD4+ T cells cultured under Th1, Th17 and Treg polarizing conditions. Data demonstrated that five miRNAs recapitulated the *in vivo* expression pattern. We will further determine the role of the miRNA candidates in T cell subsets differentiation using gain- and loss-of-function strategies, as well as establish the miRNA-target networks that may impact effector/regulatory T cell balance *in vivo*.

Abstract

27. New Roles for Old ncRNAs—Tuning the Ribosome

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The ribosome is the key interpreter of genetic information in all living organisms and is in mammalian cells responsible for a large part of the total cellular energy consumption. Hence, it is of outmost importance to understand how the ribosome is regulated. Although a highly complex structure, the ribosome was long thought be a constitutive machinery with little intrinsic selectivity on translation. However, evidence from several laboratories strongly suggest that the ribosome pool is heterogeneous and that this central biological machine comes in different flavors with distinct functionalities. Ribosome variation can arise from multiple sources – including the incorporation of different subsets of ribosomal proteins and via the high number of possible combinations of chemical modifications of the ribosomal RNA.

Using the RiboMeth-seq method, we have together with collaborators shown that the 2′-O-methylation profile of rRNA is both heterogeneous between cell types and displays dynamics during cellular processes. Based on extensive profiling of cell culture models and tissues we hypothesize that a subset of 2′-O-methylation sites are regulatory.

Examples will be presented of 2′-O-methylation sites displaying dynamic modification levels following specific cues, such as oncogene expression and cellular differentiation. As 2′-O-methylation is guided by snoRNAs, the ribosome modification pattern can be manipulated and the functional consequences of specific modification sites investigated. Results from snoRNA knockout cell lines will be presented, which strongly indicate regulatory functions for a subset of 2′-O-methylation sites.

Abstract

28. Newly Identified Small Nucleolar RNAs Play a Role in Myogenic Differentiation

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Introns represent almost half of the human genome, yet they are generally eliminated from the pre-messenger RNA by splicing and then degraded. Interestingly, we uncovered a subset of introns that escape degradation, providing precursors of small non-coding RNAs (sncRNAs). Since RNA splicing is a critical step for gene expression in eukaryotes, any disruption will lead to human diseases like the Myotonic Dystrophy type 1 (DM1). Alternative splicing (AS) of introns and the biogenesis of the sncRNAs remain poorly documented in these contexts.

We developed a RNA-seq dedicated to the systematic identification of new mid-sized ncRNAs in normal and DM1 human satellite muscle cells. As expected, we identified all known small ncRNAs at the expected abundance. In addition, we uncovered about hundred new snoRNAs.

In order to validate them as genuine and functional snoRNAs, we first analyzed their expression using discriminating primer pairs. Then, we assessed their presence in snoRNP complexes and their accumulation in the nucleolus. Since it is not possible to design probes for the specific detection of one snoRNA belonging to a particular snoRNA family by RNA-FISH, we adapted a technique for nucleoli isolation. Using PCR on nucleolar fractions, we found that newly identified snoRNAs were indeed present in the nucleolar fraction.

Among the new snoRNAs, we discovered new members of a family of snoRNA (snoX) whose expression levels increase during normal muscle differentiation but are drastically reduced in DM1 cells. Loss and gain of function experiments reveal that snoX family is involved in muscle differentiation. Indeed, reduced levels of snoX impair cell fusion in normal cells whereas overexpression of snoX promotes cell fusion in DM1 cells.

In conclusion, snoRNAs may play an unexpected role in muscle differentiation. They provide unconventional biomarkers for splicing diseases and new lines of research for therapeutic approaches.

Abstract

29. Variation, Splicing and RNA Editing from Single Cell RNA-Sequencing Data

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The vast majority of the existing models on single cell RNA-sequencing data (scRNA-seq) assess only the gene expression and typically do not consider additional transcriptome features, such as expressed genetic variation, splicing or RNA modifications such as RNA-editing. The later three features are essential components of the genomic interactive and are strongly interlinked with each other as well as with gene expression. In the single cell, these relationships can be more robustly assessed due to preservation of within-cell regulatory relationships. In this study, we applied series of analyses aiming to: (1) develop and test methods for estimation of splicing, DNA-encoded variation and RNA-editing levels from scRNA-seq data, and, (2) explored the ability to correlate the above features with gene expression levels on a genome-wide scale. To do that, for each locus of interest we assessed the frequency of RNA-sequencing reads carrying DNA-encoded or RNA-editing introduced variant, or particular splice-junction, and searched for correlation with gene expression using an eQTL-based (expression quantitative loci trait) linear regression model. To process the scRNA-seq data we adopted methods developed for bulk RNA-sequencing, including GATK, LeafCutter and ReadCounts, accounting for missing values, lower sequencing depth and overall smaller dataset size. In addition, we applied analysis-specific modules such as variation-aware alignment to address mapping bias, and reference-free splicing estimation to identify novel splice junctions. We validated selected findings on bulk RNA-sequencing data from the Genotype Tissue Expression (GTEx) project. Our results show that many bulk RNA-sequencing methods are suitable for scRNA-seq analysis after appropriate adjustments, and that the integrative variation-splicing-editing-expression transcriptome analysis holds a strong potential to identify genetic interactive networks from scRNA-seq data.

Abstract

30. Chromatin Related Functions of Long Noncoding RNAs in the Regulation of Cancer Pathways

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A major shift in our conception of genome regulation has emerged in recent years. It is now obvious that the majority of cellular transcripts do not code for proteins, being a significant subset of them long non-coding RNAs (lncRNAs). We have previously shown that some lncRNAs are functional molecules, taking active part in tumor-suppressor and oncogenic pathways through diverse mechanisms. Among those, the direct relationship between some lncRNAs and the chromatin places them in a central position in the control of gene expression and cellular identity, but while numerous studies have addressed the function of lncRNAs in gene silencing, less is known of their role at active genomic regions, where many lncRNAs are transcribed. I will present data regarding the relationship between the SWI/SNF chromatin remodeler complexes and lncRNAs transcribed *in cis*, revealing a functional interdependence between the complexes and lncRNAs in promoter-enhancer regulation with consequences in cell transformation.

Abstract

31. Single-Molecule Imaging of Transcription at Damaged Chromatin

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How DNA double-strand breaks (DSBs) affect ongoing transcription remains elusive due to the lack of single-molecule resolution tools directly measuring transcription dynamics upon DNA damage. Here, we established new reporter systems that allow the visualization of individual nascent RNAs with high temporal and spatial resolution upon the controlled induction of a single DSB at two distinct chromatin locations: a promoter-proximal (PROP) region downstream the transcription start site and a region within an internal exon (EX2). Induction of a DSB resulted in a rapid suppression of preexisting transcription initiation regardless of the genomic location. However, while transcription was irreversibly suppressed upon a PROP DSB, damage at the EX2 region drove the formation of promoter-like nucleosome-depleted regions and transcription recovery. Two-color labeling of transcripts at sequences flanking the EX2 lesion revealed bidirectional break-induced transcription initiation. Transcriptome analysis further showed pervasive bidirectional transcription at endogenous intragenic DSBs. Our data provide a novel framework for interpreting the reciprocal interactions between transcription and DNA damage at distinct chromatin regions.

Abstract

32. Epigenetic Regulation of the Imprinted *GNG12-AS1/DIRAS3* Locus by Non-Coding Transcription

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Non-protein coding RNAs are important epigenetic regulators of gene expression. Long non-coding RNAs (lncRNAs) are defined as spanning genomic regions of 200 or more basepairs, and can be spliced and post-transcriptionally processed. Emerging evidence suggests that the expression of lncRNAs has an impact on the surrounding chromatin organization and the activity of neighbouring genes; however, the molecular mechanisms that underpin this regulatory crosstalk are poorly understood. Imprinted genes are an excellent model system to study lncRNAs and their roles in coordinating the expression of multiple genes within a gene cluster. We are using the *GNG12-AS1/DIRAS3* locus to interrogate how chromatin architecture is affected by lncRNA transcription and how this impacts on the epigenetic regulation of nearby genes. *DIRAS3* is an imprinted tumour suppressor gene embedded within the *GNG12-AS1* lncRNA. Inhibition of *GNG12-AS1* transcription upregulates *DIRAS3*. Conversely, when *DIRAS3* is upregulated, *GNG12-AS1* expression levels are reduced.

To analyse the effect of the lncRNA on the chromatin architecture we have performed allele-specific capture Hi-C which has yielded insights into how *DIRAS3* and *GNG12-AS1* are topologically organized, and identified domain structures that may dictate their reciprocal transcription. Next we are addressing the potential of RNA polymerase (RNAP) collision/pausing to resolve how *GNG12-AS1* transcription interferes with the convergent transcription of *DIRAS3*. We are carrying out Global Run-On sequencing together with DNA-RNA immunoprecipitation and RNAPII chromatin immunoprecipitation to map engaged RNAPII pausing, and assess the re-annealing of nascent transcripts to the DNA template (R-loop formation).

Our combinatorial approach will elucidate the epigenetic regulation mechanisms of a key imprinted gene by its neighbouring lncRNA. This will gain novel insights into the role of lncRNAs in epigenetic regulation in complex gene regulatory landscapes such as imprinting clusters, with potential implications for our understanding of cancer etiology.

Abstract

33. Integrative Transcriptomic Analysis Suggests Novel Autoregulatory Splicing Events Coupled with Nonsense-Mediated mRNA Decay

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Nonsense-mediated decay (NMD) is a eukaryotic mRNA surveillance system that selectively degrades transcripts with premature termination codons (PTC). Many RNA-binding proteins (RBP) regulate their expression levels by a negative feedback loop, in which RBP binds its own pre-mRNA and causes alternative splicing to introduce a PTC. We present a bioinformatic analysis integrating three data sources, eCLIP assays for a large RBP panel, shRNA inactivation of NMD pathway, and shRNA-depletion of RBPs followed by RNA-seq, to identify novel such autoregulatory feedback loops. We show that RBPs frequently bind their own pre-mRNAs, their exons respond prominently to NMD pathway disruption, and that the responding exons are enriched with nearby eCLIP peaks. We confirm previously proposed models of autoregulation in SRSF7 and U2AF1 genes and present two novel models, in which (1) SFPQ binds its mRNA and promotes switching to an alternative distal 3′-UTR that is targeted by NMD, and (2) RPS3 binding activates a poison 5′-splice site in its pre-mRNA that leads to a frame shift and degradation by NMD. We also suggest specific splicing events that could be implicated in autoregulatory feedback loops in RBM39, HNRNPM, and U2AF2 genes. Taken together, these findings indicate that autoregulatory negative feedback loop of alternative splicing and NMD is a ubiquitous form of post-transcriptional control of gene expression among splicing factors.

Abstract

34. Identification of Cell-Free Circulating microRNAs for the Detection of Early Breast Cancer and Molecular Subtyping

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Early detection is crucial for achieving a reduction in breast cancer mortality. Analysis of circulating cell-free microRNAs present in the serum of cancer patients has emerged as a promising new non-invasive biomarker for early detection of tumors and for predicting their molecular classifications. The rationale for this study was to identify subtype-specific molecular profiles of cell-free microRNAs for early detection of breast cancer in serum. Fifty-four early-stage breast cancers with 27 age-matched controls were selected for circulating microRNAs evaluation in the serum. The 54 cases were molecular classified (luminal A, luminal B, luminal B Her2 positive, Her-2, triple negative). Nanostring platform was used for digital detection and quantitation of 800 tagged microRNA probes and to compare the overall differences in serum microRNA expression from breast cancer cases with controls. We identified 42 most significant (P 0.05, 1.5-fold) differentially expressed circulating microRNAs in each molecular subtype for further study. Of these microRNAs, 19 were significantly differentially expressed in patients presenting with luminal A, eight in the luminal B, ten in luminal B HER 2 positive and four in the HER2 enriched subtype. AUC is high with suitable sensitivity and specificity. For the triple negative subtype miR-25-3p had the best accuracy. Predictive analysis of the mRNA targets suggests they encode proteins involved in molecular pathways such as cell adhesion, migration, and proliferation. This study identified subtype-specific molecular profiles of cell-free microRNAs suitable for early detection of breast cancer selected by comparison to the microRNA profile in serum for female controls without apparent risk of breast cancer. This molecular profile should be validated in larger cohort studies to confirm the potential of these miRNA as early detection biomarkers to decrease unnecessary biopsy in patient with suspect of breast cancer.

Abstract

35. A Transcript-Level Analysis of Breast Cancer RNA-Seq Data Revealed a Widespread ERα Activity on RNA Alternative Splicing

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The luminal, estrogen receptor-alpha (ERα)-positive breast cancer (BC) subtype, is the most frequent form of BC. ERα is a key transcription factor in luminal BC cells, and its expression is critical for preserving an epithelial state and preventing EMT. Moreover, alternative splicing (AS), a key molecular mechanism regulated by multiple RNA-binding proteins (RBPs), is now believed to regulate the expression patterns of splice isoforms in a subtype-specific manner in BC. Deregulation of AS has also been associated with the development of resistance to endocrine therapy. In the present work, we investigate the role of ERα in regulating AS and controlling the expression of splice isoforms in the luminal BC subtype. Hence, we performed a transcript-level analysis using a paired-end RNA-seq dataset in MCF-7 cells and analyzed AS changes upon siRNA-mediated ERα silencing together with the assessment of expression changes in RBP genes and the enrichment of their binding motifs in the alternatively spliced sequences. We identified 4060 protein-coding and non-coding genes differentially expressed (DE) upon silencing ERα, including 2154 downregulated genes, related to cell cycle progression and DNA replication, and 1906 upregulated genes related to TGF-beta receptor signaling and cell migration activation. Interestingly, 305 RBP genes were also DE, comprising 230 downregulated and 75 upregulated RBP genes. The gene ontology analysis shows these DE RBP genes to be significantly related to mRNA splicing. Moreover, 713 splicing events were significantly regulated by ERα. Considering enriched motifs of RBP genes, we observed highly enriched motifs for downregulated RBP genes such as ESRP1/2 and SRSF proteins in skipping events most frequently on exons, suggesting these RBPs to enhance the inclusion of bound exons. In summary, the correlation of AS events together with expression changes in RBP genes may help us to identify central elements in the regulatory ERα-controlled network in BC.

Abstract

36. Circulating Serum miRNAs as Biomarkers in Fabry Disease: Adverse Disease Outcome and Functional Distinction between Phenotypic Groups

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Fabry disease is a monogenic X-linked genetic condition caused by mutations in the GLA gene, encoding for a galactosidase responsible for the metabolism of glycolipids. Despite of the accumulation of specific glucolipids, Fabry disease also involves life-threatening complications such as kidney damage, heart disease and stroke. Treatment for Fabry disease is based on the Enzyme-replacement therapy (ERT), by the administration of a recombinant GLA enzyme. Because classical biomarkers often failed to predict Fabry disease complications, we performed a multicenter test study to characterize the potential applications of serum circulating miRNAs as biomarkers of this condition. We quantified 31 different serum miRNAs in a large cohort of FD patients by reverse quantitative PCR using LNA primers. The diagnostic accuracy of miRNAs to identify patients with classical phenotype and its correlation with clinical variables was evaluated. In our cohort of 95 FD patients (49.5% males, mean age 51.2 years old, 84.2% under ERT) and 25 controls, a remarkable difference in the expression profile of the quantified miRNAs between patients with classical phenotype, attenuated phenotype and healthy controls, was observed. The diagnostic accuracy of miRNAs to identify patients with classical phenotype was significant (area under the curve [AUC] >0.7336 and p value 0.0001 for nine of the evaluated miRNAs). Two serum miRNAs also showed a significant direct correlation with estimated glomerular filtration rate (eGFR) stronger than the inverse correlation between albuminuria and eGFR, mainly in classical phenotype, demonstrating that circulating miRNAs may help to distinguish classical and attenuated phenotypes and may overcome albuminuria in the evaluation of nephropathy. Our results also suggested that impaired exocytosis of the miRNAs (due to disruption of the vesicle trafficking in an environment of lysosomal dysfunction) may be an alternative pathophysiological pathway in Fabry disease.

Abstract

37. LincRNA-p21-Mediated DDB2 Degradation Increases Chemosensitivity

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Chemotherapy is the most common therapeutic strategy for advanced breast cancer patients. Unfortunately, the development of chemoresistance leads to the high cancer recurrence and low survival rate, and remains a hurdle to cure this disease. p53 mutations have been reported to contribute to chemoresistance, but can not fully predict the sensitivity to chemotherapy. The roles of lincRNA-p21, a downstream regulator of p53, in regulating chemosensitivity were explored in this study.In this study, our results demonstratedthat lincRNA-p21, a p53-dependent long non-coding RNA, mediates DDB2 ubiquitination and proteasomal degradation by acting as a complex scaffold for DDB2 and Cul4 E3-ligase to reduce the DNA repair for chemosensitization. However, the repression of DDB2 by lincRNA-p21 was not found in estrogen receptor-positive cells due to the enhancement of DDB2 gene expression by the cooperation between ER and p53**.** These resultselucidate that ER tips the p53-mediated transcriptions toward to DDB2, but not lincRNA-p21, gene expressions in conferring chemoresistance.

Abstract

38. MicroRNA-221/222 Is Involved in the Acquired Lapatinib Resistance in Breast Cancer

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Lapatinib, a tyrosine kinase inhibitor targeting human epidermal growth factor receptors 1 and 2 (HER1 and HER2) has showed encouraging survival benefits in advanced HER2-overexpressing breast cancer patients, but the durable clinical response is severely limited by the emergence of drug resistance. A lot of efforts are made to elucidate the mechanism underlying acquired lapatinib resistance. In this study, we investigated the involvement of microRNA dysregulation in acquired lapatinib resistance. The microRNA expression pattern in response to lapatinib resistance was examined by microRNA microarray analysis and RT-qPCR. The involvement of microRNA in lapatinib resistance was verified by specific microRNA mimics and inhibitors. Signal pathway involved in microRNA dysregulation was evaluated by western blot.We found that the expression pattern of microRNA-221/222 was distinct between lapatinib-sensitive and lapatinib-resistant breast cancer cells with HER2 overexpression by using microRNA microarray analysis and RT-qPCR. Alteration of microRNA-221/222 expressions affected the sensitivity of HER2-overexpressing breast cancer cells to lapatinib. In the analysis of upstream regulator of microRNA-221/222 expressions, the results showed that Src tyrosine kinase is the potential regulator. Our results not only identify the involvement of microRNA-221/222 dysregulation by Src in acquired lapatinib resistance, but also suggest microRNA-221/222 as a predictor for lapatinib sensitivity in HER2- overexpressing breast cancer cells.

Abstract

39. MiR-494 Induces Sorafenib Resistance via Activation of the AKT/mTOR Pathway and Represents a Possible Biomarker in Hepatocellular Carcinoma

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Background and aims: Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death worldwide with a dismal prognosis in advanced stages. Novel systemic drugs have been approved, beside sorafenib, however no biomarkers helping the choice of personalized treatment for advanced HCC and/or predictive of treatment escape are actually available. A subgroup of HCCs showed tissue miR-494 upregulation together with a stem cell-like phenotype. This study aimed at elucidating miR-494 involvement in sorafenib resistance and investigating its possible use a biomarker of treatment response in HCC.

Methods: Two HCC animal models were used to evaluate the role of miR-494 in sorafenib resistance and to investigate the anti-tumor potential of a combined miR-494-silencing strategy. QPCR quantified miR-494 expression in tissue, sera samples and exosomal fraction from HCC patients and preclinical models. A functional analysis was performed to investigate miR-494-dependent regulation of AKT/mTOR pathway in sorafenib-treated HCC cells. MiR-494-mediated effects in sorafenib-treated HCC preclinical models was evaluated by proliferation and apoptotic assays, as well as by Western blot and cytofluorimetric analyses.

Results: MiR-494 upregulated the AKT/mTOR pathway in HCC cell lines by PTEN direct targeting, increasing cell survival of sorafenib-treated HCC cells. Higher miR-494 levels associated with bigger tumour size and reduced sorafenib response in both HCC animal models. AntagomiR-494 enhanced sorafenib efficacy in the rat HCC model, decreasing tumor progression respect to sorafenib-only treated animals. Circulating miR-494 levels correlated with tumor size in HCC animal models and predicted sorafenib response in HCC cell lines. Moreover, higher miR-494 basal levels associated with sorafenib resistance in a preliminary set of HCC patients.

Conclusions: MiR-494 plays a central role in sorafenib resistance of HCC cells through the upregulation of the AKT/mTOR pathway. MiR-494 deserves attention as a possible biomarker of prognosis and response to treatments in HCC.

Abstract

40. AP001056.1, A Prognosis-Related Enhancer RNA in Squamous Cell Carcinoma of the Head and Neck

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A growing number of long non-coding RNAs (lncRNAs) have been linked to squamous cell carcinoma of the head and neck (SCCHN). A subclass of lncRNAs, termed enhancer RNAs(eRNAs), are derived from enhancer regions and could contribute to enhancer function. In this study, we developed an integrated data analysis approach to identify key eRNAs in SCCHN. Tissue-specific enhancer-derived RNAs and their regulated genes previously predicted using the computational pipeline PreSTIGE, were considered as putative eRNA-target pairs. The interactive web servers, TANRIC (the Atlas of Noncoding RNAs in Cancer) and cBioPortal, were used to explore the RNA levels and clinical data from the Cancer Genome Atlas (TCGA) project. Requiring that key eRNAs should show significant associations with overall survival (Kaplan–Meier log-rank test, p 0.05) and the predicted target (correlation coefficient r > 0.4, p 0.001), we identified five key eRNA candidates. The most significant survival-associated eRNA was AP001056.1 with ICOSLG encoding an immune checkpoint protein as its regulated target. Another 1640 genes also showed significant correlation with AP001056.1 (r > 0.4, p 0.001), with the "immune system process" being the most significantly enriched biological process (adjusted p 0.001). Our results suggest that AP001056.1 is a key immune-related eRNA in SCCHN with a positive impact on clinical outcome.

Abstract

41. Deregulation of Lineage-Specific Alternative Splicing Subverts Tumor Suppressor Function and Promotes Gliomagenesis

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Lineage-specific alternative splicing, where splicing occurs in a tissue-regulated manner involving evolutionary conserved alternative exons, has a determinative role in brain development. The role of tissue-specific alternative exons in malignant transformation and tumor development is undefined. The impact of lineage-specific splicing on glioblastoma (GBM) tumorgenicity remains unclear but is conceptually attractive given the prevalence of this process in determining the fate of ancestral cells of potential GBM origin. We found that brain-enriched splice factor PTBP1 mediates lineage-specific alternative splicing of the ANXA7 tumor suppressor gene. PTBP1 is expressed in neural precursor cells (NPCs) and binds ANXA7 pre-mRNA to skip exon 6; this produces spliced ANXA7 isoform 2 (I2) expression. During lineage specification, PTBP1 is downregulated and this allows the expression of unspliced ANXA7 I1 (I1), which includes exon 6. We determined that the patterned expression of the I2 splice variant in the brain is restricted to lineages that represent potential GBM cells of origin but that I1 is virtually absent in these cells. Our data illustrate that lineage-specific splicing can augment genetic mechanisms to deregulate oncogenic pathways. Specifically, we showed ANXA7 I1, but not I2, targets oncogenic receptor tyrosine kinases (RTKs) such as EGFR, MET and PDGFRA for endosomal degradation. In the adult brain, PTBP1 is nearly absent; in GBM, PTBP1 aberrantly persists and RTK levels are elevated. However, we demonstrate that upon PTBP1 knockdown and/or, ANXA7 I1 re-expression, RTK signaling is reduced, and GBM tumorigenicity is diminished. This work defines a role of aberrant lineage-specific alternative splicing in GBM pathogenesis, offers a broader understanding of this process in malignant transformation in general, and provides a potential foundation for future studies to develop and therapeutically target developmentally regulated splicing factors in GBM.

Abstract

42. High Immune Cytolytic Activity in Tumor-Free Tongue Tissue Confers Better Prognosis in Patients with Squamous Cell Carcinoma of the Oral Tongue

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Immune cells and cytolytic activity within the tumor microenvironment are being intensively studied. Through transcriptome profiling, immune cell enumeration using the xCell tool and cytolytic activity quantification according to granzyme A (*GZMA*) and perforin (*PRF1*) mRNA levels, we investigated immunoreactivity in tumor and/or tumor-free tongue tissue samples from 31 patients with squamous cell carcinoma of the oral tongue (SCCOT) and 14 healthy individuals (control tongue tissues). We found significantly altered immune cell compositions (p 0.001) and elevated cytolytic activity (p 0.001) in tumor compared to tumor-free samples, and altered infiltration of a subset of immune cells (e.g. $CD8^+$ T cells, p 0.01) as well as increased cytolytic activity (p 0.001) was identified in tumor-free compared to control samples. Controlling for patient age at diagnosis and tumor stage, Cox regression showed that high cytolytic activity in tumor-free samples associated with improved disease-free survival (hazard ratio = 4.20, 95% confidence interval = 1.09–16.20, $p = 0.037$). However, the degree of cytolytic activity in tumor samples did not provide prognostic information. Taken together, our results show the presence of cancer-related immune responses in clinically tumor-free tongue in patients with SCCOT. Measuring cytolytic activity in tumor-free tongue samples contralateral to tumor might thus be an effective approach to predict clinical outcome.

Abstract

43. Identification of Cell-Free microRNAs for Breast Cancer Early Detection and Molecular Subtype: A Case Control

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Breast cancer is the leading cause of death among women and the standard screening test for this neoplasm is a mammography. However, this method has certain limitations, such as false-positive results, uncomfortable technique, reduced sensitivity in young women and women with high breast density. Considering these limitations, some studies are searching for non-invasive biomarkers that help the early diagnosis of breast cancer. MicroRNAs is a new class of biomarkers as regulators of gene expression that may be detected by liquid biopsy, such as serum. Thus, an identification of microRNAs molecular signatures associated to early detection of the breast cancer molecular subtypes is emerged. Our aim was to determine the expression profile of microRNAs and to correlate with breast cancer subtypes in Brazilian patients with early breast cancer and women without breast cancer risk. Fifty-four patients with early-stage breast cancer were selected and paired with twenty-seven women without breast cancer risk (controls). Total RNA was obtained from serum of cases and controls by miRNeasy Serum / Plasma Kit (QIAGEN). The expression profile of differentially expressed serum microRNAs was evaluated using the nCounter panel from NanoString Technology. Statistical analyzes were performed in R software. In silico analysis was determined by networks miRNAs-target genes were using Cytoscape software and the molecular pathways were identified by Reactome. It was possible to identify several miRNAs differentially expressed and specific for each molecular subtype. In addition, in silico analysis revealed target genes miRNAs of important molecular pathways associated with carcinogenesis for each molecular subtype, among them, molecular pathways of angiogenesis, proliferation, and apoptosis can be highlighted. Our results showed that there is a group of circulating miRNAs with the potential for biomarkers of early breast cancer associated with its molecular subtypes when compared to healthy women, which regulate important molecular pathways of carcinogenesis.

Abstract

44. Identification of microRNA Signature and Potential Pathway Targets Cervical Cancer Progression

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New and more accurate prevention strategies are needed to detect the premalignant lesions or cervical intraepithelial neoplasia (CIN). MicroRNA (miRNA) expression analysis can be used as molecular biomarkers to early detection of cervical cancer (CC) based on liquid-based cytology (LBC). We aimed to determine molecular signatures of miRNAs in cervical precursor lesions from LBC cervical and to identify molecular pathways potentially associated with the progression of CC. We included 99 LBC cervical samples from women previously submitted to colposcopy at the Barretos Cancer Hospital as follows: without CIN (n = 14), CIN 1 (n = 34), CIN 2 (n = 17), CIN 3 (n = 26) and CEC (n = 08). We tested high-risk HPV by Cobas X480™. We performed miRNA expression by the nCounter® miRNA Expression Assays panel (NanoString Technologies). The interaction networks of miRNA-target were constructed in the Cytoscape program, the molecular pathways related to these targets were found by REACTOME and the molecular network illustrations were obtained from KEGG. We found seven miRNAs significantly overexpressed and two miRNAs downregulated NIC 3 samples. A logistic regression analysis was performed to select a combined ROC curve analysis group. In this analysis the combination of the four miRNAs presented an AUC of 0.99 with 95% sensitivity and 100% specificity, showing how accurate the combination of these biomarkers. In addition, in the *in silico* analyzes they revealed that hsa-miR-381-3p targets the cyclin dependent kinase-2 (CDK2) gene and that hsa-miR-205-5p targets the Fibronectin-1 (FN1) gene, both described in important molecular pathways, such as cell cycle. This study showed a panel of miRNAs found in CIN samples that may discriminate patients without-CIN and CIN 3 group. In addition, we suggest that these miRNAs may be involved with the progression of CC.

Abstract

45. LncRNA and mRNA Network Reconstruction Highlights Novel Key Regulators in Breast Cancer Subtypes

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Long-non-coding-RNAs (lncRNAs) are functional RNAs with sequences longer than 200 nucleotides. Dozens of lncRNAs act as regulators of gene expression in various biological processes and their deregulation is associated with a wide variety of diseases, including breast cancer (BC). BC is the most common cancer in women worldwide, but little is known regarding the regulatory role of lncRNAs in BC transcriptional networks. Through the use of an innovative methodology, our goal was to develop a network-based approach to clarify how lncRNAs-mRNAs networks contributes to BC subtypes differentiation. LncRNAs and mRNAs expression datasets of breast cancer samples were extracted from The Cancer Genome Atlas (TCGA) and the reconstruction of transcriptional networks was performed using the R package called RTN. We computed, via mutual information, the potential regulatory network for 120,000 lncRNAs and 20,000 mRNAs, considering only lncRNAs as the major network regulator, influencing in both lncRNAs and mRNA expression. Then, we filtered the results based on network size and on a metric called differential enrichment score (dES), which quantifies the regulatory networks activity between different BC subtypes. This analysis resulted in 108 regulatory networks, that were then stratified according to greater difference in regulatory networks activity between the luminal A and basal-like subtypes due the great prognosis differences between these subtypes. We selected three lncRNAs potential regulators for expression validation, through the RT-qPCR, in Brazilian BC patients. All selected lncRNAs are differential expressed in subtypes, two were high expressed in the luminal A subtype and the other in triple negative subtype, confirming the previous results from TCGA data. Our network approach provides a footing to reveal the regulatory circuits governing breast cancer subtypes differentiation and may help to identify new therapeutic targets.

Abstract

46. Long Noncoding RNAs as Prognostic Biomarkers after Cardiac Arrest

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Introduction: More than 400,000 cases of cardiac arrest (CA) occur every year in Europe. Despite modern healthcare strategies, only 10% of these patients survive without major neurological sequelae. Predicting outcome after CA would allow adapting healthcare, maximising efforts to patients who would benefit. However, current prognostication modalities and biomarkers lack accuracy, hence the need to discover novel prognostic biomarkers. Previous studies suggest that circulating noncoding RNAs constitute a reservoir of novel biomarkers.

Purpose: To identify circulating long noncoding RNAs (lncRNAs) able to predict outcome after CA.

Methods: In this sub-study of the Target Temperature Management trial, we enrolled 2 groups of 25 age- and sex-matched patients. Patients in the first group survived with no major neurological sequelae (cerebral performance category score 1) while patients in the second group died within 6 months. A discovery phase with RNA sequencing was performed in whole blood samples collected 48h after CA in PAXgene RNA tubes.

Results: RNA sequencing provided an average of 62 million reads per sample. The mapping of these reads to the human genome detected 115,722 RNAs, 64,689 of which were considered as lncRNAs by LNCipedia database. 20 lncRNAs were identified as differentially expressed between the 2 patient groups with a p value below 0.05 and a fold-change higher than 1.5. Among these 20 lncRNAs, one in particular (lnc001) was able to predict 6-month survival. Patients with high levels of lnc001 were associated with a higher chance of survival (hazards ratio 1.25 ± 95% confidence interval [1.05/1.48], $p = 0.013$).

Abstract

47 Micro-RNA-17 Has a Minor Role in Kidney Ischemia-Reperfusion Injury in Mice

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Introduction: Ischemia-reperfusion (IR) is a common causes of acute kidney injury (AKI), a risk factor of renal fibrosis and chronic kidney disease. We previously described the activation of the pro-proliferative and anti-apoptotic miR-17-5p in IR induced AKI. Aim: to investigate the function of miR-17-5p in IR-induced renal fibrosis in mice.

Methods: AKI was induced in the left kidneys of C57BL / 6N mice with 20-minute ischemia, without the removal of the right intact kidney. One day before the IR operation, mice were given miR-17-5p silencing (17-LNA-IR group, N = 10) or scrambled sequence (SCR-IR group, N = 10) locked nucleic acid (LNA) modified antisense oligonucleotides (30 mg/kg, ip). On the 7th reperfusion day, the efficacy of the miR-17-5p inhibition was evaluated in the injured and control kidneys (qPCR). We investigated tubular damage in histological sections (PAS, HE) and KIM1 mRNA, miR-17-5p-target p21 mRNA, fibrosis (FN1 mRNA), inflammation (TNFα mRNA), oxidative stress response (NRF2 mRNA), and cell-proliferation (PCNA mRNA) with real-time PCR.

Results: At the 7th reperfusion day, specific LNA inhibition prevented the increase in miR-17-5p expression (1.62×, *p* 0.01, SCR-IR vs. non-IR) in the damaged kidneys (0.49×, *p* 0.0001; 17-LNA-IR vs. SCR-IR). The inhibition of miR-17-5p following IR had no significant effect either on the histological damage, or on KIM1 (83.2×, *p* 0.0001), FN1 (8.6×, *p* 0.0001), TNFα (5.8×, *p* 0.0001), NRF2 (1.4×, *p* 0.01), PCNA (1.3×, *p* 0.05), and p21 (3.8×, *p* 0.0001) mRNA levels.

Conclusion: While miR-17-5p could have renoprotective effects, our findings suggest that miR-17-5p inhibition has no significant effects on the IR-induced AKI outcome.

Abstract

48. MicroRNA Expression Assessment in Formalin-Fixed, Paraffin-Embedded Breast Cancer Tissues from Hereditary Breast Cancer Patients

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Background: MicroRNAs (miRNAs) are small non-coding RNAs involved in post-transcriptional gene expression regulation and have been implicated in multiple cancer-related pathways. Aberrant expression of miRNAs has been frequently reported in sporadic breast cancers (SBC), but few studies have focused on determining miRNA expression profiles and investigate their potential impact in hereditary breast cancer (HBC). **Aim:** Evaluate the miRNAs expression profile and analyze in silico the role of miRNA in hereditary breast carcinogenesis. **Methods**: We analyzed 61 FFPE breast tissue samples obtained from 33 HBC (27 *BRCA1/2* and 6 BRCAx) and 20 SBC patients, as well as 8 normal breast tissues (NBT) from healthy individuals using a NanoString technology. Subsequently, we performed a target prediction analysis for each DE miRNA using the miRDIP platform, followed by a pathway enrichment analysis on Reactome using the Cytoscape software. **Results**: We found miRNA expression profiles in HBC showing a specific deregulation pattern compared to SBC and NBT. Bioinformatic analysis revealed that these miRNAs shared target genes involved especially in cell cycle regulation. **Conclusions**: Our findings suggested that are specifically deregulated miRNAs in HBC. Moreover, the expression of their predicted target genes using tissue microarrays (TMA) can validate the role of this miRNAs to better understanding *BRCA1/2*-mediated breast carcinogenesis.

Abstract

49. Modulated Electro-Hyperthermia (mEHT) Differentially Inhibits Slow and Fast Progressing Triple Negative Breast Cancers in Mice—A Link to H19 lncRNA

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Introduction: The effective therapy of triple-negative breast cancer (TNBC) has not yet been achieved. Modulated electro-hyperthermia (mEHT) is a novel adjuvant therapeutic approach to induce selective destruction of cancer cells, based on the highly selective effects on tumor cells by a 13.56 MHz radiofrequency promoted electric field.

Method: Syngeneic mouse TNBC cells (slow processing 4T07 and fast progressing 4T1) were inoculated in female Balb/C mice. Tumor size was followed with digital caliper and ultrasound. 1.0 ± 0.5 W power was applied to reach 40 °C of the skin above the tumor maintained for 30 minutes. Treatments were repeated 1-5 times with 48 h or 72 h intervals. H19 lncRNA was determined by real-time PCR.

Results: Although short protocols (1–3 treatments) did not reduce tumor size in most protocols, TDR was elevated already by one mEHT. Only three or more mEHTs with 72 h inhibited tumor growth. Animals stopped gaining weight in each treatment protocol—including sham treatments. This effect was stronger in the fast-growing 4T1 model.

mEHT was more effective on fast progressing 4T1 tumors. The toxicity indicated by body weight loss was also higher in these mice. Tumor aggressiveness and metastatic capacity correlated with H19 lncRNA which was affected by mEHT.

Five treatments caused tumor cell death which manifested in a decreased measurable tumor size and weight. Ki67 positive nucleus count was reduced.

Summary: A single mEHT treatment increased tumor destruction, this effect manifested in measurable tumor size reduction only after 3 mEHT treatments with 72 h intervals. Repeated mEHT inhibited tumor cell proliferation by promoting cell cycle arrest and was more effective against fast progressing 4T1. H19 lncRNA mediated mechanisms may be involved in mEHT effects and in differential tumor agressivity.

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Abstract

50. Prostate Cancer Epitranscriptome: Impact of m6A Deregulation in Malignancy

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Prostate cancer (PCa) is a highly prevalent malignancy in which deaths are mostly due to advanced disease where no curative treatments are available. Thus, further elucidation of the mechanisms involved in PCa aggressiveness is imperative. Although several non-coding RNAs (ncRNAs) were associated with castration-resistant PCa, little is known about ncRNA post-translational modifications' implication in prostate carcinogenesis.

RNA modifications are far more abundant than those in DNA and new insights in this field may expand knowledge on critical mechanisms for PCa progression. The functional dissection of the proteins that modulate RNA modifications has major relevance to further unveil their biological significance. Methylation of the N6 position of adenosine (m6A) is a RNA post-transcriptional modification involved in gene regulation. Emerging evidence suggests that m6A methylation plays a critical role in cancer through various mechanisms. Although, the components of m6A modification machinery are known, the specific functions and mechanisms involved in malignancy development are still unclear, particularly in prostate cancer.

The main goal of this study is to evaluate the expression of proteins involved in m6A establishment and the potential impact of their deregulation in ncRNAs expression and ultimately in prostate cancer aggressiveness.

Differential expression of m6A methyltransferase complex (MTC) components was observed in prostate cancer cell lines, specially for VIRMA which was found overexpressed in PC-3 cell line, both at transcript and protein levels. Interestingly, PC-3 VIRMA knockout using CRISPR methodology significantly decrease m6A levels, as well as several ncRNAs involved in prostate tumorigenesis. Importantly, PC-3 VIRMA silencing attenuated malignant phenotype with significant reduction in cell viability, invasion and migration capacity.

In conclusion, our study shows MTC expression deregulation in PCa cell lines and associate lower m6A RNA modification levels with reduced PCa aggressiveness through oncogenic ncRNAs suppression.

Abstract

51. Regulatory circRNA-Centered Networks and miRNA Sponging Activity in Permanent Atrial Fibrillation

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Atrial fibrillation (AF) is the most prevalent cardiac arrhythmia in western countries responsible for an increased risk of embolic stroke and heart failure and being a cause of morbidity and mortality especially of elderly individuals. After a trigger event that starts the disease, the clinical manifestations of AF often include the transition from a paroxysmal AF (where arrhythmic crises are sporadic and reversible) to a long-lasting and chronic form of the condition (permanent AF). The factors governing the progression of AF to a permanent chronic condition are still not well characterized. Cardiac tissue requires a delicate control of its functions at the molecular level which will ensure a mechanical, electrical and chemical synchronization. In the non-proliferative myocardium, some epigenetic factors such as non-coding RNAs (ncRNAs) have been characterized as important players involved in its function but also in pathological conditions as AF. We hypothesize about the existence of additional regulatory layers in AF involving an intricate cross-talk between different ncRNA species, namely miRNAs and circRNAs for the establishment of a chronic AF condition. We have performed an unbiased study analyzing the expression profile for miRNAs and circRNAs in left-atrial biopsies from patients with paroxysmal and permanent AF by RNA-seq. The transition from paroxysmal to permanent AF is characterized by a pattern of down-regulated miRNAs, concomitant to the appearance of specific circRNA species. The analysis of the sponging activities of the circRNAs exclusively expressed in permanent AF samples, allowed us to determine that they could be responsible for the downregulation of specific miRNAs in establishment of a permanent AF condition. Sponging activity of circRNAs sequestering specific miRNAs is an important factor to be considered for the determination of the molecular mechanisms involved in AF progression.

Abstract

52. Shared RNA-Dependent Networks in Motor Neuron Diseases

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The high incidence of causal mutations affecting ubiquitously expressed proteins involved in different aspects of RNA metabolism is a striking feature of motor neuron diseases. Despite their distinctive genetic and clinical features, the motor neuron degenerative disorders Amyotrophic Lateral Sclerosis and Spinal Muscular Atrophy present multiple similarities and unexpected connections that support the concept of shared molecular mechanisms for neuronal degeneration. In order to explore this hypothesis, we generated comparable Drosophila models and transcriptome datasets for loss-of-function mutations for the fly orthologues of three hallmark genes linked to the two diseases—TDP-43, Fus and Smn. We additionally identified the nuclear and cytoplasmic mRNAs bound by these proteins in neuronal cells using tissue specific expression of GFP-fusion proteins couples to RNA immunoprecipitation and sequencing (RIP-Seq). The integrative analysis of these datasets supports a model whereby each disease gene has a direct effect on a large number of distinct cellular targets, converging through network level interactions towards the disruption of a common subset of cellular functions, defining a network signature for motor neuron disease.

Abstract

53. The Role of Aging on Renal Ischemia Induced lncRNA Profile

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Aim: Expression profile of lncRNA is hardly known in control, injured kidneys of old mice.Methods: Left renal pedicle of adult (9.4 ± 0.3 months, n = 7) and old (28.5 ± 1.2 months, n = 8) C57BL/6N mice was clamped 20 min. The right kidney was left intact. Plasma urea, urine NGAL were measured prior to, 7 days after reperfusion. On day 7 tubular injury (PAS, HE) and KIM1 mRNA, fibrosis ,senescence was analyzed (histology, qPCR). Long non-coding RNA profile was examined with qPCR array.Results: Older mice had higher baseline uNGAL (old: 285.7 ± 93.8 ng/mg Crea vs. adult: 78.0 ± 9.1 ng/mg Crea, p 0.05) , milder kidney injury (p 0.05). KIM1 (8.3×; p 0.001), FN1 (2×; p = 0.05) , p21 (3.8×; p 0.05) mRNA were higher in their non-ischemic kidneys vs the adult group. Was no difference in baseline plasma urea (old: 75.9 ± 10.2 mg/dl vs. adult: 57.6 ± 5.3 mg/dl). Following IR, tubular injury increased in both groups (p 0.05) with KIM1 (adult: 202×, old: 19.5×, p 0.0001) , FN1 (adult: 8.5×, old: 3×, p 0.001) mRNA vs contralateral control kidneys. uNGAL was higher after IR compared to the baseline (old: 3817 ± 1975 ng/mg Crea, p 0.0001; adult: 2833 ± 1201 ng/mg Crea, p 0.001). p21 mRNA increased only in ischemic kidneys (4.5×, p 0.01), vs contralateral control. The plasma urea concentration increased only in the old group at 7 days (140.7 ± 25.6 mg/dl, p 0.01). From the 81 measureable lncRNAs 8 increased, 1 decreased only by IR. Old age only influenced 1 lncRNA expression. Further 12 lncRNA expression was influenced by both IR and old age.Conclusion: demonstrated significant tubular damage, decreased renal function in old mice, in accordance with literature. Several lncRNAs differentially expressed in old and adult mice in both control, ischemic kidneys.

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Abstract

54. The T1D-Associated lncRNA *Lnc13* Modulates Pancreatic Beta Cell Inflammation by Allele-Specific Stabilization of *STAT1* and *STAT2* mRNA

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The vast majority of type 1 diabetes (T1D) association signals lie in non-coding regions of the human genome and many have been predicted to affect the expression and secondary structure of lncRNAs. However, the contribution of these lncRNAs to the development of the disease and the molecular mechanisms by which these molecules may contribute to the pathogenesis of T1D remain to be clarified.

Results of our Group have demonstrated that the expression of several T1D-associated lncRNAs in pancreatic beta cells is modified by diabetogenic stimuli, such as viral infections and pro-inflammatory cytokines. Of special interest are the results obtained in the functional characterization of *Lnc13*, a lncRNA expressed and upregulated by viral dsRNA in pancreatic beta cells that harbors a SNP associated with T1D (rs917997).

Overexpression of *Lnc13* in human pancreatic beta cells led to an increase in STAT1/2 pathway activation that correlated with increased production of pro-inflammatory chemokines. Interestingly, when the *Lnc13* harboring the risk allele for T1D (rs917997*C) was transfected, the increase in STAT1/2 signaling was more pronounced than in cells transfected with the plasmid encoding the *Lnc13* with the non-risk allele for T1D (rs917997*T). In addition, the effect of *Lnc13* upregulation on pro-inflammatory chemokine production was also allele-specific.

Our studies have shown that intracellular PIC induces *Lnc13* translocation from the nucleus to the cytoplasm, promoting the interaction between *STAT1* and *STAT2* with a protein named PCBP2 that regulates the stability of *STAT1* and *STAT2* mRNA molecules.

In conclusion, our results show that *Lnc13* participates in pancreatic beta cell inflammation via regulation of the STAT1/2 signaling pathway in an allele-specific manner.

Abstract

55. Using Blood Transcriptome Profiles of Alzheimer's Disease Patients to Diagnose Disease and Further Our Understanding of Mechanisms

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Alzheimer's dementia is a neurodegenerative condition typified by progressive cognitive decline, affecting over 5.5 million patients in North America today. Diagnosis is typically based on cognitive assessment of the patient, enhanced brain imaging, and often combined with assay of cerebral spinal fluid for levels of amyloid beta, tau and phosphorylated tau proteins. However, such approaches have challenges identifying different types of dementia, are invasive, and not always available to all patients. Therefore, we investigated whether a blood biomarker test may be able to assist in the diagnosis of Alzheimer's dementia.Patients were recruited from the Emory University Alzheimer's research center. Controls were identified from the Emory research center as non-cognitively impaired individuals. The study was subjected to IRB review at Emory University, and Morehouse School of Medicine. Blood samples were obtained in Paxgene tubes and stored at −20 °C until RNA was extracted (pre-Analytix). Whole transcriptome RNA-Seq libraries were prepared and multiplexed onto sequencing beads, using the total RNA kit v2.0 for Ton Torrent. Sequencing was performed in an Ion Torrent S5 sequencer using 540 chips. Data were aligned to the Hg19 (GRCh37) reference genome using STAR and bowtie2 and annotated using the ensembl GRCh37.87 guide. Data were further analyzed using Partek genomics suite.We investigated differential expression of RNAs and subsequent RNAs were used in cluster analysis. Hierarchical clustering shows a difference in expression patterns between patients and controls. Refining our patients, we observe clustering of the AD other dementia and MCI patients. Principle component analysis enabled a distinction between the different patient groups (PCA 60%), and showed clustering by subgroup. Gene hub analysis may provide insight into brain regulation of RNA in AD. While preliminary, these data suggest blood RNA expression patterns may assist in the diagnosis of Alzheimer's disease.

Abstract

56. Using RNA-Seq to Determine Cancer-Specific Therapeutic Targets

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Regardless of the drug being used, chemotherapy for cancer treatment is accompanied by side effects which vary from mild to severe or life threatning. Most of these manifestations result from the activity of the pharmacological substance upon endogenous cells. Although most side effects have great relevance in the clinics, very few studies have focused on the molecular basis of their triggers. In this study we investigate the similarities and differences between various types of proliferating cells and patient-derived cancer cells, based on transcript expression profile revealed by RNA-Seq experiments. Contrasting the transcriptomes of endogenous cells and cancer cells represents an alternative approach to identify better drug targets or assess existing drugs. In this sense our findings may further support the development of new forms of treatment that specifically target the malignant cells within a tumour, with less widespread side effects. As proof of principle we used our data to construct clinically relevant ASOs targeted to candidate transcripts and observed a marked decrease in proliferation of primary glioblastoma cell-lines.

Abstract

57. Exploring the Role of *Xist*-Mediated Polycomb Recruitment in the Initiation of Transcriptional Silencing of the X Chromosome

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Xist lncRNA has been established as the master regulator of X-chromosome inactivation in female placental mammals but its mechanism of action remains unclear. However, the mechanism underlying its capacity to trigger chromosome-wide silencing remains far from understood. *Xist* is an unusual big (~17 Kb) and poorly sequence conserved, with the exception of a number of tandem arranged repeats, named A-to-F. Here, we created a series of novel *Xist* inducible mutants at the endogenous locus in mouse embryonic stem cells (ESCs) and explored their capability to initiate X-linked gene silencing and to modify chromatin via recruitment of Polycomb (PcG) repressive complex 1 (PRC1) and 2 (PRC2). We will show our extensive characterization of these *Xist* mutants using several high-throughput methods to evaluate the effect of several of these repeats on the recruitment of chromatin marks (by ChIP-seq), on the *Xist* protein-interactome (by ChIRP-MS) and on chromosome-wide transcriptional silencing (by RNA-seq). Our results shed light on the molecular mechanisms on the RNA modules mediating Polycomb recruitment and their effect on transcriptional silencing in the context of the X chromosome.

Abstract

58. A Key Role for Micrornas in Regulating IL-17 *versus* IFN-γ Production by γδ T Cells

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γδ T cells are key providers of proinflammatory cytokines in various contexts of (patho)physiology. They are preprogrammed in the thymus into distinct subsets producing either interleukin-17 (IL-17) or interferon-γ (IFN-γ), which segregate with CD27 expression. In the periphery, CD27⁻γδ T cells, which usually express IL-17, can be induced to coexpress IL-17 and IFN-γ. We have previously found that miR-146a was selectively enriched in these cells and restricted their IFN-γ production by targeting Nod1 mRNA. We aim at further dissecting microRNA-mediated regulation of effector γδ T cell differentiation independently of the use of surface markers, which do not allow the isolation of pure populations of IL-17⁺ or IFN-γ⁺ γδ T cells. Thus, we isolated these pure γδ T cell populations from peripheral lymphoid organs of a double reporter IL-17-GFP:IFN-γ-YFP mouse strain and subjected them to next generation sequencing analysis of both microRNA and mRNA repertoires, which allowed us to identify miRNA and mRNA signatures directly associated with cytokine expression. Furthermore, differentially expressed miRNAs and mRNAs were bioinformatically integrated into networks that allowed the prediction of 6 and 3 miRNAs targeting key determinants of the IL-17 and IFN-γ programs of γδ T cells, respectively. Preliminary results, based on gain-of-function studies on fetal liver progenitor cells co-cultured with OP9-DL1 cells indicate that miR-326 and miR-450b may regulate γδ T cell development, inhibiting IFN-γ production. Further molecular assays are being performed on peripheral γδ T cells to provide a broader functional characterization of the impact of microRNAs on the identity and differentiation of effector γδ T cell subsets.

Abstract

59. Comparing the Relative Gene Expression Level of Hyoxia Related Genes in Different Cancer Cell Lines

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Background: Hypoxia plays a pivotal role in the process of cancer progression and dissemination. Clinical studies have clearly shown that patients with hypoxic tumors (pO2 ≤ 10 mmHg) have significantly lower overall survival or disease-free survival. Different cell types vary in their microenvironments and preferred oxygen levels. Comparing hypoxia inducible genes expressions among tumors with different oxygen requirements in their microenvironments would provide a better understanding of these mechanisms.

Objective: This study aims to identify the changes in expression of hypoxia-inducible genes in 6 different cancer cell lines that vary in their oxygen levels in an attempt to identify hypoxia biomarkers that can be targeted in therapy.

Methodology: Human cancer cell lines obtained from the American Type Culture Collection were used; MCF7 breast cancer cells, PANC-1 pancreatic cancer cells, PC-3 prostate cancer cells, SH-SY5Y neuroblastoma brain cancer cells, A549 lung cancer cells, MCF 10A normal breast cells, and Hep G2 hepatocellular carcinoma. The differences in gene expression were examined using the real-time based PCR array technology.

Results: Almost all hypoxia-inducible genes showed a pO_2-dependent upregulated expression. Noticeable gene expression differences were identified, the most important changes occurred in the HIF1, MAPK and NF-KB signaling pathways and in central carbon metabolism pathway genes such as HKs, PFKL, and solute transporters.

Conclusion: This study determined hypoxia biomarkers such as NF-KB, HIF1, HK, PFKL, and PIM1 that were expressed only in hypoxic cells. In addition, the genes expressed only in the sever hypoxic liver and pancreatic cells indicate that sever and intermediate hypoxic cancer cells vary in their gene expression. Pleotropic pathways that play role in inducing hypoxia such as HIF1 and MAPK, and NF-κB pathways were upregulated. It showed the effect of tumor's microenvironment on gene expression.

Abstract

60. Control of Endothelial Sells Phenotypic Switch by ncRNAs

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Angiogenesis is a multi-step, tightly regulated process that plays a crucial role in development, as well as in pathological conditions such as tumor growth. During sprouting angiogenesis(SA), endothelial cells(EC) are activated by VEGF-A, a potent angiogenic growth factor, which triggers the transition from quiescent to active EC. However, the gene expression events which sustain this phenotypic switch have not been elucidated so far. Previous studies have already elucidated the importance of miRNAs, in the regulation of angiogenesis. But, research on lncRNAs in angiogenesis is still in its infancy. We hypothesize that lncRNAs participate, together with miRNAs, to the regulatory network that sustains SA ultimately contributing to EC phenotype specification.To study the activation of quiescent ECs induced by an angiogenic stimulus, and the impact that lncRNAs and miRNAs may exert on this process, we exploited, by using a global transcriptomic approach, a three dimensional model—that recapitulates SA in vitro. This allowed the annotation of non-coding RNAs whose expression is modulated during sprouting. The use of miRNA target prediction tools, together with co-expression analysis between miRNAs, lncRNAs and protein-coding genes, identified biological pathways whose activity is modulated during SA, and the genes that are more subjected to post-transcriptional control. The information obtained was used to generate a non-coding RNA regulatory network that sustains EC phenotypic transition during SA.

Abstract

61. Mir-7 Replacement by Syntetic Molecules Carried by Neuromag® Magnetic Particles—A Potential Neuroprotective Strategy in the Rat Rotenone Model of Parkinson

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Parkinson's disease is a brain disorder characterized by pathological changes in nigral dopaminergic neurons along with increased oxidative stress, intracellular aggregation of alpha-synuclein and neuroinflammation (Przedborski et al., 2005; Jellinger, 2012). In this regard, a study has shown a down-expression of the microRNA-7 in PD patients associated with alpha-syn accumulation, loss of dopaminergic neurons and reduction of dopamine content (McMillan et al., 2017). Therefore, a decrease in miR-7 levels may have an essential role in Parkinson's pathobiology. In the present work, we evaluated whether the reposition of miR-7 levels by synthetic molecules of miR-7 (mimic 7) could protect rat nigrostriatal neurons against rotenone injury. For that, we injected Mimic-7 nanostructured in Neuromag® magnetic particles by stereotaxic surgery into the right lateral ventricle of rotenone-injured rats. Fluorescence microscopy at seven days after surgery revealed that the Neuromag® nanoparticles successfully transfected the molecules into striatal neurons. Behavioral tests showed that animals treated with mimic-7 presented better motor activity and coordination as compared to rotenone group. Furthermore, mimic-7 treated animals exhibited a high number of TH (+) cells in the Substantia Nigra when compared to rotenone-injured rats. Likewise, Iba1 immunostaining in the same brain region revealed a reduced number of microglial cells in the treated group, suggesting that the replacement of mirR-7 protected the nigral dopaminergic neurons against the rotenone insult. Altogether these results indicate that microRNA-7 is a possible therapeutical target and its replacement is a potential neuroprotective strategy in the rat rotenone model of Parkinson.

Abstract

62. Regulation of miRNA Turnover in T Cell Activation

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An important downregulation of the majority of the microRNAs occurs few hours after T cell activation, while certain miRNA are up-regulated. Mechanisms responsible for changes in the miRNA repertoire during T cell activation are largely unknown. Uridylated miRNAs have been described to decrease their levels upon T cell activation in previous studies from our group. Interestingly TUTase enzymes expression is also influenced by T cell stimulation. An active mechanism such an exonuclease able to degrade miRNAs with certain post-transcriptional modifications could account, at least in part, for the decrease in miRNA levels. Now, we have identified in activated T cell lysates an exonuclease which preferentially binds to uridylated miRNAs. Our results indicate that this enzyme is able to degrade miRNA and is upregulated upon T cell stimulation. Using CRISPR/Cas9 technology we have developed a silencing tool which is being currently used to explore the RNA targets of this enzyme and its role during T cell activation.

Abstract

63. Role of microRNAs in Regulation of Hunger/Satiety Center in the Mouse Brain

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The global increase in obesity and related metabolic disorders has led to an increasing need to study causative agents that may ultimately lead to the development of treatments for pathologies associated with disturbed energy balance. Energy homeostasis is achieved through strictly regulated communication between the central nervous system and peripheral organs. The hypothalamus is the most studied region of the brain associated with the energy balance due to its direct participation in both the detection of metabolic states and the control of feeding and energy expenditure. The anatomical location and the interconnected cellular composition enable this small area of the brain to regulate the energy balance of the whole body. In particular, the arcuate nucleus (Arc) in the hypothalamus is one of the basic units with localization readily available for peripheral hormonal and nutrient signals, including leptin, ghrelin, insulin, amino acids. Energy balance and food intake regulating cells in the nucleus consist of: 1—orexygenic "hunger" neurons AgRP and 2—satiety neurons—POMC secreting neurons and recently discovered OxytocinR-vglut2 neurons. AgRP neurons receive both homeostatic and 'cognitive' signals and serve as a center for monitoring and regulating food intake. Here we show that the interruption of microRNA biogenesis by removing the Dicer gene specifically in AgRP neurons in the arcuate nucleus leads to the development of hyperphagic obesity. Titration of AAV-AgRP-Cre vector causing mutation allowed us to precisely control the level of obesity phenotype. The phenotype described in mice with a voracious hyperphagia in a regular chow diet is very similar to the phenotype of patients with PWS (Prader-Willi Syndrome). This human genetic disease likewise causes severe obesity, and the people affected suffer from hyperphagia and lack of satiation.

Abstract

64. Smaug2 Drives Adipocyte Differentiation by Repressing the Alternative Translation of the C/EBPβ Inhibitory Isoform

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We previously described that both mammalian and insect Smaug, a translational repressor that binds specific RNA motifs termed Smaug recognition elements (SREs), form membraneless organelles similar to Processing Bodies (PBs), termed S-bodies. In neurons, the S-bodies localize at dendrites and respond to specific synaptic stimuli, dissolving and releasing transcripts to allow their translation (Baez et al., J Cell Biol 2011; Pimentel & Boccaccio 2014; Luchelli et al. J Cell Sci, 2015). In addition to the CNS, Smaug function is important in other cell types. Smaug1/Samd4a KO mice show strong developmental defects of mesenchymal tissues, namely bone, cartilage, muscle and fat. Here we show that Smaug2/Samd4b expression increases during adipocyte differentiation and helps differentiation. The KD of Smaug2 in 3T3L1 cells affects several molecular and cellular markers of adipogenesis, including the accumulation of lipid droplets. C/EBPβ is a key transcription factor that regulates adipogenesis. The alternative translation of C/EBPβ mRNA generates two proteins with antagonistic effect and little is known on the regulation of the alternative translation of C/EBPβ mRNA. A pro-adipogenic variant is translated from an early AUG and the translation of a downstream AUG brings about an inhibitory isoform. We found that Smaug2 knockdown provokes an increase in the translation of the C/EBPβ inhibitory isoform. The C/EBPβ mRNA contains several SREs and Smaug2 binds C/EBPβ mRNA. We propose that Smaug2 directly represses the translation of C/EBPβ inhibitory isoform, thus allowing adipocyte differentiation. As in neurons, we found that Smaug2 forms cytosolic bodies in 3T3L1 that contain repressed mRNAs. The S-bodies are highly dynamic and dissolve when mRNAs are trapped in polysomes by the action of cycloheximide. How S-body dynamics affects C/EBPβ alternative translation remains unknown.

Abstract

65. N^6-Methyladenosine: A Conformational Marker That Regulates the Substrate Specificity of Human Demethylases FTO and ALKBH5

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N^6-Methyladenosine (m6A) is currently one of the most intensively studied post-transcriptional modifications in RNA. Due to its critical role in epigenetics and physiological links to several human diseases, it is also of tremendous biological and medical interest. The m6A mark is dynamically reversed by human demethylases FTO and ALKBH5, however the mechanism by which these enzymes selectively recognise their target transcripts remains unclear. Here, we report combined biophysical and biochemical studies on the specificity determinants of m6A demethylases, which led to the identification of an m6A-mediated substrate discrimination mechanism. Our results reveal that m6A itself serves as a 'conformational marker', which induces different conformational outcomes in RNAs depending on sequence context. This critically impacts its interactions with several m6A-recognising proteins, including FTO and ALKBH5. Remarkably, through the RNA-remodelling effects of m6A, the demethylases were able to discriminate substrates with very similar nucleotide sequences. To our knowledge, this is the first report demonstrating that m6A itself serves as an important selectivity determinant for m6A demethylases. Our findings provide novel insights into the biological functions of m6A modifications. The mechanism identified in this work is likely of significance to other m6A-recognising proteins.

Abstract

66. Functional Dissection of the Conserved Sequences within *Xist* lncRNA and Its Protein Interactors in X-Chromosome Inactivation

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X-chromosome inactivation (XCI) is a remarkable example of epigenetic silencing, regulated by the *X-inactive specific transcript* (*Xist*) long non-coding RNA (lncRNA) in female eutherian mammals. This ~17,000 bp lncRNA is monoallelically upregulated from the X chromosome chosen for inactivation, coating the entire chromosome in *cis* and inducing transcriptional silencing and heterochromatin formation. *Xist* possesses six tandem-repeated regions, known as the A to F repeats, believed to act as regulatory modules, through the interaction with specific proteins (e.g., Spen, Wtap, HnrnpK, Matr3 and Ptbp1). However, the relative contribution of each of these RNA modules and its proteins interactors to XCI is not fully understood. Using the CRISPR-Cas9 approach, we deleted every single tandem-repeated region of *Xist*, in mouse embryonic stem cells, to address their impact on XCI. As a complementary approach, we knocked-down several RNA-binding proteins (RBPs) previously shown to bind to some of these regions. I will present the results of some explored features namely Xist RNA stability, capacity to coat the X-chromosome, recruitment of heterochromatin marks and induction of transcriptional silencing on both newly generated *Xist* mutants and knock-down approaches of RBPs. RNA-FISH, IF and RT-qPCR techniques were used to perform these analyses, that will shed light on how this multi-tasking lncRNA coordinates these different tasks to ensure a stable inactive state of a full chromosome.

Abstract

67. Gametophytic Transcriptomics of an Early Embryophyte (*Blasia pusilla*) and Comparative Landscape of Gametophyte Evolution

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Transcriptomics plays a central role in understanding the genetic underpinning of post-transcriptional regulation. In recent years, transcriptomics has not only acted as a role model for unraveling post-transcriptional regulatory events but has also provided a basis for species-level comparative transcriptomics. In the absence of genomic data, transcriptomics not only allows for de novo phylo-transcriptomics approaches but also enables the detection of specific genes, which are specific to certain clades of organisms. We present the first reference gametophytic transcriptome of *Blasia pusilla*sequenced using Illumina Next-Seq and PacBio-Isoseq with the aim of unravelling the gametophytic transcriptomics of an early embryophyte. We also highlight the role of transcriptomics in identifying early genes and possible phylogenetic pressures on ancestral gene evolution across land plants.

Abstract

68. Integrative Analysis Reveals Functional and Regulatory Roles of H3K79me2 in Mediating Alternative Splicing

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Accumulating evidence suggests alternative splicing (AS) is a co-transcriptional splicing process not only controlled by RNA-binding splicing factors, but also mediated by epigenetic regulators. Aberrant AS plays an important role in regulating various diseases, including cancers. In this study, we integrated AS events derived from RNA-seq with ChIP-seq of H3K79me2 across 34 different normal and cancer cell types, and found the higher enrichment of H3K79me2 in skipping exon (SE) and alternative 3′ splicing site (A3SS). Further, we unveiled two clusters mainly comprised of blood cancer cell types with a strong correlation of H3K79me2 and SE. We further showed the deletion of DOT1L1, the sole H3K79 methyltransferase, impeded the leukemia cell proliferation and switched exon skipping to inclusion isoform in two MLL-rearranged (MLLr) AML cell lines, demonstrating H3K79me2/DOT1L is involved in mediating skipping exon processing which might influence transformation and leukemogenesis. We further compared SEs before and after EPZ5676 (a DOT1L inhibitor) treatment in a pilot of 12 MLLr AML and 3 normal controls and identified 332 switched SEs mediated by DOT1L. Remarkably, those switched SEs are associated with a loss for a majority of H3K79me2 peaks in the EPZ-treated primary MLLr samples. Collectively, our work for the first time reveals H3K79me2 plays functional and regulatory roles through co-transcriptional splicing mechanism.

Abstract

69. LncRNAs Associated with Heterochromatin Function in Immune Cells in Schizophrenia Subjects

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Abstract: Psychosis is associated with chronic immune dysregulation. Many long non-coding RNAs (lncRNAs) display abnormal expression during activation of immune responses, and play a role in heterochromatic regulation of gene promoters. We have measured lncRNAs MEG3, PINT and GAS5, selected for their previously described association with heterochromatin. Peripheral blood mononuclear cells (PBMCs) were isolated from blood samples collected from 86 participants with a diagnosis of psychosis and 44 control participants. Expression was assessed in relation to diagnosis, illness acuity status, and treatment with antipsychotic medication. We observed diagnostic differences with MEG3, PINT and GAS5, and symptom acuity effect with MEG3 and GAS5. Medication effects were evident in those currently on treatment with antipsychotics when compared to drug-naïve participants. We observed that clinical diagnosis and symptom acuity predict selected lncRNA expression. Particular noteworthy is the differential expression of MEG3 in drug naïve participants compared to those treated with risperidone. Additionally, an in vitro cell model using M2tol macrophages was used to test the effects of the antipsychotic drug risperidone on the expression of these lncRNAs using quantitative real-time PCR (qRT-PCR). Significant but differential effects of risperidone were observed in M2tol macrophages indicating a clear ability of antipsychotic medications to modify lncRNA expression.

Abstract

70. Multi-Network Approach to Predict New Proteins Involved in NMD

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The mechanism of nonsense-mediated decay (NMD) selectively degrades mRNAs carrying a premature translation-termination codon and regulates the abundance of a large number of physiological mRNAs that encode full-length proteins. Although this complex process has been extensively studied along the years, the interactions and connectivity among NMD players is not completely understood. Additionally, some NMD mechanistical aspects suggest missing roles that can be played by proteins still not reported as involved in this pathway.

To tackle this hypothesis, we developed a bioinformatic network-based approach to predict new proteins involved in NMD. Our approach consists in performing several queries to different types of publicly available data, in order to explore the ability of proteins to bridge related processes, while integrating data regarding protein-protein interactions, co-expression and co-regulation.

We found that known NMD-factors have physical, regulatory and co-expression interaction signatures with related processes (mRNA translation, mRNA splicing, mRNA degradation and mRNA transport), which can be used to distinguish them from other proteins. We computed a scoring algorithm to rank NMD-neighbors according to the similarity to these signatures, generating a list of NMD candidates, that we aim to validate experimentally. Interestingly, some candidates were recently studied in NMD context and showed promising results. Furthermore, a cross-validation analysis indicated the robustness of the predictions provided by our method.

On the road to developing a tool to apply this approach to other biological processes, we observed good cross-validation results for other RNA-related processes, suggesting this method's usefulness in the RNA research area.

Abstract

71. Nurr1 Overexpression Exerts Neuroprotective and Anti-Inflammatory Roles via Down-Regulating IL-1β Expression in Both Neonatal and Adult Microglia

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Microglia play a central role in immune surveillance and modulation of neuroinflammation as well as playing a role in neurodevelopment. Microglia involve in the development of pathological pain in adults but not early in life. However little detail is known about the changing phenotype of microglia during development. We examined age-related changes in microglia following activation with pathogen- and damage- associated molecular patterns (PAMPs/DAMPs). Microglial cultures were prepared from neonatal postnatal day (P1) and adult (P40) rat brains and spinal cords. Immunocytochemistry, qRT-PCR and functional assays were used to identify age-related differences. Adult microglia display a pro-inflammatory immune profile characterized by significantly increased IL-1β mRNA levels in response to PAMPs and DAMPs. In contrast, IL-1β mRNA in neonatal microglia showed a slight increase after stimulation with DAMPs. Anti-inflammatory gene expression was significantly increased in neonatal microglia relative to adult microglia. Compared to adult microglia, neonatal cells had increased phagocytic activity when unstimulated and following activation with LPS and ATP. Moreover, the nuclear receptor Nurr1 may play a major role in reducing pro-inflammatory signalling and promoting the anti-inflammatory phenotype in neonatal microglia. Nurr1 isoforms are differentially expressed in neonatal and adult microglia, with the Nurr1a isoform being significantly elevated in neonatal cells. Using lentiviral vector-mediated expression of Nurr1 isoforms, we also show that over-expression of TINUR, a splice variant of Nurr1, in neonatal and adult microglia attenuates inflammation by trans-repression the IL-1β expression and trans-activation the IL-10 gene expression following ATP exposure. Together, these data provide evidence for the age-related difference in microglial function during postnatal development. In addition, these findings demonstrate insight into the mechanisms by which Nurr1 might act, and suggest potential therapeutic targets for the treatment of neuroinflammatory diseases.

Abstract

72. Oxygen-Sensing Ribonucleoprotein Interactions Control Hypoxic Adaptation

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Adaptive, system-wide translatome (protein output) remodeling represents a fundamental response to biological change, including evolution, development, and stimuli adaptation. Recent compelling evidence from our laboratory and others indicate that this phenomenon is controlled predominantly via global reprogramming of mRNA translation efficiencies (TE) by stimuli-specific translation machineries. These discoveries highlight the fundamental question as to the mechanisms that control the global switch in mRNA TE in response to physiological changes. We propose that this phenomenon is governed by mRNA-encoded TE determinants that interact with stimuli-activated RNA-binding proteins (RBPs). Focusing on the physiological stimulus of oxygen deficiency (hypoxia), our system-wide, unbiased MATRIX platform identified HuR, hnRNP A2/B1, PCBP1, PCBP2, and PTBP1 as oxygen-responsive RBPs that recruit differential mRNA populations for translation in an oxygen-regulated manner. This ancient RBP network allows anoxia-resistant animals and mammalian cells to survive hypoxia by operating as an oxygen-sensitive switch of glycolytic intensity. TMT-pSILAC and RNA sequencing revealed this RBP network as a translation efficiency checkpoint, actively integrating upstream mRNA signals to activate the Pasteur Effect. Hypoxia-inducible factor 2α (HIF-2α) is the oxygen-sensor of this system. Our data suggest that an oxygen-sensing RBP network controls anaerobic metabolism by TE remodeling to confer hypoxia tolerance across evolution.

Abstract

73. RNA Structure-Function Analysis of Regulatory Regions of p53 mRNA

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At least half of all tumors exhibit mutations in the tumor suppressor *p53* gene. Indeed, the fact that *p53* is frequently mutated in cancer led to its identification as an oncogene, when first described in 1979. Only later it was classified as a tumor suppressor, due to the clarification of its wild-type role in maintaining genome integrity and preventing malignant transformation. The p53 mRNA can express and regulate several p53 protein isoforms, through several mechanisms. One of those involves Internal Ribosome Entry Sites (IRES), which are RNA secondary structures that can directly recruit the ribosome to the vicinity of the initiation codon instead of bringing it to the 5′-end cap. We have shown before that Δ40p53 isoform expression is regulated by an IRES in p53 mRNA. Another regulatory function of p53 mRNA that we have discovered is its capacity to bind and sequester Hdm2 in the nucleoli, preventing Hdm2-mediated degradation of full-length (FL) p53 proteins. Here, we analyzed, structurally and functionally, a highly conserved regulatory region of p53 mRNA. This region, commonly mutated in cancer, regulates the translation of a short p53 isoform. While FL-p53 protein works as a tumor suppressor by regulating many biological processes such as cell cycle, senescence and DNA repair, we previously found that this shorter p53 isoform is implicated in cancer progression. In fact, it can promote enhanced cell survival, proliferation, adhesion and formation of invasive cell structures. Our results suggest that some mutations can promote tumorigenesis not only by inducing loss of protein function but also by acting through mechanisms at the mRNA level, promoting oncogenic p53 protein isoform expression. This may lead to new understandings of the onset and progression of some types of tumors as well as to the development of new cancer therapies.

Abstract

74. Role of the Different LSD1 Isoforms in Regulation of Transcription and Alternative Splicing of Cancer Related Genes

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The lysine specific histone demethylase LSD1 is highly expressed in a wide variety of cancer types, leading to enhanced cell growth and invasion. LSD1 participates in chromatin remodelling and represses or activates transcription depending on the interacting cofactors by modifying the histone 3 lysine 4 (H3K4) and H3K9 methylation status. LSD1 inhibition results in a prominent shift of alternative splicing. Via alternative splicing two mini exons Ex2a and Ex8a could be included or not in the LSD1 mRNA in a cell-type specific manner. LSD1 isoforms supposed to have different histone demethylation activities and different affinities to the components of CoREST or NuRD, The deregulation between expression levels of LSD1 isoforms in normal tissue could be one of the drivers of tumour initiation and progression. In this study, expression of LSD1 2a+ mRNA isoform was quantified in different cancer and non-cancer cells. Despite the different level of the expression of the total LSD1 mRNA in different cell types, the LSD1 2a+ isoform represents always 25–30% of the total LSD1 mRNA. Upon inhibition of LSD1 demethylase activity with the small molecule the percentage of LSD1 2a+ mRNA isoform was significantly reduced. RT-PCR with opposite-directed primers followed by sequencing of the PCR products revealed the expression of new circular RNAs from LSD1 locus. Expression of the LSD1 circRNA with Ex3_Ex2 back-splicing junction was found significantly downregulated in cancer cells or tumor tissues compared to the non-cancer ones. Knockdown of identified LSD1 circRNAs with siRNA, specific for the back-splicing junctions or overexpression of LSD1 circRNAs from the transfected vectors altered the expression of different isoforms of LSD1 mRNA. The understanding of the role of different LSD1 mRNA isoforms and circular LSD1 RNA species will shed light on cancer regulatory mechanisms and provide novel strategies for diagnostics and therapeutic approaches.

Abstract

75. Targeting microRNA Function by Dietary Polyphenols from Olive Oil

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Environmental and lifestyle factors (i.e. diet) are key players to control human health status. The mechanisms involved between dietary factors and our genome to produce biological effects remains poorly described. Several lines of evidence support that dietary compounds influence epigenetic mechanisms including small RNA expression. However, the mechanisms involved in these interactions remained unclear.

microRNAs (miRNAs) are small noncoding RNAs that regulate gene expression post transcriptionally. By targeting multiple genes, miRNAs can control entire biological pathway that control tissue homeostasis, offering a new therapeutic opportunity by modulating miRNA function to treat a variety of disorders.

Polyphenols (the main phytochemical present in vegetables) may influence signaling pathways involved in pathological processes through miRNA modulation. Hydroxytyrosol (HT) is the most prominent phenolic compound of virgin olive oil, with strong antioxidant activity and is involved in exerting health benefits against chronic diseases.

Here, liver samples from C57BL/6 mice supplemented with 45 mg HT/kg/day for 8 weeks were subjected to small RNA sequencing. Four miRNAs were found to be modulated by HT treatment (miR-802-5p, miR-423-3p, miR-30a-5p and miR-146b-5p) from the 247 miRNAs identified.

Gene ontology analysis reveals that modulated miRNAs are involved in Wnt signaling pathway, CCKR signaling map and inflammation mediated by chemokine and cytokine signaling pathway regulation. In addition, these miRNAs regulate genes including Ccdc117, Ntrk2, Mrpl17, Timm22, Zfp945, Ubxn7, Tmem71, Slc30a7, Gucy1a2, 4931406C07Rik, Zdhhc21, and Dclk1, which are involved in crucial signaling, i.e. Gucy1a2 in endothelium signaling pathway and Zdhhc21 in metabolic process.

Beneficial effects attributed to HT consumption might be explained through the regulation of certain miRNAs involved in different signaling pathways. Targeting miRNA levels by specific dietary components or lifestyle factors might be a viable option to accompany current pharmacological therapy to target miRNA function.

Abstract

76. *LOC339803*lncRNA Mediates Susceptibility to Intestinal Inflammation by Allele-Specific Methylation-Dependent Gene Expression Regulation

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LOC339803 is a lncRNA with unknown function that is located on the intestinal inflammatory disease-associated region 2p16.1. One of the disease-associated SNPs is close to a m6A mark, so we hypothesized that SNP-related methylation changes could affect lncRNA function. To test this hypothesis, we used an intestinal cell line heterozygous for the associated SNP together with RNA extracted from human small intestinal tissue.

When analyzing the expression of each lncRNA allele, we observed that in both, cell cultures and human tissues from intestine the risk allele transcript was present at lower levels and was less stable. To check if m6A methylation was involved in the difference of lncRNA allele levels we performed an allele-specific meRIP and observed that the G allele was preferentially methylated. Moreover, when the m6A mark was inhibited with cycloleucine, the G allele lncRNA expression was further decreased, pointing to an involvement of the methylation mark in lncRNA function. Assessment of the subcellular location of the lncRNA showed that *LOC339803*is primary nuclear in intestinal cells suggesting a role in transcriptional regulation. Analysis of three-dimensional structure of this locus using 3C technique showed that *LOC339803*region preferentially interacts with the nearby *COMMD1*gene region, and this interaction seems to be dependent of m6A methylation levels. Overexpression of *LOC339803*induced allele-dependent downregulation of *COMMD1*gene, while CRISPR-Cas9 mediated deletion of the lncRNA induced *COMMD1*expression. In addition, we also observed an alteration of NFkB levels in the overexpressed and mutated cells, in line with previously described results on *COMMD1*modified cells.

These results suggest that the lncRNA *LOC339803*has an allele-specific effect in NFkB-mediated inflammation. The allele specificity seems to be related to m6A methylation levels and to the ability of the lncRNA to regulate the expression of the nearby *COMMD1*gene.

Abstract

77. Repurposing of Affymetrix Microarray to Investigate lincRNAs: The Role of *LINC00675* in Colon Cancer Predisposition

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The most updated human ENCODE annotation lists more than 28000 different lncRNAs [1], among them lincRNAs are central players of gene regulation and are involved in cell biology and human diseases; however, the mechanisms underlying lincRNA role in cancer is not completely understood yet. Genome-wide association studies (GWAS) displayed that 80% of cancer-associated single nucleotide polymorphisms (SNPs) reside in non-coding regions of the human genome [2].

Herein, we show how by repurposing public datasets of Affimetrix U133 plus 2.0 gene expression microarray and by using GWAS datasets, we could retrieve novel lincRNAs associated with human cancer predisposition.

First, to identify microarray probes that match to lincRNAs, we reannotated the Affimetrix U133 plus 2.0 probes using GENCODEv24 annotation; by these means, we characterized lincRNA probe expression in two public gene expression datasets of human normal tissues and we identified lincRNAs that are enriched in selected normal human tissues. Next, by comparing the map of tissue specific lincRNAs with the position of SNPs (±50 kb) associated with cancer, we retrieved 3 lincRNAs (*LINC00675*, *CASC15*, *RP11-849I19.1*) that are near SNPs associated with colon, endometrial or prostate cancer and are enriched in the normal counterpart tissue.

Secondly, we evaluated the expression of these 3 lincRNAs in microarray datasets containing normal-tumor tissues, and we observed that *LINC00675* expression is decreased in colon cancer and liver metastasis tissues compared with normal colon mucosa. To further explore *LINC00675* function, we either knocked out by Cas9 or silenced by dCas9-KRAB the first exon of *LINC00675*, and investigated the impact on surrounding genes [3].

In conclusion, we present data supporting the potentiality of public gene expression datasets to explore non-coding transcripts associated with human tumor predisposition.

References

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Abstract

78. Adaptation to Extracellular Acidosis by pH-Dependent eIF5A

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System-wide remodeling of protein synthesis is an important component of cellular stress adaptation. Global translational adaptations (e.g., translation efficiency remodeling and alternative translation machineries) often predominate over transcriptional control and mRNA levels in controlling protein output. This phenomenon has been observed during evolution, development, differentiation, and especially during cellular adaptations to physiological stimuli. Acidification of the extracellular environment (extracellular acidosis) as a consequence of anaerobic metabolism is frequently observed in tumors. We, and others, have shown that extracellular acidosis induces cellular dormancy, an enigmatic phenotype involved in ischemic tolerance and cancer resistance to radiation and chemotherapy. An important question remains as to what are the sensing mechanisms and assets that enable translational adaptations in cells responding to variations in extracellular pH. Methodology: We have utilized pulse SILAC mass spectrometry and RNA sequencing technologies to uncover the proteins that are made in response to hypoxia acidosis. Using our newly-developed, mass spectrometry related, unbiased, biological activity-based MATRIX platform, we have uncovered the acidotic protein synthesis machinery. Results: Amongst the acidosis-enriched translation factors identified by MATRIX, eIF5A is, interestingly, the only one that can be traced back to the last universal common ancestor, which is believed to have relied exclusively on anaerobic metabolism. We have discovered that eIF5A operates as a pH-dependent transducer that is essential for acidosis-induced cellular dormancy. eIF5A is activated in acidosis by SIRT1, a NAD+ dependent deacetylator. eIF5A controls the production of tumor suppressor, TSC2, via translation efficiency which is a potent inhibitor of mTORC1 and energy expensive processes. Additionally, my studies have revealed unique acidosis-specific biomarkers. Conclusion: Collectively these results uncover a novel role for a highly conserved translation factor in the response to hypoxia-acidosis and a collection of markers of the acidotic state.

Abstract

79. Diurnal Transcriptomics Analysis on Heterosis Mechanism of Super-Hybrid Rice

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Heterosis or hybrid vigor refers to the complicated biological phenomenon that F_1 hybrid exhibits increased levels of growth and fitness than any of patents. Great research efforts revealing some mechanisms of rice heterosis from different aspects were successfully made during the last century. But until now the mechanism of heterosis still puzzles us. In order to reveal the molecular mechanism of rice heterosis from the perspective of circadian rhythms, a comparative transcriptome analysis between rice hybrid *LY2186* and its parents were performed employing genome-scale strand specific RNA sequencing. The samples of flag leaves were collected from 17 serial time points of two diurnal cycles during grain-filling stage in a controlled condition greenhouse. Based on the RNA regulatory data sets, we systematically analyzed and the intersected portion of differentially expressed genes and circadian-regulated genes and identified 1,155 genes exhibiting circadian expression profiles (4.4%–4.6% of expressed genes), with clear separation of transcripts peaking in the morning and evening. Morning-peaking genes associated with light harvesting, oxidation reduction, aromatic amino acid family metabolic process, transmembrane transport, isoprenoid biosynthetic process and O-acyltransferase activity; evening-peaking genes associated with response to oxidative stress, redox activity, cellular protein metabolic process and heme binding. *In silico* metabolic pathway analysis further indicated circadian regulation of photosynthesis; it also predicted circadian variation in biosynthesis of secondary metabolites such as carotenoid, phenylpropanoid, chlorophyll and cysteine. In summary, *in vivo* circadian rhythms associated with photosynthesis, redox activity and active secondary metabolites such as isoprenoid may contribute to rice heterosis.

Abstract

80. DNA Methylation Affects the Expression of Genes Encoding Vitamin K Related Proteins

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Vitamin K (VK)-related proteins participate in important extra-hepatic processes, including bone metabolism, calcification, inflammation and carcinogenesis. However, knowledge on their regulation by epigenetic factors is scarce. DNA methylation is an epigenetic modification known to regulate gene expression and be influenced by nutrition, drugs and life style, and is frequently deregulated in disease.

Here we investigated if DNA methylation could impact in the expression of VK-related genes using a cancer database, a (de)methylation *in vitro* system and zebrafish *in vivo* model.

First, we compared the methylation status (b-values) of genes coding for VK-dependent proteins (F2, BGLAP), VK cycle enzymes (VKORC1, VKORC1L1, GGCX) and VK nuclear receptor (NR1I2) in normal and tumor primary tissues from 6 cohorts at The Cancer Genome Atlas. Genes were considered differentially methylated if |Db| > 0.2 (|Db| = |Tumorb-Normalb|). F2 and NR1I2 had at least one probe differentially methylated in 5 cohorts, BGLAP in 3 cohorts and VKORC1L1 in one cohort. VKORC1 and GGCX were not differentially methylated.

The vast majority of identified probes (CpGs) had an AUC > 0.8 indicative of good diagnostic biomarkers. Kaplan-Meier curves evidenced VKORC1L1 and NR1I2 methylation as good overall-survival (OS) predictor in pancreatic cancer (log-rank *p*-value = 0.0209 and 0.006, respectively) and NR1I2 methylation as indicative of Recurrence-Free Survival (RFS) (log-rank *p*-value = 0.0125). BGLAP probe was a prognostic biomarker for OS in colorectal cancer (log-rank *p*-value = 0.0062).

Identified CpGs were located within known transcription factors binding sites and hypomethylation of promoter regions associated with gene expression. Thus, we used the HCT116 DNMT3B/DNMT1 double knockout cells (demethylation *in vitro* model) to confirm the impact of DNA methylation in the expression of VK-related genes. Finally, we used the zebrafish as an *in vivo* model to investigate if VK supplementation could impact on DNA methylation and modulate the expression of VK-related genes.

Abstract

81. Papd5-Mediated Polyadenylation of Intergenic rRNA Drives Liquid-to-Solid Amyloid Body Formation

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Amyloid-bodies are fibrous biomolecular condensates composed of immobilized proteins that adopt an amyloid-like state. The formation of Amyloid-bodies are initiated by a class of inducible low complexity long noncoding RNA derived from stimuli-specific loci of the rDNA intergenic spacer (rIGSRNA). These rIGSRNA seed a transient liquid phase in the nucleolar area that rapidly transition to a solid-like state by a physiological amyloidogenesis phase transition program. This raises the question as to what mechanisms promote the maturation of the liquid-like droplet into a fibrous solid-like amyloidogenic condensate. Here we show a role for the non-canonical nuclear poly(A) polymerase Papd5 in A-body biogenesis. On stimulus, Papd5 mediates the *de novo* polyadenylation of rDNA intergenic RNA in the nucleolus. The polyadenylated RNA species are stable and remain associated with Amyloid-bodies during stimuli. Depletion of Papd5 does not impede rIGSRNA-seeded liquid phase but prevents the transition to solid-like Amyloid-bodies. The disassembly of Amyloid-bodies after stimuli termination requires exosome-dependent degradation of polyadenylated intergenic RNA. These data identify regulated enzymatic activities that drives a liquid-to-solid phase transition to assemble Amyloid-bodies.

Abstract

82. RNA Analysis Revealed Genes Related to Cellular Cholesterol Metabolism

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Background: Transcriptome analysis and bioinformatics approach were applied to determine genes that change their expression in monocyte-derived human macrophages exposed to HDL or LDL. Furthermore, we individually knocked down revealed genes in cells and evaluated their role in cellular cholesterol metabolism.

Methods: Monocyte-derived human macrophages were cultured with native HDL or native human LDL as well as modified LDL for 24 hours, and then RNA-seq libraries were prepared using a NEBNext Ultra RNA library kit. Libraries were sequenced on an Illumina HiSeq 1500. To identify genes showing differential expression we used edgeR package.

Cholesterol accumulation was evaluated in monocyte-derived human macrophages with genes knocked-downed by siRNA. We individually knocked down these genes in THP-1 cells using gene silencing by siRNA and measured cellular cholesterol efflux to HDL

Results: Using RNAseq, we analyzed mRNAs induced by HDL in macrophages and identified four genes: *CCL4*, *FADS1*, *INSIG1*, and *LDLR*.

Knock-down of *CCL4* increased cholesterol efflux from lipid-loaded macrophages. Knock-down of *INSIG1* and *LDLR* diminished cholesterol efflux. Conversely, there was no difference in the capacity of HDL to remove cholesterol from THP-1 cells in which *FADS1* expression was knocked down.

The transcriptome from macrophages incubated with native LDL was compared with the transcriptome from macrophages incubated with modified LDLs. As a result, four genes related to cholesterol accumulation were identified: EIF2AK3, TIGIT, CXCL8, and ANXA1. Knock-down of these genes prevented cellular cholesterol accumulation. In the case of ANXA1, knock-down decreased cellular cholesterol concentration in cells cultured with modified LDL versus cells cultured without LDL

Conclusion: We have identified *CCL4*, *LDLR*, and *INSIG1* as genes involved in cholesterol efflux, whereas EIF2AK3, TIGIT, CXCL8 and ANXA1 involved in cholesterol accumulation.

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