Keystone Symposia - Poster Abstracts
Small Regulatory RNAs (D7)

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POSTER NUMBER: 1039

MicroRNA1915 modulate insulin signalling pathway by suppressing of DNAJ Heat Shock Protein Family (Hsp40) Member B3 (DNAJB3) expression

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Background: Heat shock response (HSR) is one of the key host-defense mechanism that is dysregulated in obesity-induced insulin resistance and type 2 diabetes (T2D). Our recent data demonstrated that DNAJB3 was dysregulated in obesity. Its overexpression enhanced glucose uptake in cells via increased AKT and AS160 phosphorylation. MicroRNAs are important post-transcriptional regulators of gene expression and play an important role in the pathogenesis of obesity and diabetes. Based on computational miRNA target prediction analysis a miR1915 was identified as possible microRNA regulator for DNAJB3 gene.

Aim: Our aim was to study the expression of miR1915 in blood circulation and adipose tissue in a cohort of obese and non-obese people and assess its possible role in obesity and DNAJB3 gene modulation.

Methods: The expression levels of miR1915 in obesity were investigated in 144 participants: according to their BMI, 82 were non-obese (BMI <30) and 62 were obese ((BMI >30). MicroRNA1915 level in plasma and adipose tissue were measured by RT-PCR. Overexpression and 3'UTR-Luciferase assays were used to assess the bindings and repressions of these microRNA to their target proteins.

Results: MiR1915 level was significantly increased in obese as compared to non-obese subjects. Our *in-vitro* analysis confirmed miR1915 binding and repression to DNAJB3 transcript. Furthermore, overexpression of miR1915 mitigated insulin signaling and glucose uptake.

Discussion: Obesity leads to alterations in microRNA expression of miR1915. It's up-regulation in obesity was associated inversely with DNAJB3 proteins involved in regulating insulin signaling and glucose uptake. miR1915 overexpression in HEK-293 cell line resulted in decrease insulin signaling and glucose uptake. miR1915 can be used as potential biomarker for future targeted therapy in obesity.

1

POSTER NUMBER: 1001

The Role of a Regulatory microRNA Cluster in Augmenting the Immune Response during Infection

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Successful recognition of viral infections and consequential triggering of antiviral immune responses are crucial for host persistence and survival. Traditionally, to learn more about these interactions, regulatory networks at the transcriptional and translational levels have been extensively studied. With the discovery of microRNAs- a group of small regulatory non-coding RNAs, there has been a shift in this dogma. microRNAs (miRNAs) are a class of highly conserved and abundant, small non-coding RNAs involved in post-transcriptional regulation of gene expression. miRNAs perform their function by binding to the 3′-untranslated region of target mRNAs to induce degradation and suppress translation. Herein we describe a miRNA cluster that modulate interferon production and signaling during viral infection. The identified miRNA cluster appears to regulate key activation events which are crucial to the antiviral signaling cascade. We found that the cluster enhances the innate antiviral response by augmenting the

phosphorylation of transcription factors, interferon regulatory transcription factor 3 (IRF3) and signal transducer and activator of transcription 1 (STAT1), during viral infections. We demonstrate that the cluster elicits its antiviral response by targeting negative regulators of the IFN signaling cascade and JAK-STAT signaling pathway. Overall, our work reveals a novel mode of cooperation between a miRNA cluster in the regulation of antiviral cellular responses and aid in uncovering fundamental roles for small non-coding RNAs in infection and immunity.

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POSTER NUMBER: 1002

Visualization of the Delivery and Release of Small Regulatory RNAs Using Genetic Code Expansion and Unnatural RNA-Binding Proteins

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Endogenously expressed noncoding RNAs are regulators of mRNA translation and affect diverse biological pathways. Recently, microRNAs have become an important therapeutic target with strategies that employ oligonucleotides as both mimics and inhibitors of target microRNAs, successfully altering gene expression and cellular pathways in relevant contexts. However, delivery of these exogenous effectors remains a major challenge. Here, we present a method for evaluating noncoding RNA delivery using the viral suppressor of RNA silencing (VSRS) protein p19, optimized for cellular delivery of small regulatory RNAs. Using genetic code expansion technology, *p*-azidophenylalanine (AzF) was incorporated site specifically into a recombinant p19 protein and used to develop a fluorescence resonance energy transfer (FRET) sensor. AzF was used to attach FRET acceptor moieties using bioorthogonal chemistry. We show that this strategy not only gives rise to FRET signals that report on small RNA binding, but also allows for fluorescence quenching as well, convenient for measuring RNA release. We demonstrate the successful use of a modified version of the probe to track the delivery and release of small RNAs into mammalian cells using cell penetrating peptides. The results provide a basis for development of vehicles for small RNA delivery and release for intervening in noncoding RNA biology. We expect this method to be applied as a strategy for designing high throughput tools for reporting on levels of small regulatory RNAs. The approaches presented offer the opportunity to dissect and optimize the different steps of RNA transfer from the delivery agent through other interactions leading to binding to Argonaute (Ago) proteins, where the ultimate function will take place.
Ahmed, N. De Graaf, J. Ahmed, N. Foss, D.V. Delcorde, J. Schultz, P.G. Pezacki, J.P. (2018) Visualization of the delivery and release of small RNAs using genetic code expansion and unnatural RNA-binding proteins. *Bioconjugate Chem.* 29, 12, 3982-3986.

3

POSTER NUMBER: 1003

CLIPick: a sensitive peak caller for expression-based deconvolution of HITS-CLIP signals

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High-throughput sequencing of RNAs isolated by crosslinking immunoprecipitation (HITS-CLIP, also called CLIP-Seq) has been used to map global RNA-protein interactions. However, a critical caveat of HITS-CLIP results is that they contain non-linear background noise—different extent of non-specific interactions caused by individual transcript abundance—that has been inconsiderately normalized, resulting in sacrifice of sensitivity. To properly deconvolute RNA-protein interactions, we have implemented CLIPick, a flexible peak calling pipeline for analyzing HITS-CLIP data, which statistically determines the signal-to-noise ratio for each transcript based on the expression-dependent background simulation. Comprising of streamlined Python modules with an easy-to-use standalone graphical user interface, CLIPick robustly identifies significant peaks and quantitatively defines footprint regions within which RNA-protein interactions were occurred. CLIPick outperforms other peak callers in accuracy and sensitivity, selecting the largest number of peaks particularly in lowly expressed transcripts where such marginal signals are hard to discriminate. Specifically, the application of CLIPick to Argonaute (Ago) HITS-CLIP data were sensitive enough to uncover extended features of microRNA target sites, and these sites were experimentally validated. CLIPick enables to resolve critical interactions in a wide spectrum of transcript levels and extends the scope of

HITS-CLIP analysis. CLIPick is available at: http://clip.korea.ac.kr/clipick/

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POSTER NUMBER: 1004

Deciphering miRNA regulation in the disease vector mosquito *Aedes aegypti*

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The mosquito *Aedes aegypti* is responsible for transmitting disease-causing RNA viruses that account for hundreds of thousands of illnesses per year. Reduction of mosquito population provides a cost-effective method to curb disease, and thus a greater understanding of the underlying molecular mechanism(s) that regulate mosquito reproduction is needed to develop novel inhibitory methods. Our previous studies characterized the role of miRNAs in mosquito physiology and reproduction. In adult female mosquitoes, Juvenile Hormone (JH) regulates the transcriptional cascade responsible for tissue development, termed post-eclosion (PE) stage, that is required for the reproductive cycle. Currently, the involvement of miRNAs that govern the changes in gene expression during the PE stage are unknown. Here, we characterized an insect specific miRNA, *miR-276*, that plays a critical role during this stage. We validated that *miR-276* is JH responsive via an *in vivo* JH III topical application assay to artificially induce the JH mediated response and by dsRNA knockdown of the JH receptor, *met*, to prevent the JH response. Mature miRNAs are generated from a precursor transcript termed pri-miRNAs. We silenced *drosha* to avert the cleavage and degradation of pri-miRNAs, and subsequently collected RNA for RNA-seq analysis. This approach allowed us to validate and discover novel *pri-miRNAs* that were absent from the annotation reference in the mosquito genome. We utilized *in silico* predictions to identify Met and E75 as two transcription factors that could regulate *pri-miRNA-276*. We cloned the upstream region into a luciferase vector and found an induction of luciferase activity upon the addition of E75. We further used dsRNA to knockdown E75 and found a reduction of *miR-276* expression. Collectively, both these experiments suggest that E75 is a regulatory factor of *pri-miR-276*. Future experiments will be aimed at confirming the direct binding site of E75 on the *pri-miR-276* promoter region through the use of EMSA and additional luciferase assays. Overall, this project signifies the importance of hormonal regulation on miRNA expression in the mosquito *A. aegypti*.

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POSTER NUMBER: 1005

Investigating the role of microRNAs in the hypoxic response in prostate cancer

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Introduction: Hypoxia (a pathologically low oxygen level) is a well-established driver of aggressive behaviour in prostate cancer (PCa). However, the reasons for this are not completely characterised and the role of microRNAs (miRNAs) in the hypoxic response remains unclear. In this study we investigate the expression and functional role of miRNAs in response to hypoxia in prostate cancer.

Methods: Three models of PCa hypoxia were utilised (i) *in vitro* culture at 0.1% oxygen (ii) 3D spheroid culture and (iii) an *in vivo* tumour xenograft experiment. miRNA expression was measured by RT-qPCR. miRNA overexpression was achieved by transfection and the effect on selected targets was assessed by RT-qPCR and Western blots. Cell proliferation, apoptosis, migration and invasion abilities were assessed by functional bioassays. miRNA-seq and mRNA-seq was used to examine expression patterns in a small cohort of prostate biopsies. Bionformatic analysis of prostate cancer data in The Cancer Genome Atlas (TCGA) repository was also performed.

Results: Several miRNAs were identified as being affected by hypoxia in prostate cells. Among these, miR-210 and miR-21 were shown to be upregulated by hypoxia in our various models. The subsequent effect on their respective networks of target genes was explored and we demonstrate for the first time that miR-210 targets neural cell adhesion molecule (NCAM) in prostate cells. miR-210 and miR-21 expression was positively correlated with markers of hypoxia and tumour aggressiveness in clinical samples, suggesting they may have value as novel biomarkers in this disease.

Discussion: We provide evidence that various miRNAs can contribute to the progression of PCa through hypoxia-related mechanisms. In particular, miR-210 and miR-21 appear to play key roles in the hypoxic response which can contribute to PCa progression. We propose that miRNA profiling of these and other miRNAs has great value for improving diagnostic, prognostic and potential therapeutic approaches in this disease.

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POSTER NUMBER: 3042

miRNAs in arthritis pathogenesis and therapy

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MicroRNAs (miRNAs) play critical roles in various biological processes by targeting specific mRNAs. We have reported that cartilage specific miR-140 plays a critical role both in skeletal formation and arthritis pathogenesis. However, miR-140 exists into intronic region of E3 ubiquitine ligase, Wwp2, and its knockout mice generated from Wwp2 gene trap ES line showed similar skeletal phenotype, it is unclear which molecules critically contribute to this phenotype. By generating single deletion and double deletion mice for miR-140 and Wwp2 by CRISPR/Cas9, we could obseve the exact function of both molecules and found that only miR-140 could contribute to the skeletal phenotype.
To identify miRNA's functional targets, we created a cell-based screening system using a luciferase reporter library composed of 4,891 full-length cDNAs, each of which was integrated into the 3′-untranslated region (3′-UTR) of a luciferase gene. Using this reporter library system, we conducted a screening for targets of miR-34a (a tumor suppressor miRNA) and miR-140.
Our strategy is useful for elucidation of miRNA functions and their therapeutic application.

References

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POSTER NUMBER: 1007

Determination of human pri-miRNA secondary structure by SHAPE-MaP

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MicroRNA maturation is initiated by primary microRNA (pri-miRNA) processing by the Microprocessor complex. Human Microprocessor complex recognizes both structural features and primary sequence motifs of pri-miRNAs for efficient processing. However, the secondary structures of human pri-miRNAs have not been defined systematically. Here, we determine the secondary structures of highly confident human pri-miRNAs (402 pri-miRNAs) by Selective 2′-Hydroxyl Acylation analyzed by Primer Extension and Mutational Profiling (SHAPE-MaP) and deep sequencing. The SHAPE-MaP data reveals that the lower stem of specific length (13 bp) is required for efficient processing while the upper stem and apical loop lengths do not affect processing efficiency in general. We also find that the basal UG motif and apical UGU motif are functional only in specific structural contexts. Lastly, we uncover the interplay between structural motifs and sequence elements in the pri-let-7 loop structures, which mediates differential regulation. By providing a global view of human pri-miRNA structures, our study allows a comprehensive understanding of the role of pri-miRNA structures in miRNA processing.

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POSTER NUMBER: 1006

Most RNA-binding proteins are microRNA targeting enhancers

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Argonaute is the primary mediator of metazoan miRNA targeting (MT). Among the currently identified >800 human RNA-binding proteins (RBPs), there are a few RBPs known to enhance MT mainly by improving the target site accessibility to Argonaute, while several other RBPs are reported to function as MT suppressors. To gain a global insight into the regulatory impact of RBPs on MT, we have systematically analyzed transcriptome-wide binding sites for 117 human RBPs and evaluated the quantitative effect of individual RBPs on MT efficacy. As a result, most 3′UTR-binding RBPs, if not all, significantly enhance MT when binding close to the miRNA target site, while no RBP detectably suppresses MT on a global scale. We demonstrate that these RBPs make the local secondary structure of the miRNA target site more easily accessible and therefore enhance MT. Our finding illuminates the unappreciated widespread regulatory impact of RBPs on MT, indicating that hundreds of RBPs may play key roles in the gene regulatory network governed by metazoan miRNAs and that MT should be understood in the context of these co-regulating RBPs.

POSTER NUMBER: 1008

Peptide Emanating from Circular Variant of lncRNA *PVT1* Regulate MYC Levels in Cancer

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v-myc myelocytomatosis viral oncogene homolog (*MYC*), located on human 8q24, is frequently amplified in a wide range of cancers and predicts poor outcome. Targeting MYC can provide therapeutic index even for other intractable driver mutations. However, direct inhibition of MYC in human cancer has remained a challenge. We have previously identified the long non-coding RNA (lncRNA) Plasmacytoma Variant Translocation 1 (*PVT1,* located adjacent to, and co-amplified with *MYC*), as a critical regulator of MYC in cancers with 8q24 gain (Tseng *et al*; Nature, 2014; 512:82-86). Here we present our current work elucidating the mechanism by which PVT1 regulates MYC in the Human 8q24+ cancers and propose that targeting the PVT1-MYC axis in these cancers may provide an effective therapeutic strategy.

Hu PVT1 gene encodes for twenty-five alternative splice variants. We have identified a specific PVT1 splice variant that directly contributes to augmentation of MYC. This variant is highly abundant in a subset of Group 3 Medulloblastoma tumors that are primarily driven by MYC. It is also particularly enriched in cancer cell lines harboring high genomic copy number of MYC. We have discovered that one of the exons of PVT1[hi] undergoes back-splicing and form a circular RNA (CircPVT1). Cloning the splice junction confirms that CircPVT1 originates from the specific PVT1 splice variant and not from other splice variants of PVT1. Si_RNA designed against the junction of CircPVT1 can inhibit CircPVT1, and reduces MYC levels and induces cellular apoptosis, suggesting that the product of CircPVT1 may contribute to maintaining the MYC levels in these cells. We found that upon circularization, CircPVT1 can form a protein-coding open reading frame (ORF) of 104 amino acids. We designated this potential peptide as PVT1 Encoded Peptide upon Circularization (PEP_C). Exogenous addition of PEP_C can augment MYC in 8q24 copy number neutral cells, and increase their transformation and metastatic potential. We have developed antibodies against the PEPc which can identify endogenous expression of the PEPs in cell lines as well as in patient-derived xenografts. We will discuss the structural and molecular basis by which the PEPc regulate MYC levels in cancers.

POSTER NUMBER: 1041

Rhythmic miRNA expression in the mammalian brain: importance of miRNA biogenesis and decay

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The molecular clockwork comprises a transcriptional-translational feedback loop where the generation of rhythmic mRNA and protein abundance arises from repression- and activation-mediated temporal and spatial delays that eventually control downstream rhythmic behavioral patterns and physiological functions within an approximate 24-h period. Nearly half of the circadian output proteome does not show corresponding transcript oscillations, indicating that extensive post-transcriptional mechanisms are important components of circadian rhythmicity. microRNAs are major post-transcriptional regulators, involved the control of virtually all biological pathways in mammals. However, the role of brain miRNAs in the generation and maintenance of robust circadian rhythms in animals has not been adequately studied. Here we dissected the transcriptional and post-transcriptional mechanisms of astrocyte to neuron circadian communication by using Next-Generation Sequencing technology in a tamoxifen (TM) inducible mouse model (Bmal1flx/flx;GLAST-CreERT2;R26-Tomato, *Bmal1cKO*) in which the selective *Bmal1* gene deletion in approximately 50% of astrocytes impairs brain circadian rhythms.
We identified the global oscillating miRNome from cortex, at different time points of the day of *Bmal1cKO* and control mice (Bmal1flx/flx). We found that approximately 19% of miRNAs are rhythmically expressed in mouse cortex. Remarkably, while *Bmal1cKO* animals have mild differences in miRNA expression levels compared to control mice, they showed a strong impairment in their rhythmic oscillations, indicating that the rhythmic expression of miRNAs in cortex is under the regulation of the astrocytic circadian transcription factor BMAL1. By performing and integrating circadian transcriptome analysis of cortex in control and Bmal1cKO mice, we explored the mechanisms underlying miRNA oscillations and that were subverted in Bmal1cKO mice.
Our study is the first comprehensive *in vivo* study of the mechanism of circadian oscillating miRNAs in mammalian brain.

POSTER NUMBER: 1009

Proximity-CLIP provides a snapshot of protein-occupied RNA elements in sub-cellular compartments

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Many cellular RNAs localize to specific cellular compartments but methods to systematically study RNA biology at subcellular resolution are limited and lagging behind proteomic tools. Here, we combined APEX2-mediated proximity biotinylation of proteins with photoactivatable ribonucleoside-enhanced crosslinking to simultaneously profile the proteome, as well as the transcriptome bound by RNA binding proteins in any given subcellular compartment (Nature Methods, 2018). Our approach is fractionation-independent and enables to study the localization of RNA processing intermediates, as well as the identification of regulatory RNA cis-acting elements occupied by proteins in a cellular compartment-specific manner. We applied Proximity-CLIP to study RNA and protein in the nucleus, cytoplasm and at cell-cell interfaces. Among other insights, we observed frequent transcriptional readthrough continuing for several kilo-bases downstream of the canonical cleavage and polyadenylation site and a differential RBP occupancy pattern for mRNAs in the nucleus and cytoplasm. Surprisingly, mRNAs localized to cell-cell interfaces often encoded regulatory proteins and contained protein-occupied CUG sequence elements in their 3' untranslated region, which have previously been implicated in targeting mRNAs to the membrane and in several repeat expansion neurodegenerative diseases, including myotonic dystrophy. We plan to pursue location-specific functions of RNA binding proteins in the context of cellular stress response using models of cancer and neurodegeneration.

POSTER NUMBER: 1010

Lateral septum miRNA alterations in response to social fear conditioning: Functional involvement of miR-132 in extinction and oxytocin-mediated reversal of social fear

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Known as post-transcriptional regulators of neuronal gene expression, microRNAs are fundamental factors associated in somatic and affective disorders. Especially neuronal miR-132 has repeatedly been reported as crucial regulator of synaptic plasticity and higher cognitive functions like learning and memory. Likewise, miR-124 was identified as regulator of sociability in mice by changing AMPA receptor subunit composition. Whether appetitive or aversive, social interactions are an indispensable component for prosperous survival of a species. Their dysregulation leads to devastating disorders like social anxiety disorder (SAD). Recently, we established the social fear conditioning (SFC) paradigm, an animal model resembling SAD by generating robust fear of same-sex conspecifics in mice. In this context and within the lateral septum (LS), the neuropeptide oxytocin (OXT) - known for its anxiolytic and pro-social effects - was shown to reverse SFC-induced social fear. Since the OXT-mediated orchestration of transcriptional and post-transcriptional gene expression regulation is still to be elucidated, we examined the involvement of microRNAs within the LS in SFC-induced social fear in male mice.
90min after acquisition of SFC, miR-132 and miR-124 levels were elevated in the LS of conditioned (SFC^+) mice, whereas 30min, 180min, and 24h after acquisition no differences were found. Moreover, 90min as well as 180min post-extinction miR-132 levels of SFC^+ mice were decreased compared to respective post-acquisition levels. In comparison to post-acquisition expression, miR-124 levels in unconditioned (SFC^-) mice were elevated only 90 min after extinction. Anyhow, neither repeated exposure to social vs non-social stimuli nor intracerebroventricular infusions of OXT did alter the expression of these microRNAs within the LS.
In order to reveal the behavioral relevance of miR-132, its pre-acquisition (48h) inhibition within the LS via a locked nucleic acid impaired extinction of social fear in SFC^+ compared to miR-scrambled-infused SFC^+ mice. During recall of social fear extinction no significant differences between inhibitor and scrambled-treated SFC^- and SFC^+ mice, respectively, were found. Further, pre-acquisition inhibition of miR-132 within the LS prevented the local OXT-induced reversal of social fear in SFC^+ mice. Preliminary results suggest that adeno-associated virus-mediated overexpression of miR-132 within the LS improved extinction of social fear.
These data reveal dynamic alterations of miR-214 and miR-132 during acquisition and extinction of social fear. Specifically, miR-132 seems essential for extinction of social fear, since its inhibition impaired extinction and prevented OXT-mediated reversal, whereas its overexpression seems to improve extinction of social fear. We conclude that miR-132 may underlie the social fear reversing effect of OXT.

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POSTER NUMBER: 1011

MicroRNA Functional Analysis with Correlation Support from TCGA and TCPA expression data in Pancreatic Cancer

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MicroRNAs (miRNAs) are small RNAs involved in post-transcriptional gene regulation. *In silico*-based miRNA functional analysis consists of miRNA target prediction and functional enrichment analysis of miRNA targets, and can be performed to enable discovery of novel molecular mechanisms of the disease and potential therapeutic targets. MiRNA target prediction methods generate high false positive rates, which requires further validation to narrow down interesting candidate miRNA targets. One commonly used method encounters correlation between miRNA and its predicted targets.

Finding novel non-invasive biomarkers for pancreatic cancer is highly desirable as the only clinically used biomarker today, carbohydrate antigen-19-9, is not sensitive or specific enough. MiRNAs are emerging as potential biomarkers in pancreatic cancer and a dataset of 15 circulating miRNAs previously identified as differentially expressed in pancreatic cancer was used in this study.

The aim of this study was to build a bioinformatics pipeline in R for miRNA functional analysis following correlation analyses between miRNA expression levels and its targets on mRNA and protein expression levels. The Cancer Genome Atlas (TCGA)-derived expression data of specific mature miRNA isoforms instead of the precursor miRNA gene was used.

Fifteen significantly altered circulating miRNAs detected in pancreatic cancer patients were queried separately in the pipeline. The pipeline generated predicted miRNA target genes, enriched gene ontology (GO) terms and Kyoto

encyclopedia of genes and genomes (KEGG) pathways. Predicted miRNA targets were evaluated by correlation analyses between each miRNA and its predicted targets.

Our results suggest that hsa-miR-885-5p could act as a tumor suppressor and should be further validated as a potential prognostic biomarker in pancreatic cancer. This pipeline can serve as a valuable tool in biomarker discovery involving mature miRNAs associated to pancreatic cancer and could be developed to cover additional cancer types.

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POSTER NUMBER: 1012

Combinatorial targeting of processes and pathways by microRNAs

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MicroRNAs (miRNAs) have an established role in controlling epithelial-mesenchymal transition (EMT), a reversible phenotypic program underlying both normal and pathological processes. Many studies demonstrate the role of individual miRNAs using overexpression at levels greatly exceeding physiological abundance. This can influence transcripts with relatively poor targeting and may in part explain why well over 100 different miRNAs are directly implicated as EMT regulators. Analysing a human mammary cell model of EMT, we found evidence that a set of miRNAs, including miR-200 family members, cooperate to promote an epithelial phenotype at concentrations much closer to endogenous levels than is typically used and do so with less off-target effects. This has potential therapeutic application, as the lower levels of miRNAs required in combination may be both safer and easier to achieve *in vivo*. The effects mediated by miRNAs are a result of both direct multi-targeting of pathway components, and the indirect, co-ordinated actions of genes that are themselves controlled by miRNA-regulated transcription factors.

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POSTER NUMBER: 1013

Mechanistic analysis of enhanced RNAi activity by 5'-end modification of the siRNA guide strand

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Since the discovery that siRNAs can specifically silence target genes in humans, therapeutic application of RNAi has been long awaited and, just recently, the first siRNA-based drug was finally approved. However, siRNA drugs still have room for improvement, particularly in their efficacy and stability. A key for such improvement is chemical modifications of siRNAs. We have recently performed an in-silico screening of modifications at the 5'-end base of the guide strand that enhances the affinity to the 5' nucleotide-binding pocket of human Argonaute2 (Ago2), the core protein of the effector complex called RNA-induced silencing complex (RISC). As a result, an adenine-derived compound with a hydrophobic moiety was identified to enhance the RNAi activity by ~3 fold in cultured human cells as well as in vivo mouse models. Nevertheless, it remained unclear how this chemical modification enhances the siRNA potency. Here, we used a series of biochemical approaches including an in vitro target cleavage assay and a native gel analysis of siRNA-Ago2 complex formation to quantitatively evaluate the effect of the modification at each step in RISC assembly and function. We found that the modification improves the formation of mature RISC in multiple ways, including the fixation of the loading orientation of siRNA duplexes and the increased stability of mature RISC after passenger strand ejection. Our data will provide a molecular platform for further development of siRNA therapeutics.

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POSTER NUMBER: 1045

Identification of circulating microRNA as a biomarker of sleep disorder: RNA sequencing and quantitative PCR

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Hypothesis: Persistent sleep disorder leads to inability to daily activity, weakness of optimal cognitive performance, and/or immunocompromised state. Therefore, early state diagnosis of sleep dysfunction would be necessary. However, diagnostic biomarkers of sleep disorder have been still lacked. Here, we aimed to find circulating microRNAs (in serum) associated with persistent sleep disorder.
Methods: Next-generation sequencing (NGS) of small RNAs was performed with serum of 10 dyssomnia subjects (Pittsburgh sleep quality index > 5) and of 10 healthy controls (matched for age, gender, and body mass index). After selecting microRNAs presenting high differential expression levels between cases and controls, dyssomnia-associated microRNAs were validated using reverse transcription quantitative PCR (RT-qPCR) in two populations (1st validation set: 30 cases and 30 controls; 2nd validation set: 49 cases and 50 controls). Receiver operating characteristic (ROC) curve analysis was conducted in order to show discrimination ability of microRNA as a biomarker of dyssomnia.
Results: Of 37 circulating microRNAs from NGS analysis, differential expression of six microRNAs was validated in 1st RT-qPCR analysis. Of 6 microRNAs, two circulating microRNAs were re-confirmed in 2nd validation. Area under the curve values of two validated microRNAs for dyssomnia obtained by ROC curve analysis were over 85% (when combined two microRNAs) in both two validation populations.
Conclusions: We identified circulating microRNAs which could discriminate dyssomnia subjects from healthy people. The findings provided clinical potential in circulating microRNA as a potential source of biomarkers of sleep disorder.

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POSTER NUMBER: 1014

The NuRD complex mediates piRNA-guided heterochromatin formation in metazoans

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In eukaryotes, trimethylation of lysine 9 on histone H3 (H3K9me3) is associated with the transcriptional silencing of transposable elements (TEs). In Drosophila ovaries, this heterochromatic repressive mark is thought to be deposited on TE genomic loci after the initial recognition of their nascent transcripts by PIWI-interacting RNAs (piRNAs) that guide a piRNA-induced transcriptional silencing (piRITS) nuclear complex the composition and mechanisms of action of which are still elusive. Here, we present the first biochemical and genetic characterization of the Drosophila piRITS complex. We show that the piRNA-dependent H3K9me3 deposition by the Eggless/SetDB1 histone methyltransferase requires Nucleosome Remodeling and histone Deacetylase (NuRD) complex. Moreover, we found that the mouse NuRD subunit CHD5 also interacts with the MIWI2 PIWI protein in mouse embryonic testes, suggesting a conserved mechanism for the repressive role of NuRD in metazoan piRITS complexes.

POSTER NUMBER: 1015

Characterization the Role of miR-34/449 in the Central Nervous System

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Although the function of miRNAs in the central nervous system has been intensively studied in recent years, how their regulations might affect animal behaviors remain largely unexplored. By the microarray profiling and small RNA sequencing, several miRNAs that are enriched in the central nervous system have been systematically identified in our and others' laboratories. Among such, the *miR-34/449* family, which comprises six miRNA members locates at three separate genomic loci, manifest dynamical expression pattern in the developing spinal cord and brain. Although *mir-34/449* has been shown to play important role during ciliogenesis in epithelia cells, the biological function of *miR-34/449* in the nervous system remains enigmatic. Here, we generated the triple knockout (KO) mouse model of *miR-34/449*, and surprisingly uncovered several phenotypes that have not been reported from both the *miR-34/449* triple KO and *miR-34bc/449* double KO mice, including peculiar motor behaviors and eyelid

ptosis. These striking findings highlight the critical roles of *miR-34/449* family in the central nervous system *in vivo*. We will illustrate the ongoing characterizing behavioral phenotypes and the potential underlying mechanisms lead to the unique behavior deficit in this meeting.

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POSTER NUMBER: 1017

Safety and Efficacy of double strand RNA against Sacbrood Virus Infection in *Apis cerana*

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Sacbrood disease is a viral disease of honeybee, caused by Sacbrood virus (SBV) and characterized by larvae decay. It has resulted in the extreme damage in India, Thailand, Vietnam and China Oriental Honeybee (*Apis cerana*) since the 1970s. In Korea, it has occurred since 2009 and to date, has been threatened as a devastating disease in *A. cerana*. Therefore, the preventive and treatment measures are urgently needed. RNA inference (RNAi) is a gene-silencing technology by which small double strand RNAs are used to degrade the target RNA with complementary sequences and also a natural immune factor in honeybees. Previously, SBV RNAi medicine has been applied in laboratory condition and shown positive effect to SBV infection. In this study, we synthesized double strand RNA (dsRNA) corresponding to a segment of structural protein VP1, and tested the safety and efficacy at artificial and natural infections in laboratory and field apiaries. VP1 dsRNA was ingested with sugar solution by feeding biweekly. The mortalities and viral loads were observed and analyzed by real-time PCR at RNAi treatment period. In the laboratory test, the viral loads of VP1 dsRNA-treated larvae and adult were significantly decreased to a level similar to pre-infection on 10 days after the artificial infection. The survival rate in treated larvae was increased upto 40%. In the field test, we failed to recover the healthy status at the treated hives in currently Sacbrood disease-occurred apiaries. However, the treated hives in surrounding apiaries around Sacbrood disease-occurred apiaries kept the healthy status without clinical signs of Sacbrood disease, such as larvae decay, pulled-out larvae, and finally colony collapse disorder. Meanwhile, the untreated hives in surrounding apiaries showed colony collapse disorder. All treated group in laboratory and field tests showed the safety on larvae and adult honeybees. Conclusively, we confirmed the safety and efficacy of SBV VP1 dsRNA against Sacbrood virus in *A. cerana* treated at surrounding apiaries which have just SBV-infected larvae at incubation period. It will be very useful for the prevention of Sacbrood disease in *A.cerana* to block the spread of SBV infection to surrounding apiaries.

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POSTER NUMBER: 1018

Rg6, a rare ginsenoside, controls systemic inflammation through the induction of microRNA-146a

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The immunobiological functions of Rg6, a rare ginsenoside from ginseng, have been largely unreported. In this paper, we demonstrate that Rg6 has a significant immunosuppressive function on Toll-like receptor (TLR) 4-induced systemic inflammatory responses through induction of miRNA-146a (miR-146a), a key posttranscriptional regulator of inflammation. Rg6 negatively regulated pro-inflammatory responses and severity in vivo, and thus induced recovery in mice with lipopolysaccharide (LPS)-induced septic shock and cecal ligation and puncture (CLP)-induced sepsis. We then hypothesized that Rg6 could directly induce miR-146a. We found that miR-146a expression was increased until 48 h after treatment of BMDMs with Rg6. To assess miR-146a expression under the inflammatory condition, cells were treated with Rg6 and LPS and harvested 6 and 18 h after LPS activation. We found that the addition of Rg6 to cells in the LPS-induced inflammatory state increased the expression of miR-146a 6 h after LPS treatment. In addition, Rg6 and LPS co-treatment resulted in an increase in the expression of miR-146a, whereas the addition of Rg6 or LPS caused little increase in the expression of miR-146a at the 18-h time point. Further studies showed the anti-inflammatory function of Rg6-induced miR-146a upon TNF-α and IL-6 secretion in cultured macrophages. Thus, our findings indicate that ginsenoside Rg6 not only induces miR-146a expression, but also

amplifies its production under inflammatory conditions, causing a reduction in pro-inflammatory cytokines, such as TNF-α and IL-6, in murine macrophages.

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POSTER NUMBER: 1019

miRNAs in Planarian Regeneration

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Planarian, with the robust regeneration ability, has been used as a model organism for stem cell research in recent years. Regeneration in planarian is mediated by adult stem cells, namely neoblasts, which can proliferate and differentiate into almost all cell types for replacing the whole body. MicroRNAs are 20-24 nt small non-coding RNAs that are associated with Argonaute proteins and form miRNA-induced silencing complex (miRISC) to regulate gene expression at post-transcriptional level. Our study showed that a specific population of miRNAs is highly expressed in regenerating tissues, implicates the potentially important roles of miRNAs in neoblasts during regeneration. Interestingly, depletion of planarian Argonaute 2 (DjAgo2) results in a dramatic effect of head degeneration, suggesting that miRNAs are also required for planarian homeostasis. In this study, we further examined the roles of specific miRNAs in planarian. Successful inhibition of several candidate miRNAs results in a degeneration phenotype in planarian treated with miRNA inhibitors. This result proved our hypothesis that miRNAs indeed participate in the self-renewal of stem cells and the differentiation process in planarian.

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POSTER NUMBER: 1020

Dynamics of piRNA-related granules in silkworm cells

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In animal germ cells, PIWI proteins and PIWI-interacting RNAs (piRNAs) protect the genome by silencing deleterious transposons. To study the conserved piRNA biosynthesis pathway called the ping-pong cycle, we use silkworm ovary-derived BmN4 cell line as a model. In BmN4 cells, two PIWI proteins, Siwi and BmAgo3, are involved in this pathway, where they bind piRNAs to cleave complementary transposons and piRNA precursor transcripts. The 3' cleavage products are handed to the other PIWI protein, forming PIWI-piRNA precursor complex. Subsequently, the 3' ends of precursor piRNAs are trimmed and methylated to form mature PIWI-piRNA complex. Therefore, the ping-pong cycle couples target cleavage to piRNA biosynthesis, amplifying piRNAs for efficient transposon silencing.

It is known that both Siwi and BmAgo3 proteins reside in cytoplasmic membrane-less RNP granule called nuage, together with numerous co-factors including Tudor domain-containing proteins and ATP-dependent RNA helicases. Meanwhile, a list of additional piRNA factors including nucleases Zucchini and Trimmer that are required for piRNA maturation reside on the outer surface of mitochondrial membrane. Of note, the nuage-mitochondrial compartmentalization is a feature of piRNA pathway conserved across species. Accordingly, it is believed that a robust exchange of RNAs and proteins occurs at the nuage-mitochondrial intersection in the piRNA pathway. However, the molecular interaction and dynamics of these subcellular compartments remain poorly understood. In the current study, we utilize deconvolution fluorescence microscopy to study dynamics of nuages upon knockdown or mutant over-expressions of various piRNA-related factors in live BmN4 cells. We would like to discuss our recent results in this conference.

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POSTER NUMBER: 1021

A negative feedback loop between Dicer and miR-144 dampens canonical microRNA biogenesis and maximizes the Ago2-dependent processing of miR-451 during vertebrate erythropoiesis

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Although several small RNAs are known to bypass the first committed step of microRNA processing catalyzed by Drosha, miR-451 is the only known example of a microRNA whose biogenesis is strictly Dicer-independent and instead relies on the slicer activity of Ago2 and further trimming by PARN. miR-451 is a vertebrate-specific microRNA and the most abundant in erythrocytes. It is expressed together with the Dicer-dependent miR-144 as a cluster and its demise leads to incomplete erythrocyte differentiation and anemia.

While the unique biogenesis of miR-451 and its role in erythropoiesis has been extensively studied, little is known about why the Ago2-dependent processing of miR-451 became essential for erythropoiesis in the first place. Our analysis of microRNA abundance indicates that miR-451 is 5-fold more abundant than miR-144 in zebrafish erythrocytes, even though the processing of pre-miR-451 is much slower than the processing of an engineered Dicer-dependent pre-miR-451.

Using miR-144 zebrafish mutants, together with biochemical and sequencing analysis, we uncovered a negative feedback loop that involves miR-144 targeting its own processing enzyme Dicer. Repression of Dicer in turn induces a global dampening of canonical microRNA production that clears the field to maximize miR-451 production.

In addition, we uncovered a second negative feedback loop involving miR-451 and Ago2 to limit miR-451 production. In absence of miR-451, Ago2 mRNA levels double and lead to a global stabilization of canonical microRNAs in erythrocytes.

Overall, these results ii) uncover the mechanism that drives the imbalance between miR-144 and miR-451, ii) indicate how microRNAs from a cluster can influence their processing in *trans*, and iii) suggest why Ago2-dependent processing of miR-451 is ultraconserved in vertebrate erythropoiesis.

POSTER NUMBER: 2038

Potential influence of N6-methyladenosine (m6A) modification on miRNA-mediated gene regulation

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N6-methyladenosine (m6A) is the most prevalent RNA modification on messenger RNAs (mRNA) in eukaryotes. There are ~140,000 reported m6A modification sites in human transcriptome. Potential functions of m6A modification on mRNAs are not well understood. microRNAs (miRNAs) are an abundant class of small endogenous non-coding RNAs (ncRNAs), which bind to target mRNAs to induce either translational repression or target degradation. We performed computational analysis to explore potential interactions between m6A modification and gene regulation by microRNAs. We utilized miRNA binding data from the CLASH study that reported ~18,500 high confidence miRNA:mRNA interactions. For functional data on microRNA regulation, we used data from high throughput proteomics and microarrays.
Analysis of CLASH data confirmed previous reports that m6A modification was enriched in the 5' and 3' untranslated regions. For miRNA target binding sites with m6A modification, the miRNA:target hybrid stability is significantly higher than those without modification. The most interesting observation is that, the evolutionary conservation for miRNA binding sites with m6A modification is significantly higher than that for miRNA binding sites without modification. This particularly suggests functional significance of m6A modification on mRNAs. In analysis of miRNA functional data, we observed that the presence of m6A modification within miRNA binding sites tend to exert significantly higher levels of regulation. We also found that for miRNA targets with seed or only seedless bindings sites, the level of regulation is significantly higher when m6A is present on the transcript. In addition, among targets with m6A present, the regulation is significantly stronger when the m6A is within or near the miRNA binding site.

These findings strongly suggest functional significance of m6A modification in posttranscriptional gene regulation by miRNAs. We have compiled transcriptome scale data on miRNA binding sites with m6A modification. This dataset can be used for experimental investigation on potential interactions between miRNA and m6A modification.

POSTER NUMBER: 1022

A RNAi-based transgene suppression in plants via external application of synthetic dsRNAs and siRNAs

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RNA interference (RNAi) is widely known as a phenomenon mediating resistance to undesirable nucleic acids and regulating expression of endogenous protein-coding genes by small RNAs. Recent investigations show that exogenously applied small interfering RNAs (siRNA) and long double-stranded RNAs (dsRNA) can be taken up and translocated in plants to induce RNAi in the plant. The question of whether genes in the plant genome can undergo suppression as a result of exogenous RNA application on the plant surface is almost unexplored. This study analyzed whether it is possible to influence transcription of transgenes, as more prone sequences to silencing, in Arabidopsis genome by direct exogenous application of target long dsRNAs and synthetic siRNAs. We introduced the neomycin phosphotransferase II (*NPTII*) and enhanced green fluorescent protein (*EGFP*) genes under the control of the double CaMV 35S promoters into *Agrobacterium tumefaciens* and transformed *Arabidopsis thaliana* by floral dip. *In vitro* synthesized *NPTII*- and *EGFP*-dsRNAs and siRNAs were applied on the surface of four-week-old transgenic *Arabidopsis* by spreading with sterile individual brushes. qRT-PCR data revealed that the dsRNAs and siRNAs suppressed transgene transcription levels in Arabidopsis. Confocal microscopy and western blotting showed that EGFP fluorescence and protein levels sharply decreased post-treatment with the EGFP dsRNA. The fact that simple exogenous application of polynucleotides can affect transcription of plant transgenes opens new opportunities for the development of new scientific techniques and crop improvement strategies. This work was supported by a Grant from the Russian Science Foundation (17-74-10083). Laser scanning microscopy that was done at the Far Eastern Center for Electron Microscopy, National Scientific Center of Marine Biology, Russian Academy of Sciences.

POSTER NUMBER: 1023

PAREsnip2: a tool for high-throughput prediction of small RNA targets from degradome sequencing data using configurable targeting rules

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Small RNAs (sRNAs) are short, non-coding RNAs that play critical roles in many important biological pathways. In plants, they can target and suppress the translation of messenger RNAs (mRNAs) through cleavage and subsequent degradation. Cleavage typically occurs between positions 10 and 11 of the sRNA and the residual mRNA degradation fragments can be captured on a massive scale using high-throughput sequencing methods such as Parallel Analysis of RNA Ends. The sequenced snapshot of the mRNA degredation profile is often called the degradome which can be leveraged to provide evidence of sRNA mediated cleavage activity. Currently, a typical degradome dataset contains millions of unique reads. However, the current computational methods available to leverage the degradome to support sRNA target prediction do not scale with the increasing size of current datasets. Furthermore, current computational methods use a stringent set of sRNA-mRNA targeting rules which are based on a small number of experimentally validated microRNA mediated cleavage events. Over time, the targeting rules are likely to change as more targets become validated and may cause the current degradome assisted target prediction methods to become obsolete. Here, we present a new user-friendly, fast and low-memory computational method which can be used to accurately and efficiently predict sRNA targets using degradome sequencing data. Our method provides a flexible and user-configurable target prediction rule set and our results show that our algorithm is over two orders of magnitude faster with greater prediction accuracy when compared to the current state of the art.

POSTER NUMBER: 1024

Characterization of a new ALG-1 interactor involved in the specificity of miRNA loading

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MicroRNA are small non-coding RNA that regulate gene expression. Formed by the association of an Argonaute (AGO) protein with a miRNA, the effector complex called miRISC binds to its mRNA target leading to translational repression frequently coupled with mRNA degradation or, in some biological conditions, stabilization. Studies so far have mainly focused on miRNA biological functions while the regulation of their biogenesis pathway and their

loading onto a specific AGO selected among the several found in animals and plants are less understood. To answer what confers the proper loading of miRNA onto specific AGO, we used diverse screening approaches and identified a DnaJ chaperone protein as a new interactor of a C. *elegans* miRNA-specific AGO called ALG-1. Different genetic assays indicate that the DnaJ chaperone is important for the miRNA pathway in animals. Interestingly, recent observations made in *Drosophila* and human cells suggested that Heat Shock Proteins (HSP) are required for small RNA loading onto AGO proteins. It is also known that DnaJ are important for client protein binding to HSP to guide them to specific proteins. We thus hypothesize that the DnaJ chaperone interacting with ALG-1 recruits HSP proteins for specific loading of miRNAs. To test this model, we endogenously tagged the DnaJ gene and demonstrated that the DnaJ protein interacts with ALG-1 *in vivo*. Using size exclusion chromatography, we observed that the DnaJ protein interacts with the miRISC loading complex rather than with the effector miRISC. Accordingly, the DnaJ knockdown causes a decrease in the loading of miRNAs onto ALG-1, demonstrating its implication in miRISC formation. Studies are underway to test whether the identified DnaJ protein confers small RNA loading specificity to miRNA-specific AGO. Altogether, this study will provide mechanistic insights on how miRNA (and potentially others small RNAs) are loaded specifically onto the proper Argonautes. This work is supported by the Canadian Institutes of Health Research and a scholarship from Fonds de Recherche du Québec-Santé (PMF).

POSTER NUMBER: 2039

The metazoan microRNA complement

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Non-coding RNAs (ncRNA), a significant part of the increasingly popular '*dark matter*' of the human genome, have gained substantial attention due to their involvement in animal development, including ageing, and human disorders such as cardiovascular diseases and cancer. Among the many different types of regulatory short noncoding RNAs, microRNAs (miRNAs) are the only class with individual gene sequences conserved across the animal kingdom. Utilizing a set of unique features, *bona fide* miRNAs can also clearly be distinguished from the myriad of small RNAs generated in eukaryotic cells. Unfortunately, recognition and utilization of these clear and mechanistically well-understood criteria has not been a common practice, which made comparative miRNAs analyses difficult and often resulting in incorrect or misleading conclusions.
We have addressed this by manually reannotating the full miRNA complements of 40 animal species representing the majority of metazoan groups. We employed more than 500 small-RNAseq datasets of different organs, tissues and cell-types to arrive at more than 9,000 manually curated metazoan miRNA entries in total that are summarized in our database MirGeneDB2.0. We show that metazoan miRNA complements are very homogenous between closely related species and conduct evolutionary analyses that elucidate the phylogenetic origin of miRNA families as key determinant for the expression of miRNA genes in all animals. We identify novel structural features that help define mature arm selection; and we show that miRNA editing is a rare event across Metazoa.
MirGeneDB2.0 represents a robust platform for providing deeper and more significant insights into the biology of miRNAs, and their roles in development and disease toward more light to the '*dark matter*' in animal genomes.

POSTER NUMBER: 1025

KSRP-encoded miR-3940-5p regulates the mTORC2 co-factor Rictor and has tumor suppressive function in glioblastoma cells

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The canonical pathway for producing microRNAs involves the cleavage of RNA hairpin structures by the Microprocessor enzymes Drosha and DGCR8 in the nucleus followed by Dicer cleavage in the cytoplasm. Alternative pathways include processing by the spliceosome to produce mirtrons. The intronic miR-3940 is not dependent on processing by either Drosha or the spliceosome. We are currently characterizing the mechanisms through which this miRNA is processed. Redundant pathways for producing miR-3940 suggests that it has functional significance. miR-3940 is a primate-specific miRNA. It is generated from an intron of the RNA-binding protein KSRP and is expressed similarly to KSRP in human tissues. KSRP is an important regulator of both mRNA stability and miRNA maturation. We have shown that KSRP is critical for neuronal development and that it post-transcriptionally inhibits the expression of growth-associated proteins. KSRP is altered in several cancers, and in glioblastomas its expression is significantly associated with improved patient survival. KSRP inhibition enhances the migration and invasion of glioblastoma cells. Using LNA mimics and inhibitors, we tested the effects of miR-3940-5p modulation on glioblastoma cells. We found that the transfection of miR-3940-5p mimics reduced both glioblastoma cell migration and proliferation. Accordingly, transfection of miR-3940-5p inhibitors enhanced glioblastoma cell migration and proliferation. In addition, we identified the mTORC2 co-factor Rictor as a novel putative target of miR-3940-5p. We found that miR-3940-5p overexpression significantly decreased Rictor expression, while miR-3940-5p inhibition significantly increased Rictor expression in glioblastoma cells. We are working to further characterize the role of miR-3940-5p in normal brain tissue and in glioblastoma multiforme. Our studies suggest that miR-3940-5p functions as a tumor suppressor in glioblastoma and may provide novel strategies for treating this fatal disease.

POSTER NUMBER: 1026

Identification of piRNAs and functional investigation of piRNA-pathway in adult mammalian Neural Progenitor Cells

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PIWI-interacting RNAs (piRNAs) are small, 26-32 nt single-stranded noncoding RNAs interacting with PIWI proteins in many organisms. In mammals, piRNAs and PIWI proteins are thought to be restricted to germline cells, where they have been shown to repress mainly transposable elements (TE) to maintain genomic integrity. Recent evidence suggests that PIWI proteins and piRNAs are also expressed in the adult Central Nervous System (CNS). However, their functions in the CNS are largely unknown. Here, using both in vivo and in vitro murine models, we report that PIWI proteins and piRNAs are highly enriched in adult neural progenitor cells (NPCs). Moreover, we found that expression of PIWI proteins and piRNAs changes along neural lineage, suggesting a role of CNS-piRNAs in NPC differentiation program. Indeed, by knockdown of a PIWI protein and depletion of piRNAs in NPC, we provide the first evidence of a functional role of the piRNA-pathway in mammalian CNS. Preliminary target analysis of the CNS-piRNA clusters reveals mRNAs, long noncoding RNAs known for their roles in neural differentiation, various classes of TEs and other noncoding RNAs. A potential role of CNS-piRNAs in the general control of translation in adult NPCs will be discussed.

POSTER NUMBER: 1027

MiRViz, a web service application to visually identify and interpret large scale microRNA datasets

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MicroRNAs (miRs) are small non-coding RNAs. Many protein coding genes are regulated by multiple miRs, highlighting the redundancy of miR modes of action. An example is the existence of miR families, groups of miRs sharing the same seed (nucleotides 2-7 from the 5' end), which regulate almost identical genes. To evaluate the redundancy of miR-target genes relationships in large-scale datasets, such as miR-seq or miR phenotypic screening data, we propose to use miR networks. We built miRViz (http://mirviz.prabi.fr/), a freely available web service in which users can load, interpret and visualize their datasets by superposition onto predefined networks.
MiRViz proposes five human miR networks, in which each node is a given miR: Diana50-TargetScan54-TargetScan78, connect nodes if the corresponding two miRs share more than 50-54-78% of predicted mRNA targets using either Diana microT or TargetScan as target prediction algorithms [1]. Seed2_7 links two miR-nodes if they share the same seed, thus displaying groups of miR families. Genomic Distance links two miR-nodes if the miR genes are closer than 50kb on DNA.
We show the advantages of miRViz using experimental datasets. First, we propose a pipeline using miRViz to rapidly identify and represent differentially expressed miR families that are common or specific between two experimental conditions. Reanalyzing a public dataset, we highlight the miR-320 family as significantly enriched in exosomes secreted by a colon cancer cell line. Second, we visually show that miRs belonging to the same family tend to be co-expressed. Third, using a miRnome-wide screen dataset, we identify embryonic stem cell miRs as a cluster of functionally active miRs in the regulation of breast cancer stem cells equilibrium.

[1] Bhajun *et al.*, Scientific Reports 2015.

POSTER NUMBER: 1028

PIWIL3 plays an important role in early embryogenesis of golden hamsters

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The PIWI-piRNA pathway is a key cellular pathway that silences transposable elements (TEs) in animal gonads. In mice, three PIWI proteins (PIWIL1, PIWIL2 and PIWIL4) are almost exclusively in testes and little in ovaries. Consistent with this, Piwi knockout male mice exhibit defects in spermatogenesis with an activation of TEs, leading to infertility, but Piwi knockout female mice show no discernible phenotype. However, recent genome research indicated that the vast majority of mammals have four Piwi genes and three Piwi genes (Piwil1, Piwil2 and Piwil3) were highly expressed in the ovary except for mouse and rat.
The expression of Piwil3 was found to be restricted to the ovary. To understand possible roles of PIWIL3 in female germ cells, we have used the golden hamster (Mesocricetus auratus) in which Piwil3 is expressed in the ovary. With the CRISPR/Cas9 system, we succeeded to produce Piwil3 mutant hamster lines. We found that Piwil3 mutant females are sub-fertile as a result of abnormal development of pre-implantation embryos. Our results revealed that PIWIs have important roles even in female germ cells.

POSTER NUMBER: 1029

Tolerance for bulges in mature miRNAs, siRNA duplexes and target-bound guide strands

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Small non-coding RNAs are key players in gene regulation. Whereas miRNAs act as endogenous regulators of gene expression, siRNAs are well established as molecular biology tools and emerging as therapeutic agents. The siRNA guide strand is typically designed to form perfectly complementary duplexes both with the passenger strand and the target mRNA transcript. In contrast, nature designs miRNAs to contain elements that interrupt the perfect duplex, for example mismatches or bulges. Here, we tested if bulges of the guide strand are accepted in siRNA duplexes. Bulges of different lengths were introduced into siRNA duplexes by deleting one or more nucleotides from the passenger strand. Bulge tolerance at different positions was examined by systematically walking the bulge through the siRNA duplex. *In vitro* data were retrieved with unmodified and modified siRNAs of different nucleobase sequences. We also evaluated *in vivo* activity of selected, bulge-containing GalNAc-siRNA conjugates. Taken together, we find restrictions on bulge length and position in functional siRNAs and compare our insights to

observations in the miRNA field. Finally, we asked if guide strand bulges may also form upon target RNA binding and evaluated this as a possible off-target risk.

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POSTER NUMBER: 1030

Loss of *PIWIL4* and *L1TD1* disrupts somatic piRNA, methylome and genome stability

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The silencing of endogenous retrotransposons like *LINE-1* is critical for the maintenance of genomic stability and the somatically expressed Piwi-like RNA-mediated gene silencing 4 (*PIWIL4*) and *LINE-1* type transposase domain containing 1 (*L1TD1*) gene were hypothesized to regulate the activity of *LINE-1* through histone modifications and DNA methylation. To test this hypothesis, we used CRISPR/Cas9 system to knockout (KO) either gene in gastric, colon, breast cancer cell lines and in mesenchymal stem cells (MSCs). We observe that the KO of either gene blocks the entrance of SUV39H1 into cell nuclei and suppresses the tri-methylation of histone H3 at lysine 9. A genome-wide demethylation and reduced HP1α recruitment were also detected. Accompanied with the distorted piRNA and active/silencing (bivalent) histone marks, the KO of either gene disoriented the global CTCF binding which indicated a disorganized chromosomal conformation and was confirmed by the aneuploidy phenotype detected by G-banding. Evidences including the aneuploidy, uneven cell division, loss of cell stiffness (detected by atomic force microscope) and loss of MSC stemness altogether indicate the loss of genome stability in KO cells. We also found that overexpressed *Notch-1* increased DNA methylation within *LINE-1* promoter, and this DNA hypermethylation was attenuated by either *PIWIL4* or *L1TD1* KO. Moreover, tissue arrays confirmed abnormal PIWIL4 expression in gastric (n=45), colon (n=30) and breast (n=30) cancer samples and abnormal *L1TD1* promoter methylation was also detected in these three types of cancer. We thus conclude that the somatic loss of either *PIWIL4* or *L1TD1* disrupts somatic piRNA, DNA methylome and genome stability. (Supported by MOST 107-2320-B-194-002, 106-2320-B-194-001-MY3, and CGMH CMRPG6H-0321, Taiwan)

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POSTER NUMBER: 2040

Structural insights into Rhino-Deadlock complex for germline piRNA cluster specification

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PIWI-interacting RNAs (piRNAs) silence transposons in germ cells to maintain genome stability and animal fertility. Rhino, a rapidly evolving Heterochromatin Protein 1 (HP1) family protein, binds Deadlock in a species-specific manner and so defines the piRNA-producing loci in the *Drosophila* genome. Here, we determine the crystal structures of Rhino-Deadlock complex in *Drosophila melanogaster* and *simulans*. In both species, one Rhino binds the N-terminal helix-hairpin-helix motif of one Deadlock protein through a novel interface formed by the beta-sheet in the Rhino chromoshadow domain. Disrupting the interface leads to infertility and transposon hyperactivation in flies. Our structural and functional experiments indicate that electrostatic repulsion at the interaction interface causes cross-species incompatibility between the sibling species. By determining the molecular architecture of this piRNA-producing machinery, we discover a novel HP1-partner interacting mode that is crucial to piRNA biogenesis and transposon silencing. We thus explain the cross-species incompatibility of two sibling species at the molecular level.

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POSTER NUMBER: 1031

An adaptive stress-induced tRNA depletion response mediates codon-based gene repression and growth

suppression

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Eukaryotic transfer RNAs (tRNAs) can become fragmented upon various cellular stresses, generating tRNA-derived RNA fragments (tRFs). Though this process has been observed for numerous cellular stresses and in many species ranging from plant cells to yeast and human cells, it is still poorly characterized and understood. Such tRNA fragmentation has previously been thought to affect a small fraction of the tRNA pool and was thus presumed to not affect the role of tRNAs in translation. We report that in human cells, oxidative stress can rapidly generate tRFs derived from tyrosyl tRNA $_{GUA}$—resulting in a significant depletion of the precursor tRNA molecule and mature tRNA while also leading to elevated levels of the tRF. Proteomic and ribosomal profiling of tyrosyl tRNA $_{GUA}$-depleted cells revealed impaired expression of a gene-set enriched in its cognate tyrosine codons, comprising growth and metabolic genes. Consistent with these affected pathways, depletion of tyrosyl tRNA $_{GUA}$ or its downstream genes EPCAM, SCD, or USP3 repressed growth—revealing a tRNA-dependent growth suppressive pathway for oxidative stress response. A synthetic mimetic of the tRF induced upon oxidative stress was used to identify interactions with RNA binding proteins through mass spectrometry. High-throughput sequencing of RNA isolated by crosslinking immunoprecipitation (HITS-CLIP) of one protein, SSB, confirmed the mass spectrometry results and identified endogenous interactions between the protein and tRF. Thus, tRNA fragmentation can both deplete a precursor tRNA molecule with codon-dependent regulatory consequences and also generate small-RNAs that can interact with and potentially regulate RNA binding proteins.

POSTER NUMBER: 1042

Non-Templated Additions of miRNAs Indicates Various Roles that a miRNA-Loading Argonaute Could Play in Mammals

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Non-templated additions (NTAs) or tailing at the 3' end of miRNAs is one of the miRNA modification events that has been shown to be involved in miRNA biogenesis, maturation, stability, and targeting effectiveness. miRNAs are loaded into AGO clade proteins to exert their regulatory functions. There are four AGO clade proteins in mammals, each of which has distinct involvement in biological processes and preference to cellular localization. Mounting evidence has suggested that NTAs and four AGO clade proteins are strongly relevant to the functions of miRNAs, however, in most of the studies they were largely left unexplored (e.g., only AGO2 and canonical miRNAs were examined). In this work, we revisited 39 human and mouse Argonaute RIP- and small RNA-seq datasets and discovered several novel structural and sequence features and related NTAs to selective Argonaute loading (a.k.a., miRNA-Argonaute sorting), isoMir biogenesis, cellular localization, extracellular miRNA distribution, and differentiation stages. We discovered many evidences suggesting that NTAs, including 3' adenylation and uridylation, may reflect distinct functional roles that a miRNA-loading Argonaute could play. In addition, we carried out in vivo experiments and confirmed that one of the most abundant miRNAs in the mouse embryos and developing neurons, miR92a-1, is enriched in nucleus-located AGO1 and has mono- and poly-A tails in a developmental stage-dependent manner. Together, we demonstrated that the NTAs may reveal possible associations to many important biological mechanisms and should be examined in every miRNA sequencing analysis.

POSTER NUMBER: 1032

Identification of a unique RNA motif sequence that regulates an off-target effect by small RNA

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We have previously reported that a control siRNA (none-targeting siRNA) forms a complex with IFI16, a DNA sensor and RIG-I, an RNA sensor (IFI16/siRNA/RIG-I) and enhances DNA-mediated IFN-1 induction by near 10-fold as an innate immune response. This augmentation is associated with a dissociation of IFI16 from the IFI16/siRNA/RIG-I (Nucleic Acids Res, 2014). Here we provide a novel function of siRNA. To elucidate a mechanism of the enhancement by the control siRNA (c-siRNA) in IFN-α-stimulated HeLa cells, we co-transfected a series of siRNAs targeting IFN-stimulated genes with the siRNA. We found that some siRNAs inhibited the enhancement, however, several siRNAs containing a unique 5-nucleotide (nt) motif sequence (motif-siRNA) abolished the IFN-1 induction. In the presence of 10 nM motif-siRNA, the c-siRNA-enhanced IFN-1 induction was suppressed by > 95%. Using a series of variants of the motif-siRNA, we elucidated that the suppression was dependent on the location of the motif within siRNA. The motif-siRNA only inhibits IFN-1 induction when the motif was located at the 3' or 5' terminus of sense strand of siRNA. AlphaScreen proximity analysis clarified that affinity of the motif-siRNA to IFI16 was 2.5-fold higher than that of siRNA without the motif ($p < 0.05$) and in the presence of motif, siRNA and IFI16 persists to form IFI16/siRNA/RIG-I complex. The Inhibition of the dissociation of IFI16 from the complex suppressed induction of IFI-16-mediated down-stream signaling. Collectively, these findings may provide a new strategy to regulate the innate immune system's over-response to microbial infections and may shed light on the novel function of siRNA with the unique 5-nt motif as a quencher of innate immunity.

POSTER NUMBER: 1033

Role of the RNA helicase, Armitage, in the primary piRNA biogenesis pathway

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PIWI-interacting RNAs (piRNAs) repress transposons to maintain germline genome integrity. Recent studies showed that artificial tethering of Armitage (Armi) to RNAs induced piRNA biogenesis. However, lack of female sterile (1) Yb (Yb) in *Drosophila* ovarian somatic cells (OSCs) impaired production of transposon-targeting piRNAs even in the presence of Armi. Here, we show that specific interaction of Armi with *flamenco* transcripts, the primary source of transposon-targeting piRNAs in OSCs, is strictly regulated by Yb. Lack of Yb allowed Armi to bind RNAs promiscuously, leading to the production of piRNAs unrelated to transposon silencing. The ATP-hydrolysis-defective mutants of Armi failed to unwind RNAs and so were retained on them, abolishing piRNA production. Therefore, both Yb and ATP-dependent, RNA-unwinding activity of Armi are necessary for accumulating transposon-targeting piRNAs and for silencing transposons. Analysis of the 5′ end of Armi-bound piRNA precursors suggested direct involvement of Armi but not of Yb in Zucchini-dependent piRNA phasing.

POSTER NUMBER: 1034

Diverse mechanisms of translation inhibition by tRNA-derived regulatory RNAs

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The survival of mammalian cells exposed to adverse environmental conditions requires reprogramming of protein translation. While stress-activated kinases target different components of translation machinery to inhibit general translation, recent data suggest that tRNA and tRNA-derived fragments also play active roles in the regulation of protein synthesis under stress. The ribonuclease angiogenin (ANG)-mediated tRNA cleavage promotes a cascade of cellular events that starts with production of tRNA-derived stress-induced RNAs (tiRNAs) and culminates with enhanced cell survival. This stress response pathway partially relies on a subset of tiRNAs that inhibit cap-dependent translation initiation and induce assembly of stress granules (SGs), RNA granules with pro-survival and cytoprotective properties. In addition to these SG-promoting tiRNAs, we have identified other subsets of tiRNAs that inhibit translation through other non-overlapping mechanisms. Here, I will overview multiple modes of translation modulation by different subsets of tiRNAs, discuss their structural properties and describe their interactions with binding partners.

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POSTER NUMBER: 1035

Piwi-piRNA silencing-coupled changes of nuclear architecture in *Drosophila*

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The piRNA (PIWI-interacting RNA) pathway in *Drosophila* ovarian somatic cells (OSCs) silences transposable elements (TEs) to maintain the genome integrity. Piwi-piRNA complexes (PIWI-piRISCs) transcriptionally repress their target TEs by modifying the chromatin state, such as establishment of H3K9me3 marks. Additionally, we have recently shown that the linker histone H1 and nuclear export factor variant are necessary for Piwi-piRNA to repress TEs. We proposed a model in which Piwi-piRISCs exert TE silencing through H1 association with specific chromatin regions, resulting in modulation of chromatin accessibility. Also, involvement of nuclear export factor suggests the possible regulation of the nuclear localization of PIWI-piRISC silencing machinery. In line with these and the fact that some non-coding RNAs are known to effect chromatin boundaries, we have analyzed the involvement of the Piwi-piRNA in regulation of the 3D chromatin organization of OSCs, using Hi-C (Chromatin conformation capture sequencing) method. This revealed that depletion of Piwi induces long-range interactions within a chromosome arm. We also analyzed TADs (Topologically associating domains) of OSCs using Hi-C data, and revealed the decrease of intra-TAD interaction upon loss of Piwi. Importantly, the decrease of intra-TAD interaction correlated with the density of Piwi-piRNA target TEs in each TAD. Further analysis revealed that the modulation of TADs observed upon Piwi depletion correlated with active/repressive histone marks and transcription levels. These results suggest that Piwi is essential for maintaining intra-TAD interaction, which possibly is mediated by the regulation of target TEs, and forming compact chromatin structure. Also, ChEP (Chromatin enrichment for proteomics) analysis suggested that Piwi affects on the chromatin binding of factors including insulator proteins, which are known as the genetic boundary elements. We will further discuss the impact of Piwi-piRNA silencing on the global nuclear architecture.

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POSTER NUMBER: 1036

A piRNA-mediated regulatory pathway based on short genomic sequences conserved throughout evolution

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PIWI-interacting RNAs (piRNAs) target transcripts by sequence complementarity serving as guides for the inactivation of transposable elements in animal germ cells. The piRNA pathway is increasingly recognized as critical for essential cellular functions such as germline development or reproduction. In the *Anopheles gambiae* ovary, as much as 11% of piRNAs match protein-coding genes. These piRNAs result from different types of piRNA biogenesis. They may be produced and amplified through a ping-pong mechanism, but large amounts of piRNAs from single genes may also be produced through phased piRNA biogenesis without leading to ping-pong. The latter process is initiated by piRNAs originating from distant loci, either protein-coding genes or long non-coding RNAs (lncRNAs). We show that networks are established between protein-coding genes and lncRNAs. Genes and lncRNAs involved in the same network share related short ~40-nucleotide genomic sequences. These short sequences give rise to piRNAs, which may target one or several other transcripts to induce their slicing and production of novel targeting piRNAs, thus leading to a chain reaction. This then may result in concerted degradation of transcripts that are members of the same network. Interestingly, the short ~40-nucleotide sequences are conserved throughout evolution from insects to mammals. Our study brings to light a new type of regulatory pathway named snetDNA-pathway conserved throughout evolution, by which short sequences can place independent genes in the same biological pathway.

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POSTER NUMBER: 1037

Stress-activated miRNA governs senescent phenotypes of chondrocytes to promote osteoarthritis development

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A progressive loss of cartilage matrix leads to the development of osteoarthritis (OA). Matrix homeostasis is disturbed in OA cartilage as the result of reduced production of cartilage-specific matrix and increased secretion of catabolic mediators by chondrocytes. Chondrocyte senescence is a crucial cellular event contributing to such imbalance in matrix metabolism during OA development. Here, we identify a senescence-associated microRNA (miRNA) which is markedly upregulated in OA cartilage. The upregulated miRNA simultaneously targets multiple components of the sulfated proteoglycan (PG) biosynthesis pathway, effectively shutting down PG anabolism. Ectopic expression of the miRNA in joints triggers spontaneous cartilage loss and OA development, whereas inhibition of the miRNA ameliorates experimental OA, with concomitant recovery of PG synthesis and suppression of inflammatory senescence-associated secretory phenotype (SASP) factors in cartilage. Collectively, we unravel a stress-activated senescence pathway that underlies disrupted matrix homeostasis in OA cartilage.

POSTER NUMBER: 1038

Potential therapeutic application of NRAS depletion by microRNA-708 in NRAS mutated melanoma

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Malignant melanoma is mostly associated with BRAF and NRAS mutation. In contrast to BRAF mutation, there is currently no effective targeted therapy specific for NRAS mutated melanoma. Most patients responded to treatments poorly and drug resistances emerged over a remarkably short period of time. Glucocorticoids (GCs) are known to inhibit metastasis in ovarian cancer through upregulating one of the tumor suppressive microRNAs, miRNA-708. Our preliminary data indicate that NRAS is a novel target of miRNA-708 and depletion of its expression may have an impact to NRAS mutated melanoma. In order to test the therapeutic potential of GCs or miRNA-708 in targeting NRAS, we examined the cellular effects mediated by miR-708 in NRAS mutated melanoma cell line, SK-MEL-2. We found proliferation and migration were reduced in SK-MEL-2 cells with miR-708 overexpression, and both functions are critical for tumor growth and metastasis. Overall, our data may provide an alternative approach to treat patients with NRAS mutated melanoma and we hope that our approach to induce miR-708 expression can also apply to other NRAS mutated cancers in the future.

POSTER NUMBER: 2041

Indexing more than a million experimentally supported miRNA targets

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DIANA-TarBase (Karagkouni D *et al*, Nucleic Acids Res., 2018) is a reference database devoted to the indexing of experimentally-supported microRNA (miRNA) targets. Its 8th version is the first database to index >1 million entries, supported by more than 33 experimental methodologies, applied to 592 cell types/tissues under ~430 experimental conditions. Approximately 1,160 publications have been manually curated and more than 330 high-throughput datasets have been analysed. TarBase database has been cited >2,000 times since its first version, according to Google Scholar. Thousands of users are accessing the database on a monthly basis, while >300 labs from 5 continents have downloaded the database for local use.
Over the last 15 years, a multitude of experimental methodologies have emerged, aiming to determine miRNA interactome. However, this invaluable information is dispersed in numerous publications and raw high-throughput data.
More than 600,000 of TarBase entries are derived from the analysis of AGO-CLIP-Seq experiments. AGO-PAR-CLIP is considered one of the most powerful high-throughput methodologies for miRNA target identification.
microCLIP (Paraskevopoulou MD *et al*, Nature Communications, 2018) is an innovative framework that combines deep learning classifiers under a super learning scheme for CLIP-Seq-guided detection of miRNA interactions. Former AGO-CLIP-guided implementations depend strongly on the T-to-C conversions to define miRNA bindings, while the efficacy of neglected interactions remained unknown. By analyzing miRNA perturbation experiments and structural sequencing data we showed that the previously neglected non-T-to-C clusters exhibit functional miRNA binding

events and strong accessibility. Contrary to former implementations, microCLIP operates on every AGO-enriched cluster providing an average 14% increase in miRNA-target interactions per PAR-CLIP library, uncovering previously elusive regulatory events and miRNA-controlled pathways.

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POSTER NUMBER: 2001

Enhancer RNAs delineate adaptive immune cell identity and function

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Enhancer RNAs (eRNAs) are non-coding RNAs transcribed from active enhancer regions with an increasingly-recognized role in regulating gene transcription. Several studies have illustrated the role of eRNAs in tissue development and human diseases. However, their function in adaptive immune cells has been poorly explored. Here, we identify >2000 reproducible intergenic eRNAs in B cells, CD4 $^{+}$, and CD8^{+} T cells by integrating genome-wide DNA accessibility (ATAC-seq) and ribo-depleted stranded RNA sequencing data. These eRNAs were specific to immune cells and not present in other tissues. Nearly 40% of eRNAs were only expressed in B cells, 40% were expressed in either or shared (~20%) between CD4^{+} and CD8^{+} T cells, but absent in B cells. We show that eRNAs are distinct from non-transcribed enhancers and promoters by having the highest level of active and the lowest level of repressive histone modifications. eRNAs were highly enriched in super-enhancer architecture. As expected, eRNA expression correlated strongly with the transcription factor (TF) ChIP-seq signal of histone acyltransferases (e.g. GCN5), RNA polymerases (e.g. TAF7, GTF2F1), and chromatin regulators (e.g. MTA3, BRG1). We also identified other TFs (e.g. PML, NFATC1) that were highly correlated with eRNA expression, suggesting that they also potentially regulate eRNA expression. The genes neighboring eRNAs were involved in lymphocyte activation pathway and had >30-fold higher expression when compared to genes adjacent to non-transcribed enhancers, indicating that these genes are likely targeted by eRNAs themselves. Collectively, our work provides a comprehensive characterization of eRNAs in human adaptive immune cells.

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POSTER NUMBER: 3038

A subunit of the 26S proteasome regulates the production of transgene-derived siRNAs

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MicroRNA (miRNA) and small interfering RNA (siRNA) belong to 20- to 24- nucleotide-long small non-coding RNAs and act as post-transcriptional and/or transcriptional gene silencers in plants. Despite exciting findings made in the past decade, a mechanistic view of miRNA and siRNA in plant development, defense, and genome stability remains to be further elucidated. Here, we report a cell type-specific genetic screen system, PORI, in which expression of an artificial miRNA (amiRLuc) and its target *GFP-Luciferase* (*GFP-Luc*) is temporally overlapped but spatially separated. Our data suggest that PORI is regulated by both amiRLuc and transgene-derived siRNAs generated from the *GFP-Luc* transgene (PORI-siRNA) but not by DNA methylation pathways, indicating that PORI provides a system to study regulatory mechanisms by both miRNA and siRNA. A genetic mutant, CS838, was isolated and found to contain a 72bp deletion in the gene encoding a subunit of proteasome complex. In the CS838 mutants, *GFP-Luc* expression from the PORI was restored, suggesting that a subunit of the 26S proteasome plays a role in RNA silencing. Interestingly, the accumulation of PORI-siRNA was significantly reduced in the CS838 harboring PORI, whereas endogenous miRNAs and hc-siRNAs were not affected. These results show that a subunit of the proteasome complex plays a role in the production of transgene-derived siRNAs in plants.

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POSTER NUMBER: 2007

Distant effects of miR-34a and miR-34b/c on the inhibition of lung adenocarcinomas

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Three miR-34 family members (miR-34a, miR-34b, and miR-34c), well-known tumor suppressive miRNAs, are clustered on two different chromosomal loci, Mir34a and Mir34b/c.
These miRNAs have identical seed-sequences and are predicted to target the same set of genes. However, miR-34a and miR-34c have different sets of negatively correlated genes in the lung adenocarcinoma The Cancer Genome Atlas (TCGA) data. Therefore, we hypothesized that miR-34 family members have differential effects on lung tumorigenesis. To prove this, we overexpressed each miR-34 cluster in murine lung cancer cells. miR-34b/c enhanced cancer cell attachment and suppressed cell growth and invasion compared with miR-34a. miR-34b/c also decreased the expression of mesenchymal markers (*Cdh2* and *Fn1*) and increased epithelial markers (*Cldn3, Dsp*, and *miR-200*) better than did miR-34a. In RNA sequencing analysis, miR-34a- and miR-34b/c-overexpressed cells showed different gene expression patterns. Furthermore, knockout of all three miR-34 members promoted mutant Kras-driven lung tumor progression in mice. And combined expression of all three miR-34 members were a good prognosis marker for lung cancer patients in TCGA data. Collectively, we suggest that miR-34b and miR-34c are more effective tumor suppressors than is miR-34a in lung adenocarcinoma and combination of miR-34a and miR-34b/c inhibits lung cancer development.

POSTER NUMBER: 2002

DROSHA isoform associated with Golgi apparatus

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DROSHA is a 160 kDa RNase III endonuclease mainly found in the nucleus, where it initiates microRNA (miRNA) maturation by cleaving primary miRNAs. While DROSHA has been studied extensively as a nuclear protein, its cytosolic localization and function have been described under stress and viral infection. Here we report a novel isoform of DROSHA that specifically localizes in the Golgi apparatus. A smaller fragment of DROSHA ("sDROSHA") is formed by post-translational proteolytic cleavage of full-length DROSHA at near the N-terminus. Subcellular fractionation experiment indicates that sDROSHA is highly enriched in the membrane fraction. Immunocytochemistry confirms the localization of DROSHA in the Golgi apparatus. Like full-length nuclear DROSHA, sDROSHA forms the Microprocessor complex with DGCR8 and is enzymatically active *in vitro*. Thus, DROSHA exists as both intact and truncated forms which localize inside and outside the nucleus, respectively. We are currently carrying out formaldehyde crosslinking, immunoprecipitation, and sequencing (fCLIP-seq) to identify the substrates of sDROSHA in order to investigate the unexpected function of Microprocessor in the Golgi apparatus.

POSTER NUMBER: 2003

Estrogen regulation of miR-9-5p/ -3p degradation kinetics in the rat brain

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Circulating levels of 17β-estradiol (E2) rapidly decline during menopause, and hormone replacement therapy (HRT) is used to alleviate negative symptoms associated with the systemic loss of E2. However, large scale, longitudinal studies revealed a critical period of time immediately following the onset of menopause where HRT is clinically beneficial. Previous research in the lab showed that miRNAs (miR), small non-coding RNAs that regulate gene expression, might mediate the temporal switch in E2 action. Specifically, E2 administration significantly increased the mature form of two miRs, miR-9-5p and -3p, in the brain of aged female rats; however, this regulation was lost following extended lengths of E2 deprivation. Interestingly, the primary and precursor forms of miR-9-5p and -3p

did not correlate with E2-mediated changes in the mature form, suggesting that E2 did not alter miR biogenesis. Therefore, we hypothesized that E2 stabilized the mature forms of miR-9-5p and -3p in an age dependent manner. We tested this hypothesis using a miR degradation assay, as reported by Chatterjee and Großhans (Nature, 2009). Briefly, mature miR-9-5p and -3p sequences were radiolabeled with 32P and incubated for varying lengths of time in cell lysate prepared from neuronal-derived cell lines (in vitro) or hypothalamic tissue (in vivo) from ovariectomized rats treated with E2/vehicle. Densitometry values corresponding to the full length miR were normalized to T0, plotted on a scatterplot, and fit with an exponential decay function to determine its half-life. Interestingly, E2 stabilized miR-9-3p, but not miR-9-5p, suggesting that E2-mediated regulation of miR stability was specific to different transcripts derived from the same precursor. Additionally, E2 did not extend the half-life of other neuronal-enriched miRs (495,125,7a, and let-7i), with the exception of miR-181a. These data suggest that E2-mediated miR stabilization is specific to a select subset of miRs in the aging brain. Future studies will investigate the downstream molecular pathways of estrogen receptor activation that imparts differential miR stabilization. These findings advance the current understanding of miR biology in the aging female brain and allow for the development of more effective hormone replacement paradigms.

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POSTER NUMBER: 2004

QKI is targeted by miR-200 and promotes epithelial-to-mesenchymal-transition and tumorigenesis

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The microRNA-200 (miR-200) family members repress epithelial-to-mesenchymal-transition (EMT) by targeting the transcription repressors ZEB1 and ZEB2. To find novel miR-200 targets, we analyzed TargetScan and The Cancer Genome Atlas head and neck squamous cell carcinoma (HNSCC) dataset. Through this approach, we chose a RNA-binging protein, quaking (QKI) as a candidate target for miR-200. QKI negatively correlates with miR-200 family members *in silico* and *in vitro*. Consistently, QKI expression was also suppressed by miR-200 over-expression, and the 3'-UTR of QKI mRNA was directly targeted by miR-200 in luciferase reporter assays. Unexpectedly, shRNA-mediated knockdown of QKI promoted migration, invasion, and EMT in cancer cells and increased tumor growth in a xenograft mouse model. In addition, QKI regulated the expression of inflammation-related genes in gene set enrichment analysis of RNA sequencing data, implying that inflammatory signaling activated by QKI knock-down may promote EMT and invasiveness of cancer cells. Furthermore, high expression of QKI protein was associated with favorable prognosis in surgically resected HNSCC and lung adenocarcinoma. In conclusion, QKI increases during EMT and is targeted by miR-200; while, it suppresses EMT and tumorigenesis. We suggest that QKI and miR-200 form a negative feedback loop to maintain homeostatic responses to EMT-inducing signals.

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POSTER NUMBER: 2005

Regulation of alternative microRNA processing and strand selection by terminal uridylyltransferases

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Alternative processing of microRNAs (miRNAs) is an important mechanism to increase the diversity of mature miRNAs and expand the target repertoire. However, it remains poorly understood if it is a regulated process and has any biological significance. Here we demonstrate that the mir-324 locus produces three mature miRNAs (5p and two 3p isoforms) with distinct functions through alternative Dicer processing regulated by uridylation. Terminal uridylyltransferases, TUT4 and TUT7, uridylate pre-mir-324, which causes a shift of the Dicer cleavage site by 2 nt. This in turn leads to arm switching, producing a 3p isoform over 5p. We identified that TUT4/7 are upregulated and consequently the 3p isoform dominates in glioblastoma. Disruption of the alternative maturation of mir-324 was sufficient to impair glioblastoma cell growth. This study offers the first example of regulated alternative miRNA processing and uncovers a novel role of TUT4/7 in alternative miRNA maturation which is implicated in glioblastoma maintenance.

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POSTER NUMBER: 2008

The Atlas of DROSHA Cleavage Sites on Primary MicroRNAs

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The cleavage of primary microRNA (pri-miRNA) by DROSHA initiates canonical miRNA biogenesis. The choice of cleavage sites is critical as it defines the seed sequence and the target repertoire. Nevertheless, the DROSHA cleavage sites of individual miRNAs and the mechanisms underlying alternative processing remain largely unexplored. Here, we investigate DROSHA processing for all annotated human miRNAs (miRbase v21). To this end, *in vitro* transcribed pri-miRNAs are incubated with the recombinant Microprocessor consisting of DROSHA and its cofactor DGCR8. The cleavage sites are determined by sequencing of the processed RNA fragments. This high-throughput approach allows us to establish the list of DROSHA-dependent miRNAs and to map their DROSHA cleavage sites. About one-fifth of DROSHA-dependent miRNAs are cleaved at multiple sites *in vitro*. Comparison with the *in vivo* sequencing data suggests that some pri-miRNAs require trans-acting co-factors for cleavage site determination. Using our data, we also evaluate the role of primary sequence motifs and secondary structures for the cleavage site choice. The coordination between the mGHG motif and lower stem length is critical for the homogeneity of processing. Lastly, we uncover the 3′ end modification events such as uridylation on precursor miRNAs. Our study establishes a comprehensive map of DROSHA cleavage sites, which provides the basis for the investigation of RNA modifications and regulatory factors on miRNA biogenesis.

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POSTER NUMBER: 2006

The Role of MicroRNA-125a-3p in Inhibition of Autophagy and Antimicrobial Responses during Mycobacterial Infection

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MicroRNAs (miRNAs) are small noncoding nucleotides that play critical roles in the regulation of diverse biological functions, including the response of host immune cells. Autophagy plays a key role in activating the antimicrobial host defense against *Mycobacterium tuberculosis*. Although the pathways associated with autophagy must be tightly regulated at a posttranscriptional level, the contribution of miRNAs and whether they specifically influence the activation of macrophage autophagy during *M. tuberculosis* infection are largely unknown. In this study, we investigated the function of miRNA-125a-3p (miR-125a) in macrophages infected with *M. tuberculosis*. *M. tuberculosis* infection of macrophages leads to increased expression of miR-125a, which targets UV radiation resistance-associated gene (UVRAG), to inhibit autophagy activation and antimicrobial responses to *M. tuberculosis*. Over-expression of miR-125a significantly blocked autophagy and phagosomal maturation in macrophages, and inhibitors of miR-125a counteracted these effects, during *M. tuberculosis* infection. Silencing of UVRAG increased intracellular growth of *M. tuberculosis*. Both TLR2 and MyD88 were required for biogenesis of miR-125a during *M. tuberculosis* infection. Ongoing study is being conducted to identify the mycobacterial antigen(s) to modulate miR-125a in macrophages. Collectively, these data indicate that miR-125a suppresses innate host defense through inhibition of autophagy and antimicrobial effects against *M. tuberculosis* through targeting UVRAG.

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POSTER NUMBER: 2009

Molecular basis for the single-nucleotide precision of primary microRNA processing

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Microprocessor, composed of DROSHA and its cofactor DGCR8, initiates microRNA (miRNA) biogenesis by processing the primary transcripts of miRNA (pri-miRNAs). We here investigate the mechanism by which Microprocessor selects the cleavage site with single-nucleotide precision, which is crucial for the specificity and functionality of miRNAs. By testing ~40,000 pri-miRNA variants, we find that for some pri-miRNAs the cleavage site is dictated mainly by the mGHG motif embedded in the lower stem region of pri-miRNA. Structural modeling and deep sequencing-based complementation experiments show that the double-stranded RNA-binding domain (dsRBD) of DROSHA recognizes mGHG to place the catalytic center in the appropriate position. The mGHG motif as well as the mGHG-recognizing residues in DROSHA dsRBD are conserved across eumetazoans, suggesting that this mechanism emerged in an early ancestor of the animal lineage. Our findings provide a basis for the understanding of miRNA biogenesis and rational design of accurate small RNA-based gene silencing.

POSTER NUMBER: 2010

Development of a novel mouse model for the reversible, temporally and spatially controlled inhibition of miRNA activity *in vivo*

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Repression by miRNAs is mediated by a large multi-component ribonucleoprotein known as the miRNA-Induced Silencing Complex (miRISC) whose core components are members of the Argonaute and TNRC6 families.
We have previously reported that in many post-mitotic tissues from adult mice miRNAs are associated with Argonaute proteins in a complex that lacks members of the TNRC6 family and does not appear to be engaged in mediating mRNA repression. We have also shown that mitogenic and oncogenic stimuli can activate the miRISC leading to recruitment of TNRC6 proteins. An interpretation of these observations is that miRNA function is dynamically regulated *in vivo* and preferentially required in specific cell states (for example during cell proliferation).
To study in detail the temporal windows during which miRNA function is required, we have generated a novel mouse model that allows to acutely and reversibly inhibit miRNA function *in vivo*. This model utilizes the doxycycline-inducible expression of a YFP-tagged peptide (T6B), which specifically disrupts the interaction between Argonaute and TNRC6 proteins, thus leading to the reversible disassembly of the miRISC upon doxycycline administration.
Using this mouse model, we show that the temporal inhibition of the miRISC reversibly impairs miRNA-mediated gene repression *in vitro* and *in vivo*. Interestingly, while T6B expression does not appear to cause obvious toxicity in adult mice, it profoundly inhibits the development of primary lung adenocarcinomas and sensitizes them to radiation. Our work suggests that the development of small molecules targeting the integrity of the miRISC may be an effective addiction to the current repertoire of anti-cancer drugs.

POSTER NUMBER: 2011

Genomic clustering aids nuclear processing of suboptimal pri-miRNA loci

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Nuclear processing of most miRNAs is mediated by Microprocessor, compromised of the RNase III enzyme Drosha and its double stranded RNA binding (dsRBD) cofactor DGCR8. While a variety of non-canonical substrates can bypass Microprocessor to generate mature miRNAs, this complex is normally the gatekeeper for identifying bona fide canonical miRNA substrates. Thus, most miRNAs have presumably evolved features that optimize recognition and cleavage by Microprocessor. On the other hand, many precedents for regulation of Drosha cleavage exist, indicating

this reaction does not always proceed optimally. We uncovered a hidden layer of Microprocessor regulation via studies of the Dicer-independent locus *mir-451*, which is located in an operon with canonical *mir-144*. Although *mir-451* is fully dependent on Drosha/DGCR8, we unexpectedly find that its nuclear processing is blocked as a solo miRNA. Productive maturation of miR-451 requires a nearby canonical miRNA hairpin, although the identity and relative orientation can be changed, and their separation can be altered within a certain range. Structure-function analysis of the *mir-451* hairpin identifies its small terminal loop and short stem as both contributing to its suboptimal Microprocessor features. To ask if this principle extends to canonical miRNAs, we surveyed other mammalian miRNAs and identified several examples of short-loop miRNA hairpins whose biogenesis and function is enhanced by normal positioning within an operon. Overall, we demonstrate a new strategy for proximity-based enhancement of nuclear processing of suboptimal miRNA hairpins, and provide a rationale for the genomic retention of certain miRNA operons.

POSTER NUMBER: 2012

A randomized, controlled, cross-over clinical study investigating the bioavailability of dietary fruit microRNAs (miRNAs) in humans

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Exogenous microRNAs (miRNAs) have been reported to regulate mammalian gene expression, however there have been conflicting results regarding the bioavailability of dietary plant miRNAs in humans. To investigate the bioavailability of known plant miRNAs from a serving of Apple (*Malus domestica*), Kiwifruit (*Actinidia deliciosa*), cooked Rice (*Oryza sativa*) or Water (Control) in eight healthy male participants, we designed a randomized, controlled, cross-over feeding study. Overnight-fasted participants were administered 300g of each treatment as a breakfast (0800 h), followed by a standard lunch (1200 h), snacks throughout the day and an evening meal that contained no plant material. Participants attended four treatment sessions with a one-week wash-out period between treatments. Blood samples were taken by venipuncture at 0h, 1h, 3h, 6h and 24h after a serving of the treatment breakfast. Small RNAs were extracted from the plasma samples and study food, and then sequenced using Illumina HiSeq2000. The bioinformatics workflows developed for this study consisted of identifying exact miRNA matches present in both our treatments and plasma samples. A total of 8315 known plant miRNAs were found in the three plant food treatments, with 78 detected in plasma and approximately four showing a dose profile. Of the small percentage of plant miRNAs we have identified as bioavailable, extensive variability was observed in the read counts and between individuals. As with many bioactive compounds such as polyphenols, plant miRNA dietary absorption appears to be a limited process and approaches that enhance bioavailability may be required for the therapeutic delivery of dietary miRNAs.

POSTER NUMBER: 2016

21-22 nt easiRNA-dependent regulation of retrotransposition in the plant germline

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LTR retrotransposon is the major class of transposon for heterochromatin in plants. DNA methylation and H3K9 methylation have been identified as major chromatin components for silencing heterochromatin. A SNF2 family protein DECREASE DNA METHYLATION (DDM1) is known as a master regulator of DNA methylation in plants. *ddm1* plants display massive transcription of retrotransposons eventually leading to 21-22 nt epigenetically activated siRNAs (easiRNAs) via miRNA-dependent pathways. *EVADE* is the most active LTR copia retrotransposon in Arabidopsis [1], and produces easiRNAs from the *GAG* gene when it is de-silenced. We investigated whether easiRNAs affect translation and reverse transcription processes of retrotransposition. Our translatome data suggest easiRNAs do not generally regulate translation of transposon transcripts. To study the reverse transcription step, we purified virus-like-particles (VLPs) assembled with genomic RNA and enzymes of LTR retrotransposons. Using short read and long read sequencing technologies, we identified functional LTR retrotransposons that made full length VLP DNA. Interestingly, there were partial VLP DNA fragments from non-functional *ATHILA* elements accumulated only in *ddm1rdr6*, suggesting potential roles of easiRNAs in controlling reverse transcription. Lastly, we will discuss how an active LTR retrotransposon *EVADE* can be silenced through easiRNA pathways in female reproductive tissues. Two miRNA target sites flank the easiRNA cluster in the *GAG* gene, but no cleavage sites were detected from

PARE-seq and RACE PCR, suggesting RDR6 might be recruited without cleavage, as reported previously [2,3]. *EVADE* easiRNAs were not detected in pollen, even in a *ddm1* mutant, consistent with maternal, but not paternal silencing [4]. *EVADE* copy number and RNA levels in *ddm1rdr6* were strikingly higher than *ddm1*, suggesting easiRNAs are important to control retrotransposition.

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POSTER NUMBER: 2019

Doxorubicin induces Protein Kinase R-mediated apoptosis via suppressing a non-coding RNA

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Doxorubicin has been an important treatment option for malignancies for a long time, but its cytotoxic mechanism is still in debate. Here we have discovered a novel mechanism that involves nc886, a cellular non-coding RNA (ncRNA), and Protein Kinase R (PKR) that is a pro-apoptotic protein in cellular immune response and cancer. nc886 is transcribed by RNA polymerase III (Pol III), binds to PKR, and prevents it from aberrant activation. Doxorubicin evicts Pol III from DNA and thereby shuts down nc886 transcription, leading to an acute decrease of nc886 enabling PKR activation and ultimately to apoptosis. These events are the main cause of cytotoxicity in nc886/PKR-expressing cells, after a short (~30 min) pulse treatment of doxorubicin at a therapeutic dose. Herein the nc886/PKR apoptotic pathway is recapitulated in a three-dimensional culture system as well as the monolayer cultures. Our study has identified nc886 as a molecular signal for PKR to sense doxorubicin and is of future clinical potential by providing a selective treatment regimen with minimal side effects depending upon the nc886/PKR status of cancer cells.

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POSTER NUMBER: 2014

miR-92b-3p regulates mTOR signaling in vascular smooth muscle cells upon hypoxia

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Pulmonary artery smooth muscle cells (PASMCs) undergo proliferation by the mammalian target of rapamycin (mTOR) signaling pathway under hypoxia. Hypoxia induces expression of a specific set of microRNAs (miRNAs) in a variety of cell types. We integrated genomic analyses of both small non-coding RNA and coding transcripts using next-generation sequencing (NGS)-based RNA sequencing with the molecular mechanism of the mTOR signaling pathway in hypoxic PASMCs. These analyses revealed hypoxia-induced miR-92b-3p as a potent regulator of the mTOR signaling pathway. We demonstrated that miR-92b-3p directly targets the 3' UTR of a negative regulator in the mTOR signaling pathway, TSC1. mTOR signaling and consequent cell proliferation were promoted by enforced expression of miR-92b-3p but inhibited by knocking down endogenous miR-92b-3p. Furthermore, inhibition of miR-92b-3p attenuated hypoxia-induced proliferation of vascular smooth muscle cells (VSMCs). Therefore, this study elucidates a novel role of miR-92b-3p as a hypoxamir in the regulation of the mTOR signaling pathway and the pathological VSMC proliferative response under hypoxia. These findings will help us better understand the

miRNA-mediated molecular mechanism of the proliferative response of hypoxic VSMCs through the mTOR signaling pathway.

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POSTER NUMBER: 2015

Molecular synergism between microRNAs and small molecules to accelerate neuronal reprogramming of human fibroblasts

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Neuronal microRNAs, miR-9/9*and miR-124 (miR-9/9*-124) exhibit reprogramming activities that when ectopically expressed, can directly convert human dermal fibroblasts to neurons. These microRNAs induce an extensive reconfiguration of the chromatin state by targeting multiple components of chromatin modifiers leading to a ground neuronal state permissive to transcription factors to guide the neuronal conversion to specific neuronal subtypes. An essential element of activating the neuronal program during reprogramming is the repression of the anti-neurogenic EZH2-REST axis, accounting for the opening of chromatin regions harboring REST binding sites. In fibroblasts, EZH2, the lysine methyltransferase, functions independently of Polycomb Repressive Complex 2 to directly methylate and stabilize REST, a transcriptional repressor of neuronal genes. Upon miR-9/9*-124 expression, miR-9 and miR-124 synergistically target and repress USP14, an ubiquitin-specific peptidase that stabilizes EZH2, leading to the consequent destabilization of REST. The repression of the EZH2-REST axis by miR-9/9*-124 is essential for opening the heterochromatin regions that contain neuronal genes encompassing REST binding sites. Further, miR-9/9*-124 were shown to repress DNA-methyltransferase *DNMT1* and *DNMT3B* mRNA levels, underlying the dramatic shift of genome-wide DNA methylation patterns during neuronal reprogramming. From these studies, we reasoned that employing small molecules in addition to miR-9/9*-124 to synergistically repress the methyltransferase activity of EZH2 and DNMTs would accelerate the neuronal reprogramming. We found that the combination of EZH2 and DNMT inhibitors drastically accelerated miR-9/9*-124-mediated conversion generating mature human neurons by two weeks of reprogramming. Our results highlight the synergism between microRNAs and small molecules targeting the same genetic pathway to facilitate and accelerate the adoption of the neuronal identity.

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POSTER NUMBER: 2013

Poly(A)-specific ribonuclease sculpts the 3′ ends of microRNAs

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The 3′ ends of metazoan microRNAs (miRNAs) are initially defined by the RNase III enzymes during maturation, but subsequently experience extensive modifications by several enzymatic activities. For example, terminal nucleotidyltransferases (TENTs) elongate miRNAs by adding one or a few nucleotides to their 3′ ends, which occasionally leads to differential regulation of miRNA stability or function. However, the catalytic entities that shorten miRNAs and the molecular consequences of such shortening are less well understood, especially in vertebrates. Here, we report that poly(A)-specific ribonuclease (PARN) sculpts the 3′ ends of miRNAs in human cells. By generating *PARN* knockout cells and characterizing their miRNAome, we demonstrate that PARN digests the 3′ extensions of miRNAs that are derived from the genome or attached by TENTs, thereby effectively reducing the length of miRNAs. Surprisingly, PARN-mediated shortening has little impact on miRNA stability, suggesting that this process likely operates to finalize miRNA maturation, rather than to initiate miRNA decay. PARN-mediated shortening is pervasive across most miRNAs and appears to be a conserved mechanism contributing to the 3′ end formation of vertebrate miRNAs. Our findings add miRNAs to the expanding list of non-coding RNAs whose 3′ end formation depends on PARN.

POSTER NUMBER: 2018

RNA Nanoparticle Based miRNA Modulation for Targeted Cancer Therapy

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Aberrant expression of certain microRNAs (miRNAs) has been implicated in cancers as a promising druggable target. Systemic administration of small RNAs to modulate therapeutically useful microRNAs for targeted treatment of cancers requires robust and efficient cancer cell-specific delivery platform without immunogenicity. Here we report newly emerged multivalent naked RNA nanoparticle (RNP), named pRNA-3WJ, based on pRNA 3-way-junction (3WJ) from bacteriophage phi29 to target glioblastoma cells through folate (FA) ligand and folate receptor mediated endocytosis and deliver microRNA inhibitors for tumor cell killing. Systemically injected FA-pRNA-3WJ RNPs efficiently reduced target gene expressions leading to improved survival rate in patient-derived glioblastoma xenograft mice model. Recently, we also showed that the siRNA-loaded pRNA-3WJ RNPs can be efficiently delivered into cancer cells in various patient-derived cancer xenograft models via cancer-specific aptamer coated exosomes. These studies demonstrated the therapeutic potential of RNP-based miRNA modulation, which can provoke an immediate translation into clinical trials for the development of safe and efficient targeted cancer therapy.

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POSTER NUMBER: 2017

Roles of Cancer-Associated Fibroblast-specific microRNAs in Invasion and Metastasis of Lung Cancer Cells

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Cancer-associated fibroblasts (CAFs) in cancer microenvironment are known to promote epithelial-mesenchymal transition, invasion, and metastasis of lung cancer cells. To investigate whether this CAF activity is controlled by microRNAs (miRNAs), we performed a miRNA profiling in mouse normal lung fibroblasts (LFs) or CAFs. Using a miRNA microarray, we identified miRNAs up-regulated (miR-34b, 34c, 196a, and 224) or down-regulated in CAFs (miR-182, 183, 96, 200a, and 200b), which were then overexpressed in LFs and CAFs, respectively. LFs transduced by lentivirus with miR-34b/c and miR-196a enhanced invasion and migration of lung cancer cells. On the other hand, CAFs transduced by lentivirus with miR-182, miR-183/96, and miR-200b/200a/429 lost their activities. We are now studying the downstream target genes of these miRNAs which mediate the activation of CAFs. Our results suggest that miRNAs can regulate CAF activity in cancer microenvironment and modulation of CAF-specific miRNAs can be a good strategy for suppression of lung cancer aggravation.

POSTER NUMBER: 1043

Short hairpin gene silencing of pyruvate dehydrogenase kinase attenuates the adipogenesis in 3T3L1

differentiation cell culture model

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Pyruvate dehydrogenase kinases (PDKs) regulate the activity of the mitochondrial pyruvate dehydrogenase (PDH) complex, a central metabolic node that catalyzes the conversion of pyruvate into acetyl-CoA at the expense of lactate formation. Our previous reports have shown that Pdk knockout mice are resistant to diet-induced obesity-associated metabolic dysfunction, indicating that metabolic remodeling resulting from induction of PDK may play a critical role in the development of metabolic diseases. Therefore, we investigated the specific role of PDK on both adipogenesis process using 3T3-L1 differentiation culture system. We found that mRNA expressions of Pdk 1 and 2 were dramatically induced in a time dependent manner, while Pdk 3 and 4 transcripts were not changed. Interestingly, PDK2 protein expression was evident from day 4 to day 8, and PDK1 expression was significantly increased over time. But, the other isozymes were consistently expressed during the time. Either PDK1 or PDK2 overexpression by retrovirus delivery up-regulated the adipogenic marker proteins including SREBP1c, FASN, C/EBPα and PPARγ. Likewise, short hairpin gene silencing of PDK1 and 2 resulted in the decrease in adipgenic markers. Consistent with the genetic manipulations of PDK, pharmacological ablation of PDK by dichloroacetate prevented the metabolic reprogramming indispensable for adipocyte differentiation, confirmed by Oil Red O staining. Taken together, PDK might play an important role in adipogenesis which has been considered for the therapeutic target in obesity related diseases.

POSTER NUMBER: 2042

Small Nuclear RNA Termination Factors are Required for piRNA Production in *Drosophila*

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In *Drosophila,* germline piRNAs derive from both strands of transposon-rich genomic clusters. Transcription of these "dual strand" clusters requires a fly-specific, heterochromatin protein 1 paralog, Rhino (Rhi), which binds the heterochromatin mark trimethylated lysine 9 on histone 3 and assembles an RNA polymerase II (RNA pol II) transcriptional initiation complex, bypassing the need for promoter sequences. Rhi also collaborates with the protein Cutoff (Cuff) to suppress splicing and CPSF-mediated cleavage and polyadenylation of cluster transcripts. Like piRNA precursor transcripts, the small nuclear RNAs (snRNA) transcribed by RNA pol II are also not polyadenylated, relying on alternative mechanisms to generate their 3′ ends. First, the nuclear exosome targeting complex (NEXT) has been proposed to trigger exosome-mediated snRNA termination. Ars2, a core component of the complex, links NEXT to the cap binding complex, promoting the exosome to process the 3′ ends of snRNAs. Second, the Integrator complex (INT), a homolog of CPSF, the protein complex that initiates standard polyadenylation of mRNAs, also acts in snRNA processing, traveling with RNA pol II until it cleaves the snRNA precursor, terminating transcription and preventing read-through into downstream genes. We tested the hypothesis that piRNA precursors and snRNAs share a common mechanism of 3′ processing. Here, we report that the components of both NEXT—Ars2, CG1677 (ZC3H18), Dis3, and Rrp6—and INT—Ints11—are indispensable for germline piRNA production and transposon silencing. Moreover, *cuff* mutants, which fail to produce piRNAs from dual-strand clusters, show reduced snRNA steady-state abundance. Our data suggest that piRNA and snRNA 3′ ends are produced by the same mechanisms.

POSTER NUMBER: 2020

Ribosomes guide initial piRNA processing on long single strand precursor RNAs

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Ribosomes translate open reading frames encoded in nucleic acids into proteins. They also bind to non-protein-coding regions, and they are involved in several non-translational roles in RNA metabolism. Here, we demonstrate that ribosomes guide the formation of piRNAs—a class of small non-coding RNAs essential for germline genome integrity and gamete production. We found that in mice, 80S ribosomes are recruited to the 5′-proximal AUG of long non-coding piRNA precursors. Ribosomes do not dissociate after traversing the short open reading frame(s) but continue into the 3´-UTRs, a process requiring the MOV10L1 RNA helicase. The bound ribosomes guide fragmentation of the piRNA precursor 3´-UTRs, with the processed ribosomal-protected-regions becoming piRNAs. Ribosome-guided piRNA biogenesis is also present in rooster, and occurs in the 3´-UTR of some mRNAs, suggesting that the mechanism is conserved and common. These results identify a function for ribosomes on non-coding regions of RNAs, and delineate the mechanism by which piRNA precursors are converted into piRNA sequences.

POSTER NUMBER: 2022

The effect of FTH1 and FTH1 pseudogenes in colorectal cancer progression

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Non-coding RNAs play a vital role in diverse cellular processes. Pseudogenes, which are non-coding homologs of protein-coding genes, were once considered non-functional evolutional relics. However, recent studies have shown that pseudogene transcripts can regulate their parental transcripts by sequestering shared microRNAs, thus acting as competing endogenous RNAs (ceRNAs). We have shown that the ferritin heavy chain 1 (FTH1) transcript and multiple FTH1 pseudogenes could be targeted by the same pool of oncogenic microRNAs in prostate cancer. We also demonstrated the role of the FTH1 pseudogenes as ceRNAs of FTH1 to maintain its expression and function as a tumor suppressor that is critical in maintaining iron balance and inhibiting cancer growth. We recently identified additional FTH1 pseudogenes that are downregulated in colorectal cancer. Preliminary data show that they exhibit tumor suppressive functions in colorectal cancer and could potentially regulate the expression and functions of FTH1 in colorectal cancer. Further investigations are underway to investigate the role of the FTH1 gene:pseudogene network in the development and progression of colon cancer.

POSTER NUMBER: 2043

CRISPR-Cas9 Screen of MicroRNA Function in Cell Fitness and Cell Fate: miR-151a in Cell Cycle Regulation

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MicroRNAs (miRNAs) play important roles in the control of cell fitness, differentiation, and development. We have constructed a miRNA-focused CRISPR-Cas9 library that targets 1,594 (85%) annotated human miRNA stem-loops (*Kurata and Lin, 2018, RNA*). The sgRNAs in our LX-miR library are designed to have high on-target and low off-target activity, and each miRNA is targeted by 4-5 sgRNAs. We used this sgRNA library to screen for miRNAs that affect cell fitness by monitoring the change in frequency of each sgRNA over time. By considering the expression in the tested cells and the dysregulation of the miRNAs in cancer specimens, we identified pro-fitness and anti-fitness microRNAs. Some of those miRNAs are known to be oncogenic or tumor-suppressive, but others are novel. We have also used this strategy to identify microRNAs that affect retinoic-acid induced cell fate changes in acute promyelocytic leukemia cells. These studies indicate that our LX-miR library (deposited to *addgene*) may be useful for genome-wide unbiased screening of various microRNA functions. A plan to build a sgRNA library focusing on mouse miRs is underway.

We have also investigated the cell fitness-regulating miRNAs identified in our screen through creating mutant cell clones with decreased miRNA expression. In miR-151a mutant cells, there was an increase in the G1-phase and the

increase in G1 corresponded to an increase in p53 (TP53) and p21 (CDKN1A) protein levels. We found that miR-151a-3p was able to directly suppress p53 expression, while the miR-151a-5p suppression of p53 was likely indirect. Interestingly, ectopic expression of miR-151a-5p significantly decreased the p53 and p21 protein levels as well as the percentage of cells in G1, while re-expression of miR-151a-3p only had a modest effect. These results suggest that both 5p and 3p of miR-151a are involved in p53/p21 regulation and responsible for the observed phenotypes. We also analyzed the TCGA database and discovered that increased miR-151a expression occurs in virtually all tumor types examined. In many cancers, there was an inverse correlation between miR-151a and p21 expression, and high miR-151a expression was often associated with poor overall survival. Taken together, this study identifies a previously underappreciated role of miR-151a in cancer through its regulation of the cell cycle.

POSTER NUMBER: 2023

Drosophila tsRNAs preferentially suppress general translation machinery via antisense pairing and participate in cellular starvation response

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Transfer RNA-derived small RNAs (tsRNAs) are an emerging class of small RNAs, yet their regulatory roles have not been well understood. Here we studied the molecular mechanisms and consequences of tsRNA-mediated regulation in *Drosophila*. By analyzing 495 public small RNA libraries, we demonstrate that most tsRNAs are conserved, prevalent and abundant in *Drosophila*. By carrying out mRNA sequencing and ribosome profiling of S2 cells transfected with single-stranded tsRNA mimics and mocks, we show that tsRNAs recognize target mRNAs through conserved complementary sequence matching and suppress target genes by translational inhibition. The target prediction suggests that tsRNAs preferentially suppress translation of the key components of the general translation machinery, which explains how tsRNAs inhibit the global mRNA translation. Serum starvation experiments confirm tsRNAs participate in cellular starvation responses by preferential targeting the ribosomal proteins and translational initiation or elongation factors. Knock-down of AGO2 in S2 cells under normal and starved conditions reveals a dependence of the tsRNA-mediated regulation on AGO2. We also validated the repressive effects of some representative tsRNAs on cellular global translation and specific targets with luciferase reporter assays. Our study suggests the tsRNA-mediated regulation might be crucial for the energy homeostasis and the metabolic adaptation in the cellular systems.

Reference:
Luo S[#], He F[#], Luo J[#], Dou S[#], Wang Y[#], Guo A, Lu J* (2018). *Drosophila* tsRNAs preferentially suppress general translation machinery via antisense pairing and participate in cellular starvation response. *Nucleic Acids Research*. 46(10):5250-5268.

POSTER NUMBER: 2024

Genome-wide Analysis of Small RNA-controlled Gene Networks in Maize Leaf Development

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In plants, stem cell niches serve as a stable source of cells for postembryonic growth and development. The shoot apical meristem (SAM) gives rise to all aerial organs of a plant, and its activity throughout the plant's lifetime therefore has to be tightly controlled in a spatiotemporal manner. To gain insight into gene regulatory networks behind stem cell maintenance and organogenesis, we generated a high-resolution gene expression atlas of 12

distinct domains within the vegetative maize shoot apex using laser microdissection and RNA deep sequencing. We also generated small RNA sequencing data that informs on the role of miRNAs in the maize shoot apex. Together these data reveal a subfunctionalization of miRNA family members across the SAM subdomains, and the regulation of miRNA accumulation in the stem cell containing SAM tip and vasculature. In addition, miRNA degradome sequencing data were produced, combined with information from the SAM atlas, we predict the presence of mechanisms that further fine-tune the accumulation and activity of select small RNAs to regulate key meristem genes.

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POSTER NUMBER: 2025

Profile of small non-coding RNAs and their machinery during Xenopus Laevis oocytes meiotic maturation

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Due to publication restrictions we cannot provide more information on this abstract. We will provide full detail at our poster session. We invite you to join us. Thank you.

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POSTER NUMBER: 2026

Correlations between miRNAs and their gene targets in bronchial epithelial cells differ between asthmatics with and without an asthmatic mother

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Asthma is a common chronic inflammatory disease of the airways, affecting over 230 million people worldwide. One of the strongest risk factors for childhood onset asthma is maternal asthma. One possible explanation for this maternal risk may be altered in utero epigenetic programming. In particular, miRNA-mediated gene regulation has been suggested as a possible mechanism for the maternal effect on asthma risk in her children. We performed the first genome-wide analysis of miRNAs using miRNA-sequencing in freshly isolated bronchial epithelial cells (BECs) from 83 adults with asthma (cases) and 43 adults without asthma (controls). Annotation and quantification of miRNAs were performed using miRge2.0. Raw read counts were transformed to log2 counts per million and then quantile normalized. Technical sources of variation were identified and predicted using principal components analysis and surrogate variable analysis, respectively. Twenty of the 350 miRNAs that passed QC were differentially expressed (DE) between cases and controls (limma; FDR < 1%). Bioinformatic prediction (TargetScanHuman 7.2) of the 20 DE miRNAs (FDR <1%) yielded 10,242 unique mRNA gene targets. 218 of the target genes (RNA-seq) were also DE (73 cases, 38 controls; FDR < 1%; 892 miRNA-mRNA pairs). Two miRNAs, let-7c-3p and 106b-3p, were correlated with the expression of 13 and 2 DE mRNA targets, respectively, in the full sample (n = 111). Surprisingly, all 13 let-7c-3p and 2 106b-3p miRNA-mRNA pairs were more significantly or only correlated in cases without a mother with asthma (n = 44) compared to cases with a mother with asthma (n = 27). In contrast, none of the 15 miRNA-mRNA correlations were significant in all cases combined (n = 73) or in controls (n = 38). Overall, these results revealed dysregulation of miRNA-mediated gene regulation in BECs from adult cases that differ between those with and without an asthmatic mother, suggesting that binding of miRNAs to their mRNA targets is altered in a maternal asthma in utero environment. Supported by U19 AI095230. KMM is supported by F31 HL143891.

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POSTER NUMBER: 2027

Converging on Drosha in Stress Response and Neurodegenerative Diseases

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MicroRNAs (miRNAs) regulate the translational potential of their mRNA targets and control numerous cellular processes. Dysregulation of this network either at individual miRNA or pathway level has been linked to significant changes in numerous key molecular signals and reported to underlie many pathological processes. The key step in canonical miRNA biogenesis is the cleavage of the primary transcripts by the nuclear RNase III enzyme Drosha. Emerging evidence suggests that the miRNA biogenic cascade is tightly controlled to maintain proper cellular homeostasis. However, how Drosha itself is regulated remains to be illustrated. Our recent study uncovers an important mechanism by which cellular stress signals to inhibit Drosha function and therefore reduces miRNA biogenesis at global level. This process involves a phosphorylation-dependent modulation of Drosha stability and activity. Our new studies and a series of unpublished findings reveal that Drosha is a converging regulatory point targeted by multi signals. Signals can either enhance or inhibit Drosha, which involves multi mechanisms including novel modifications and modulation of its affinity with key partners. Furthermore, our data suggest that Drosha is sensitive to various pathologic stress conditions. Inhibiting Drosha may represent a common and important mechanism underlying neuronal vulnerability under pathologic stress and play a role in the pathogenic process of acute neuronal injury as well as chronic neurodegeneration including both Alzheimer's and Parkinson's diseases.

POSTER NUMBER: 2028

Analysis of silencing domain of GW182 in *Drosophila melanogaster*

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MicroRNAs (miRNAs) direct mRNA decay and translational repression of their complementary targets via RNA-induced silencing complex (RISC) containing AGO subfamily proteins. In *Drosophila melanogaster*, miRNAs predominantly act through Ago1, one of the two fly AGO proteins. Fly Ago1-RISC mediates mRNA decay via the scaffold protein GW182. GW182 directly binds Ago1-RISC via its N-terminal region and further recruits the CCR4-NOT deadenylase complex mainly through its C-terminal region called the “silencing domain.” Although the molecular interactions between Argonaute, GW182 and the CCR4-NOT complex have been well studied in recent years, their functional importance in the context of miRNA-mediated gene regulation during animal development remains largely unexplored. This is mostly because the fly GW182 gene locates in the 4th chromosome, which is extremely challenging to manipulate by classical genetics.
Here, we describe the generation of *gw182*-null mutant flies by the CRISPR-Cas9 system. *gw182*-null flies are lethal at an early point of the 2nd instar larval stage. Unexpectedly, this larval lethality can be largely rescued by expressing a large C-terminal truncation mutant of GW182 that lacks the entire silencing domain. On the other hand, the intact silencing domain is required for flies to further develop into the adult stage. These results together suggest that the functional requirement of GW182 and its interacting proteins is surprisingly variable during fly development.

POSTER NUMBER: 2029

Growth Regulatory miRNAs in neuronal function

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Growth regulatory miRNAs, including the Let-7 miRNA family, have evolutionarily conserved roles in mediating post-transcriptional control of pro-growth mRNAs. Functions of the Let-7 family have been extensively characterized during development, pluripotency, and in oncogenesis. Recent work indicates that Let-7 miRNAs can also undergo signal-responsive regulation in healthy adult tissues, such as the nervous system. Research in our lab has demonstrated that a MAPK-dependent induction of the Lin28 RNA binding protein from low basal levels can occur in response to neurotrophin and excitatory activity in mammalian neurons. Lin28 induction lowers Let-7 family miRNA levels and promotes enhanced protein synthesis from pro-growth plasticity-related mRNAs, allowing

growth of neuronal processes and synaptic connections. At the molecular level, defects in neuronal protein synthesis are implicated in the cellular, synaptic, and cognitive features of the neurodevelopmental disorder, autism spectrum disorder (ASD). We observe aberrant control of Let-7 family miRNAs in mice carrying ASD-linked mutations and shed light on the molecular mechanism of disrupted miRNA regulation. Bioinformatics analyses of human data are used to test the hypothesis that a chronic loss of appropriate gene target specificity in protein synthesis, by dysregulation of growth regulatory miRNAs, could occur in the context of diverse genetic mutations associated with the neurodevelopmental disorder ASD.

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POSTER NUMBER: 2030

Extracellular vesicles in culture medium of *Tribolium castaneum* TcA cells can spread the RNA interference response

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The potential utility of RNA interference (RNAi) to control insect pests and viral infections depends largely on the target organism's ability to systemically spread the RNAi response. The efficacy of systemic RNAi varies among insects; the red flour beetle, *Tribolium castaneum* has been shown to be highly responsive to injected long dsRNA. We identified an extracellular RNAi signal that is present in the culture medium of *T. castaneum* (TcA) cells after treatment with long dsRNA coding for a luciferase reporter gene. Small RNA sequencing showed the presence of luciferase-specific siRNAs in extracellular vesicles (EVs) that were purified from the cell culture medium. These EVs have also been shown to deliver their cargo of small RNAs and to induce cellular responses. Indeed, by measuring the silencing of luciferase expression, we observed that the siRNA-containing EVs can act as an RNAi signal for recipient TcA cells. We have therefore shown that a systemic RNAi response upon dsRNA treatment can be spread through EVs.

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POSTER NUMBER: 1044

FilTar: A cell type specific miRNA target prediction pipeline incorporating transcriptomic data to reannotate 3' UTRs and filter non-expressed targets

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miRNA-mediated repression is a dynamic process requiring both the miRNA and the miRNA response element (MRE) to come into physical contact in the cell for regulation to occur. Typically, researchers are interested in the function of a particular miRNA in a specific cell or tissue of interest. However, current computational target prediction algorithms will return every possible target genome-wide as they have no knowledge of which genes are expressed in a particular tissue or cell line. It is also well known that 3' UTRs are not static entities and that alternative cleavage and polyadenylation is an important process that can give rise to cell type specific 3' UTRs that are either truncated or elongated when compared with the standard database annotations.

In order to address these issues and provide researchers with a tool that can be used to give more specific and accurate target predictions in their study system of interest, we have created a novel computational pipeline, FilTar (https://github.com/TBradley27/FilTar). FilTar incorporates transcriptomic data from RNA-Seq experiments as input to the tool and using these data will remove any predicted targets that do not have evidence of expression in a particular sample of interest. In addition, FilTar will truncate or elongate default Ensembl 3' UTR annotations based on read coverage from RNA-Seq data, thus helping to remove false positive targets from truncated 3' UTRs and identifying additional novel MREs in 3' UTR extensions. Tests on miRNA mimic and antagomiR transfection experiments from multiple human cell lines show significant improvements in accuracy versus initial unfiltered TargetScan results.

POSTER NUMBER: 2031

Nuclear RNA export factor variant triggers Piwi-piRNA-mediated co-transcriptional silencing

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piRNAs are a class of small noncoding RNAs that associate with PIWI proteins and guide piRNA-induced silencing complexes (piRISCs) to target and repress TEs for the maintenance of genomic integrity. Among three PIWI proteins expressed in the *Drosophila* ovary, only Piwi is located in the nucleus of both germline cells and surrounding somatic cells. Within the nucleus, Piwi-piRISCs repress TEs transcriptionally, by spreading H3K9me3 marks and recruiting the linker histone H1 to form heterochromatin. Among factors identified as necessary for the effector phase of Piwi-mediated silencing, Panoramix (Panx; also known as Silencio) plays an essential role downstream of Piwi. Panx interacts with Piwi and promotes the deposition of H3K9me3 marks on target TE chromatin. However, it is unclear how Panx promotes the silencing of target TEs through the association with Piwi-piRISCs. Here, we identified Nxf2, a member of the nuclear RNA export factor (NXF) family highly expressed in the ovary, as a protein that forms a complex with Panx and Piwi. Nxf2 further associates with p15 (Nxt1), a co-adaptor for nuclear RNA export. Unlike Nxf1, the other NXF variant that plays a major role in mRNA export, Nxf2-p15 instead transcriptionally regulates TEs in the Piwi-piRNA pathway and also stabilises the protein level of Panx. The LRR domain of Nxf2 harbouring RNA binding activity is essential for recruitment of the Piwi-piRISC complex to target TEs. Notably, ectopic targeting of Nxf2 could initiate co-transcriptional repression of the target reporter gene in a manner independent of H3K9me3 marks or H1. These results suggest that Nxf2 is required to enforce the association of Piwi-piRISC complexes with the nascent transcript of target TEs and trigger co-transcriptional repression, prior to heterochromatin formation in the nuclear silencing pathway.

POSTER NUMBER: 3040

Investigating the Role of Small RNAs in the Replication and Pathogenesis of Mouse Mammary Tumor Virus (MMTV)

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The seminal role played by small RNAs in the regulation of gene expression, normal cell growth/development, and pathogenesis has propelled these miniscule molecules at the forefront of research. In particular, we are studying the involvement of microRNAs (miRNAs) in the replication and pathogenesis of mouse mammary tumor virus (MMTV), a retrovirus that causes breast cancer in mice. Our recent work has shown that MMTV disrupts the expression of host miRNAs, specifically the oncogenic miR-17-92 cluster and several others, during the process of infection and tumorigenesis. Data will be presented revealing dysregulation in the expression of a number of host genes in both MMTV-infected cells and MMTV-induced tumors that have important roles in cell proliferation, differentiation, cell death, angiogenesis, etc. Interestingly, the pattern of dysregulation of host gene expression is similar in the infected cells and tumors, which suggests that MMTV infection itself predisposes the cells to oncogenic transformation. Considering that MMTV-induced tumors have similarity with human breast tumors and MMTV-like sequences are being detected in human patients with breast cancer, these results have relevance to human breast cancer as well.

POSTER NUMBER: 2032

UPF1/SMG7-dependent MicroRNA-mediated Gene Regulation

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The stability and quality of metazoan mRNAs are under microRNA (miRNA)-mediated control and nonsense-mediated control, respectively. Although UPF1, a core mediator of nonsense-mediated mRNA decay (NMD), mediates the decay of target mRNA in a 3′ untranslated region (UTR)-length-dependent manner, the detailed mechanism remains unclear. Here, we suggest that 3′UTR-length-dependent mRNA decay is not mediated by nonsense mRNAs but rather by miRNAs that downregulate target mRNAs via Ago-associated UPF1/SMG7. Global analyses of mRNAs in response to UPF1 RNA interference in miRNA-deficient cells reveal that 3′UTR-length-dependent mRNA decay by UPF1 requires canonical miRNA targeting via the CUG motif. The repression of miRNA targets is additively or synergistically accomplished by the combination of Ago2 and UPF1 through UPF1-associated SMG7, which may recruit the NOT1/3 deadenylase complex in a TNRC6-independent manner. Indeed, the loss in the SMG7-deadenylase complex interaction increased the level of transcripts regulated by UPF1-SMG7. This new miRNA-mediated mRNA decay pathway may enable miRNA targeting to become more predictable and expand the miRNA-mRNA regulatory network.

POSTER NUMBER: 2033

MiR-139-3p is a negative regulator of MYC in colorectal cancer

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Elevated *MYC* transcript levels in colorectal cancer (CRC) patients highlight the importance of MYC regulation in CRC development. Research on post-transcriptional regulation showed that microRNAs are important regulators of transcript expression and could potentially drive cancer development. I identified miR-139-3p as a negative regulator of MYC expression via its targeting of the *MYC* CDS. Overexpression of miR-139-3p decreased MYC transcript and protein levels. Furthermore, biochemical analysis by dual-luciferase reporter assay confirmed the specific regulation of miR-139-3p on the *MYC* CDS to negatively affect MYC expression. Most importantly, upregulation of miR-139-3p expression decreased CRC cell line growth. The tumor suppressive property of miR-139-3p can potentially be used for microRNA replacement therapy to suppress the elevated MYC expression in CRC.

POSTER NUMBER: 2035

Association of single nucleotide polymorphisms in miR-146a, miR-196a2, miR-608, and miR-17HG with breast cancer susceptibility in a Vietnamese population

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Background
MicroRNAs (miRNAs) are a novel class of endogenous, non-coding, single-stranded RNAs capable of regulating gene expression by suppressing translation or degrading mRNAs. Single nucleotide polymorphisms of the genes coding miRNA (miRNA SNP) can alter miRNA expression, resulting in diverse functional consequences. Previous studies have examined the association of miRNA SNPs with breast cancer (BC) susceptibility. This study explored the association between eleven miRNA SNPs (MIR27A rs895819, MIR146A rs2910164, MIR423 rs6505162, MIR196A2 rs11614913, MIR605 rs2043556, MIR608 rs4919510, MIR145 rs353291, MIR101 rs1053872, MIR618 rs2682818, MIR221/222 rs34678647, and MIR17HG rs4284505) and BC risk in a Vietnamese population.

Methods
The SNPs were genotyped in 300 breast cancer cases and 300 controls. The Real-time PCR High Resolution Melting and Tetra-Arms-PCR method were used to analyse all miRNA SNPs genotype. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to estimate associations.

Results and Discussion
The rs2043556 is monomorphic in the Vietnamese population. The rs895819, rs6505162, rs353291, rs1053872, rs2682818, and rs34678647 were not related to breast cancer risk ($P > 0.05$). The MIR196A2 rs11614913-C allele, MIR17HG rs4284505-A allele, MIR146A rs2910164-CG genotype, and MIR608 rs4919510-CG genotype were associated

with an up to 2-fold increased risk of breast cancer (P < 0.05). The combination of genotyping and microRNA profiles will be able to significantly differentiate individuals in the risk group. Therefore, these miRNA SNPs could be considered as potential markers for breast cancer predicting and early prognosis in population of Vietnamese.

Conclusion
Our results suggest that MIR196A2 rs11614913, MIR17HG rs4284505, MIR146A rs2910164, and MIR608 rs4919510 likely contribute to increased susceptibility to breast cancer. Furthermore, studies with larger sample size are still necessary to further elucidate the association between miRNA SNPs and cancers risk.

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POSTER NUMBER: 2034

Nicking activity: A novel activity of human Microprocessor

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The human Microprocessor complex cleaves primary microRNA (miRNA) transcripts (pri-miRNAs) to initiate miRNA synthesis. The Microprocessor consists of DROSHA, an RNase III enzyme, and DGCR8. The two conserved RNase III domains of DROSHA make double cuts on either strand of pri-miRNAs. In this study, we show that the Microprocessor has an unexpected nicking activity that creates a single cut on only one of the pri-miRNA's strands using one of the two DROSHA's RNase III domains. This cleavage does not lead to miRNA production and thus downregulates miRNA expression. Furthermore, we observed certain RNA elements to facilitate the nicking activity, and by manipulating these elements, we could regulate the ratio of nicking to double-cut activities, thus controlling miRNA production both in vitro and in vivo. The nicking activity implicates a novel level of regulation of miRNA expression and offers a new approach to miRNA knockdown.

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POSTER NUMBER: 2036

Novel players regulate pri-miRNA processing

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The human Microprocessor complex cleaves primary microRNA (miRNA) transcripts (pri-miRNAs) to initiate miRNA synthesis. The Microprocessor consists of DROSHA, an RNase III enzyme, and DGCR8. The two conserved RNase III domains of DROSHA make double cuts on either strand of pri-miRNAs. Here, we show that the Microprocessor has an unexpected nicking activity that creates a single cut on only one of the pri-miRNA's strands using one of the two DROSHA's RNase III domains. This cleavage does not lead to miRNA production and thus downregulates miRNA expression. Furthermore, we observed certain RNA elements to facilitate the nicking activity, and by manipulating these elements, we could regulate the ratio of nicking to double-cut activities, thus controlling miRNA production both *in vitro* and *in vivo*. We also observe new RNA elements that control the efficiency and accuracy of the Microprocessor activity. The findings implicate novel levels of regulation of miRNA expression and offer a new approach to miRNA knockdown.

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POSTER NUMBER: 2037

MicroRNA 760 regulates the expression of Atxn1 via interaction with its 5'untranslated region

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MicroRNAs (miRNAs) are small non-coding RNAs known to function as posttranscriptional regulators of gene expression. For protein-coding transcripts, miRNAs are usually thought to regulate target gene expression via binding to the 3'untranslated region (3'UTR) thereby inducing RNA degradation and/or translational repression. Interestingly, studies also suggest the potential binding of miRNAs to the 5'untranslated region (5'UTR) and the coding sequence (CDS). So far only few examples exist whereby the miRNA indeed down-regulates the expression of its target gene via binding to the 5'UTR. Here we describe the 5'UTR-mediated regulation of Ataxin-1 (Atxn1), a dosage sensitive gene whose increase in protein stability is known to cause the neurodegenerative disease Spinocerebellar Ataxia Type 1 (SCA1). Atxn1's mRNA contains an intriguingly long 5'UTR, which consists of 8 exons that span over 400kbp. Generally speaking, 5'UTRs are commonly much shorter and less complex, indicating a potential important regulatory role. Recent work from our lab showed that Atxn1 expression is indeed negatively regulated via its 5'UTR. Using the miRNA prediction tool miRdB, we identified multiple miRNAs with the potential to regulate Atxn1's expression via the binding to different regions of its 5'UTR. We tested the effect of the identified miRNAs on Atxn1 expression *in vitro* by transiently transfecting miRNA mimics and miRNA inhibitors in cell culture. From our initial analysis, we identified miR760 as a negative regulator of Atxn1 expression. We discovered that MiR760 can bind and decrease Atxn1's RNA levels in an Argonaute 2 dependent manner. We further confirmed that miR760 indeed regulates Atxn1 expression via interaction with its 5'UTR and identified its exact binding site. Given the importance of Atxn1 levels in SCA1, we will next deliver miR760 virally into the brain of an SCA1 mouse model as well as to iPSC-derived neurons from SCA1 patients to reduce Atxn1 levels and investigate miR760's effect on SCA1 pathogenesis. Taken together, this work will not only provide insight into the regulation of gene expression via 5'UTR binding of miRNAs, but also shed light on the regulatory mechanisms of Atxn1 expression in hope to uncover novel risk factors and therapeutic entry points for SCA1.

POSTER NUMBER: 3001

HITS-CLIP: Elucidating the MiRNA Regulatory Network in Adipose Tissue

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Adipose tissue is a complex organ that engages in a variety of metabolic processes, including energy storage, non-shivering thermogenesis, and the secretion of signaling factors. Much of the regulation of these pathways occurs post-transcriptionally by microRNAs (miRNAs). In this study, we utilized high-throughput sequencing of RNA isolated by cross-linking immunoprecipitation (HITS-CLIP) on brown adipose tissue (BAT) and white adipose tissue (WAT) from C57BL/6J mice. Immunoprecipitated RNA from cross-linked ago-RNA complexes, containing both mature miRNA and miRNA targets, was purified and sequenced. Mapping of the sequenced fragments to the genome resulted in 411 unique ago-associated mature miRNAs and 6738 unique ago-associated miRNA targets. The top 8 miRNA families accounted for 82% of all ago-associated miRNA in adipose, consistent with our data from total miRNA sequencing. Among miRNA targets, 87% mapped to protein coding regions with a majority targeting 3'-UTRs. Those targets most enriched in BAT include the well-characterized thermogenic uncoupling protein 1 (UCP1), while those most enriched in WAT include the circulating hormone leptin. Our data identify several miRNA regulators of UCP1, leptin, and hundreds of other transcripts. As the first reported use of an ago-CLIP sequencing approach in adipose tissue, this study provides a wealth of information on previously undiscovered miRNA regulatory interactions.

POSTER NUMBER: 3002

Loss of germ granule integrity during the oocyte-to-embryo transition disrupts small RNA homeostasis in *Caenorhabditis elegans*

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Germ granules are RNA granules conserved in the germlines of metazoans that contain many components of the small RNA (smRNA) machinery (Updike and Strome, 2010). In *C. elegans*, germ granules are inherited from mother to progeny by a process that requires the intrinsically-disordered proteins MEG-3 and MEG-4 (Wang et al., 2014). In *meg-3/4* mutants, germ granules disassemble in early embryos causing release of their contents into the cytoplasm. Surprisingly, *meg-3/4* mutants also display an RNAi-defective phenotype that is gradually acquired over several

generations. High-throughput smRNA sequencing of *meg-3/4* mutants has revealed misregulation of hundreds of smRNAs, including a striking eight-fold upregulation of endo-siRNAs targeting a known component of the RNAi machinery, *rde-11* (Yang et al., 2012; Zhang et al., 2012). In wild-type animals, *rde-11* transcripts are targeted heavily by piRNAs but only modestly by endo-siRNAs. In *meg-3/4* mutants, the entire *rde-11* operon is targeted by endo-siRNAs, and *rde-11* mRNA transcript levels are greatly reduced. We have found that *rde-11* silencing, upregulation of endo-siRNAs, and the RNAi defect of *meg-3/4* mutants are all suppressed by mutations in *hrde-1*, a nuclear argonaute required for trans-generational inheritance of smRNAs (Buckley et al., 2012). These results suggest that the RNAi-defective phenotype of *meg-3/4* mutants is due to inappropriate amplification of piRNA-primed, endo-siRNAs at the *rde-11* locus. We propose that germ granules function as an isolating compartment in early embryos to prevent run-away amplification of endo-siRNAs by the trans-generational small RNA machinery.

Buckley et al., Nature. 2012; Updike and Strome, J Androl. 2010; Wang et al., eLife. 2014; Yang et al., Genes Dev. 2012; Zhang et al., Curr Biol. 2012

Funding: NIH, HHMI

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POSTER NUMBER: 3003

Epigenetic suppression of microRNA-708 to promote breast cancer progression and metastasis

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Breast cancer is the second leading cause of cancer death in women and cancer metastasis are accountable for almost 90% of related deaths. To date, there is no targeted therapy available in treating breast cancer metastasis. Previous study indicated that glucocorticoids (GCs) suppress cancer metastasis in ovarian cancer through induction of miR-708. Here we propose to investigate the therapeutic potential of GCs in treating metastatic breast cancer. We observed that miR-708 promoter region that is epigenetically silenced by both DNA methylation and histone methylation among metastatic breast cancer cells. Co-treatment of GCs, together with DNA-demethylation agents, Decitabine, significantly increased expression of miR-708, leading to the synergistic suppression of tumor growth. Clinical analysis further revealed that miR-708 and EZH2, the histone methyltransferase (HMT), was inversely correlated in breast tumor samples. Overall, our data reveal a therapeutic strategy of using GCs together with epigenetic drug in suppression of breast cancer progression and metastasis.

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POSTER NUMBER: 3004

Targeting NRAS by MicroRNA-708 in Cancers with NRAS mutation

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NRAS, a well-known oncogene belongs to the Ras family, plays important roles in cell proliferation, differentiation and apoptosis of cells. Mutation of NRAS at codons 12, 13 and 61 has been reported as leading causes to several cancer types, including melanoma (15-20%), leukemia (10%), lung cancer, colorectal cancer and thyroid carcinoma. However, a precise strategy targeting cancers with NRAS-mutation has not yet been established. Here we identified NRAS as a novel target of miRNA-708. Both mRNA and protein were depleted in the presence of miRNA-708 in NRAS-mutated melanoma cell line, SK-MEL-2. Treatment of synthetic glucocorticoids (GCs), which previously proven to mediate miRNA-708 induction, downregulated both NRAS mRNA and protein levels. We proposed to prove that NRAS is the direct target of miRNA-708 by 3'UTR luciferase assay. We will also try to investigate the underlying mechanism by analyzing the activities of NRAS-downstream effectors, including Raf-MEK-ERK cascade or PI3K-AKT-mTOR pathway. By using cell lines SK-MEL-2, THP-1 and H1299, those of which were derived from the NRAS mutated melanoma, leukemia, and lung cancer, respectively, we propose to evaluate the therapeutic potential of GCs or miRNA-708 in cancers with NRAS mutation. Overall, our study will provide a new aspect of whether the combination of synthetic GCs with other drugs, such as MAPK inhibitors, can efficiently control the

progression of cancers with NRAS mutation.

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POSTER NUMBER: 3007

Dysregulation in microprocessor processing of a primary miRNA, mir-21, leads to downregulation of a tumor suppressor, GHR, progressing hepatocellular carcinoma

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Improper miRNA biogenesis produces the 5' variants of miRNA with a shifted seed region (also called isomiRs). Although the presence of stably abundant isomiRs were repeatedly reported, it is still unclear whether the generation of isomiRs results either from the mistake of microprocessor or from the regulation by other intrinsic or extrinsic factors, and whether the isomiRs participate in critical cellular processes, particularly in cancer progress. Here, we performed both high-throughput RNA sequencing (RNA-seq) and small RNA sequencing (sRNA-seq) from 75 hepatocellular carcinoma (HCC) and normal samples (fresh frozen) of Korean HCC cohort. Combined analysis with other publicly available sequencing data, showed that although the expression signatures of isomiRs largely follow those of canonical ones, the relative abundance of some isomiRs are dynamically changed over cancer progression, forming new isomiR-target interactions. Particularly, the biogenesis of highly abundant miR-21 is dysregulated by interaction with HNRNPC to primary mir-21 during cancer progress, producing relatively more isomiR-21-5p in HCC. Introducing isomiR-21-5p mimic or antagomir against isomiR-21-5p, and the dysregulation of HNRNPC expression perturbed the relative abundance of isomiR-21-5p, leading to dysregulation of miRNA targeting on a tumor suppressor gene, GHR, and affecting cancer progress. Our study not only describes that isomiR-21-5p is an independent prognostic marker and a potential therapeutic target of HCC but also presents that the production of isomiRs is tightly regulated by other extrinsic factors in cells.

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POSTER NUMBER: 3006

siAbasic: ascertaining potent siRNA-6Ø sequences without off-target effects

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Small interfering RNA (siRNA) is widely used to specifically silence target gene expression, but its microRNA (miRNA)-like function inevitably suppresses hundreds of off-targets. Recently, complete elimination of the off-target repression has been achieved by introducing an abasic nucleotide to the pivot (position 6; siRNA-6Ø), of which impaired base pairing destabilizes transitional nucleation (positions 2-6). However, siRNA-6Ø varied in its conservation of on-target activity (~80%-100%), demanding bioinformatics to discover the principles underlying its on-target efficiency. Analyses of miRNA-target interactions (Ago HITS-CLIP) showed that the stability of transitional nucleation correlated with the target affinity of RNA interference. Furthermore, interrogated analyses of siRNA screening efficiency, experimental data, and broadly conserved miRNA sequences showed that the free energy of transitional nucleation (positions 2-5) in siRNA-6Ø required the range of stability for effective on-target activity ($-6 \leq \Delta G[2:5] \leq -3.5$ kcal mol^{-1}). Taking into consideration of these features together with locations, GC content, nucleotide stretches, single nucleotide polymorphisms and repetitive elements, we implemented a database named “siAbasic” (http://clip.korea.ac.kr/siabasic/), which provided the list of potent siRNA-6Ø sequences for most of human and mouse genes (≥ ~95%), wherein we experimentally validated some of their therapeutic potency. siAbasic will aid to ensure potency of siRNA-6Ø sequences without concerning off-target effects for experimental and clinical purposes.

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POSTER NUMBER: 3008

The role of small regulatory RNAs in rice epigenetic regulation

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Epigenetic regulation has been implicated in plant development and stress responses. The underlying mechanisms of epigenetic regulation include DNA methylation, histone modification, and non-coding RNA-mediated regulation of gene expression. Of these, non-coding small RNAs, including microRNAs and small interfering RNAs, play a crucial role in negative regulation of gene expression at both transcriptional and posttranscriptional levels. microRNA820 is a small RNA produced from transcripts originated from a region inside CACTA DNA transposons in rice. It targets *OsDRM2*, which is involved in *de novo* DNA methylation of CG and non-CG sequences in the rice genome through a RNA-dependent DNA methylation mechanism to suppress transposon activity. Interestingly, both miR820 and *OsDRM2* are down-regulated by drought stress treatment. To explore the function of miR820, transgenic rice plants over-expressing miR820 was generated. The transgenic plants exhibited drought-resistant phenotype compared with wild type plants. In addition, several transposable elements, including RIRE7, CACTA and Tos17, were up-regulated in these transgenic plants. We also confirmed that those transposons were less-methylated in the miR820 over-expressing plants. These results might be due to down-regulation of *OsDRM2*, which is responsible for the suppression of those transposable elements. Possible roles of these epigenetic regulation by miR820 and OsDRM2 as well as their agricultural impacts on drought stress resistance will be discussed.

POSTER NUMBER: 2044

Deciphering the role of miRNAs during mouse embryonic development

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MicroRNAs (miRNAs) are small RNA species that play a critical role as the post-transcriptional regulators of the genome. As part of ENCODE consortium, we collected miRNA expression profiles of multiple tissues at 7 embryonic time points as well as p0 and found 581-687 miRNAs expressed in each tissue. 48% of the top expressed miRNAs are tissue specific in at least one of the developmental stages and/or increase in their tissue specificity as the embryo develops. We utilized the dynamics of miRNA expression in order to cluster the miRNAs in a functionally meaningful manner. We compiled a list of predicted targets for each miRNA cluster and looked for mRNA clusters that are enriched for these targets. Finally, by considering both the correlation and enrichment analyses, we identified the miRNA and mRNA cluster that interact with each other and found that some miRNA clusters have a predicted role in down-regulation the expression of developmentally important genes in tissues outside their expected specific tissue during embryonic development.

POSTER NUMBER: 3009

***In vitro* recapitulation of the secondary siRNA biogenesis in plants**

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In many eukaryotes including worms, fungi and plants, RNA-dependent RNA polymerases (RDRs) convert targets of small RNAs into double-stranded forms to generate secondary small interfering RNAs (siRNAs). This so called “siRNA amplification pathway” is known to play important roles not only in suppression of selfish entities but also in regulation of gene expression. However, the molecular mechanisms by which RDRs find the templates and synthesize the complementary strands are poorly understood. This has been partly due to the lack of a biochemical framework for studying siRNA amplification pathways.

Here, by using a plant cell-free system, we recapitulated an endogenous secondary siRNA biogenesis pathway

called “trans-acting siRNA biogenesis pathway” in vitro. We show that a combination of 1) specialized RNA-induced silencing complexes, 2) the double-stranded RNA binding protein SGS3, and 3) SDE5 with an unknown function, is required for recruitment of RDR6 to the template RNAs. Furthermore, we demonstrate that removal of the poly(A) tail from the template RNAs greatly enhances the efficiency of complementary RNA synthesis by RDR6. Taken all together, our new in vitro system provides a mechanistic basis for RDR-mediated secondary siRNA biogenesis in plants.

POSTER NUMBER: 3010

Aging, Adipose function, and small non-coding RNA editing

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Adipose dysfunction with aging alters insulin sensitivity and increases risk to insulin resistance. We previously showed functional changes in microRNAs involved in adipocyte differentiation with aging. There was a loss of pre-adipocyte differentiation during aging leading to adipose dysfunction. The mechanisms involved in the functional changes in adipose microRNAs during aging is not well understood. We investigated the differential expression of both the genes involved in microRNA biogenesis as well as its editing in the adipose tissue during aging.

Metabolic changes, and adipocyte dysfunction was studied in Fisher 344 x Brown Norway hybrid rats (FBN) at ages 3mo, 6mo, 15mo, 25mo and 30mo (male and females, n=4-7). Fat red-O staining was performed for measuring adipocyte differentiation. Circulating levels of lipid profile, glucose and insulin were determined using Cholestech kits or ELISAs. qPCR was used to measure the mRNA expression of biogenesis and editing genes in the peri-gonadal fat. Sanger sequencing followed by alignment using Bio-Edit was used to determine microRNA editing with aging. We observed significant increases in body weights and fat mass of both male and female rats with aging. The increase was observed in both sexes around 15 mo of age. Blood triglyceride increased significantly around 15 mo in male rats but not much increase was observed in females until the age of 30 mo. Blood glucose levels increased with age with a slight dip at 30 mo. However, insulin levels in both the sexes dropped significantly around 15 mo of age. A decrease in pre-adipocyte differentiation as seen by Fat-red-O staining was observed with age in both sexes. Differential expression of adipose tissue microRNAs involved in differentiation was altered with aging. In males, the levels of DROSHA and DICER involved in microRNA biogenesis decreased but editing enzymes increased with age. A possible increase in splice variants of the ADARs were observed with aging which alters the editing function with age.
We conclude that the increased splice variants of ADARs with age might be contributing to the differential expression or adipoMiRs leading to adipose dysfunction and insulin resistance with aging.

POSTER NUMBER: 3011

Argonaute-miRNA Complexes Silence Target mRNAs in the Nucleus of Mammalian Stem Cells

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In mammals, gene silencing by the RNA-induced silencing complex (RISC) is a well-understood cytoplasmic posttranscriptional gene regulatory mechanism. Here, we show that embryonic stem cells (ESCs) contain high levels of nuclear AGO proteins and that in ESCs nuclear AGO protein activity allows for the onset of differentiation. In the nucleus, AGO proteins interact with core RISC components, including the TNRC6 proteins and the CCR4-NOT deadenylase complex. In contrast to cytoplasmic miRNA-mediated gene silencing that mainly operates on cis -acting elements in mRNA 3' untranslated (UTR) sequences, in the nucleus AGO binding in the coding sequence and potentially introns also contributed to post-transcriptional gene silencing. Thus, nuclear localization of AGO proteins in specific cell types leads to a previously unappreciated expansion of the miRNA-regulated transcriptome.

POSTER NUMBER: 3012

Egret, a novel factor required for producing piRNAs from the dual-strand clusters in Drosophila ovary

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The piRNA pathway controls transposons to maintain germline genome integrity in animal gonads. piRNAs arise from piRNA clusters, distinct genomic regions largely occupied by transposon remnants. Drosophila possesses two types of the piRNA clusters, uni- and dual-strand clusters. Both clusters are transcribed by Pol II, although they harbor the recessive histone mark H3K9me3. A proposed model suggests that Rhino (Rhi), a paralog of heterochromatin protein HP1a, binds specifically with H3K9me3 on the dual-strand clusters and forms a trimeric complex with Deadlock and Cutoff, termed the RDC complex, to trigger transcription of the clusters. However, its underlying mechanism remains largely unknown. To understand the mechanism, we have focused on a piRNA factor that was previously identified in genome-wide screening by Czech et al. (Czech et al. 2013). We first produced monoclonal antibodies against the protein and found that it co-localized with Rhino in the ovaries. We thus named this protein Egret (Egt). The egt mutant flies generated by CRISPR-Cas9 failed to express piRNAs from dual-strand, but not uni-strand, clusters. However, the Rhi foci remained in the nucleus as in wildtype ovaries, suggesting that Egt acts downstream of Rhi. We are currently engaged to understand the functional contribution of Egt in the piRNA biogenesis pathway. The outcome will be discussed at the meeting.

POSTER NUMBER: 3013

Mechanistic Insights and Progress on the GalNAc-siRNA Conjugate Platform for Targeted Delivery of RNAi Therapeutics to the Liver

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The past few years have seen major advances toward achieving safe and effective siRNA delivery, moving the field closer to the realization of RNAi Therapeutics. We have developed a siRNA conjugate platform to achieve targeted delivery to hepatocytes utilizing a synthetic multivalent N-acetylgalactosamine (GalNAc) ligand. Progress in design features and chemistry has led to GalNAc-Enhanced Stabilization Chemistry (ESC) siRNA conjugates, which have demonstrated potent and durable activity against multiple liver targets in investigational clinical studies across multiple programs. Ongoing efforts to maximize the potential therapeutic benefit of GalNAc-siRNA conjugates focus on improving our mechanistic understanding of the interaction of conjugates with the RNAi pathway and the effects of chemical modifications on potency and duration of effect by examining liver exposure, intracellular trafficking and extracellular vesicle content, Ago2 loading and target RNA silencing.

POSTER NUMBER: 3014

A novel sperm-specific compartment secures an Argonaute protein for paternal epigenetic inheritance

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Germ cells possess specialized perinuclear compartments that comprise the mRNA surveillance machinery responsible for transposon silencing and fertility. In the nematode *Caenorhabditis elegans*, two granular, phase-separated structures, P granules and *Mutator* foci, are home to RNA-interference (RNAi)-related pathways, driven by a highly diversified Argonaute sub-clade (WAGO) that mediates gene silencing. Intriguingly, it has been shown that WAGO-mediated gene silencing can be inherited via both oocyte and sperm. Especially the inheritance via sperm is remarkable, since mature sperm only has little cytoplasm. In fact, we and others have seen that Argonaute proteins get expelled from maturing spermatids, into so-called residual bodies. How then can sperm-mediated inheritance of RNAi work? Here, we genetically identify WAGO-3 as a major Argonaute protein required for the paternal inheritance of endogenous small RNAs. During spermatogenesis, WAGO-3 is in P granules, just like other Argonaute proteins like WAGO-1 and ALG-3. However, before P granules start to disappear, and the residual body starts to form, WAGO-3 accumulates in distinct cytoplasmic foci within the maturing spermatocyte, and remains like that until mature sperm. In contrast, we show that WAGO-1 and ALG-3 are displaced into the residual body. Using immunoprecipitation experiments on WAGO-3 followed by label-free quantitative mass spectrometry, we identified the protein PEI-1. PEI-1 is expressed specifically during late spermatogenesis, and always colocalizes with WAGO-3. Without PEI-1, WAGO-3 follows the same fate as WAGO-1 and ALG-3, and paternal inheritance of endogenous small RNAs is affected. Our results identify a new sub-cellular, sperm-specific structure, which we call PEI-granules. While not essential for spermatogenesis, PEI-granules are required for paternal inheritance of small RNAs and represent a novel mechanism for the proper sorting of an Argonaute protein during spermatogenesis.

POSTER NUMBER: 3015

tRNA as molecular chaperone for folding and assembly of virus-like particles

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tRNA, representative of non-coding small RNAs, has long been recognized as an adaptor for translation of genetic information into proteins. Besides the canonical function in protein synthesis, a moonlighting function for protein folding is being recognized in tRNA molecules, which sometimes outperform previously known protein-based molecular chaperones. The Chaperna (chaperone + RNA) function, RNA as molecular chaperone, is intrinsic to ribozymes, and is also in operation at the virus-host interface facilitating HIV infections. An RNA-interacting domain (RID) is chosen from tRNA synthetase as a transducer of Chaperna function for *de novo* folding and assembly of virus-like particles (VLPs) and nanoparticles (NPs). tRNA binding to RID triggers the folding and assembly of norovirus VP1 into VLPs (180 monomers) or ferritin nanoparticles (24mers) displaying MERS-CoV protective antigen. Mutations that affect tRNA binding greatly increased the soluble aggregation defective in regular assemblage. The results suggest that tRNA governs the overall kinetic network of antigen folding pathway in favor of enhanced assemblage into immunologically relevant conformation. Harnessing novel chaperna function, the present folding vehicle provides a robust prophylactic and diagnostic platform for viral infections. Further extending previously recognized function of RNAs as gene, messenger (mRNA), enzyme (ribozyme), and gene regulators (miRNAs), the chaperna may operate in *de novo* folding of proteins independently or in concert with protein-based molecular chaperones.

POSTER NUMBER: 3041

Integrated miRNA and mRNA expression profiling of ATF3 transgenic mouse mammary gland tumors reveals similar gene expression and pathway signatures to human basal-like breast cancer

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ATF3 is a potent oncogene in the mouse mammary gland that is aberrantly expressed in most human breast cancers. In the BK5.ATF3 mouse model, overexpression of ATF3 in basal cells produces tumors that are characterized by activation of the Wnt/b-catenin and MAPK signaling pathways. We have demonstrated the disregulation of at least 34 miRNA genes by microarray and the differential expression of 4739 mRNA genes by RNA-Seq in tumors compared to adjacent normal transgenic mammary gland. Molecular profiling of mRNA at the gene level indicated the low expression of Rb1, Esr1, and Pgr and the high expression of keratins 5, 6, and 17, Erbb2 and Egfr, which are all expression features of basal-like tumors. Further analysis of miR-21, miR-143 and miR-145 identified several known downstream targets that are differentially expressed in the expected manner. This includes down-regulation of genes that suppress the MAPK pathway (Pdcd4 and Spry1), up-regulation of genes that activate the Kras pathway and up-regulation of genes that regulate stem cell function (Klf4 and Sox2). Loss of function studies *in vitro* suggest that ATF3 directly modulates miR-143/145, but not miR-21. At the pathway and/or network level, individual and/or integrated analysis of miRNA and mRNA by Ingenuity Pathway Analysis and Gene Set Enrichment Analysis showed the enrichment of epithethial mesenchymal transition, cell cycle, p53 pathway, TGF beta signaling, basal cell carcinoma, Wnt beta catenin signaling, human embryonic stem cell plurpotency/role of nanog in mammalian embryonic stem cell, and axonal guidance signaling. Many of these enriched pathways are also characteristically enhanced in three other basal-like murine breast cancer models, C3Tag^{Ex}, Myc^{Ex}, and p53null-Basal^{Ex}. These studies and further comparison of the BK5.ATF3 model to 27 different mouse models [Pfefferle AD et al [Genome Biology (2013) 14:R125] and the TCGA human breast cancer molecular subtypes strongly suggest the BK5.ATF3 mouse model resembles the human basal-like breast cancer subtype.

POSTER NUMBER: 3016

Bioinformatic analysis on the 3' end formation of silkworm piRNAs

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PIWI-interacting RNAs (piRNAs) and PIWI proteins play important roles in defending animal germline cells against transposable elements. PIWI proteins are first loaded with long piRNA precursors and then their 3' end is processed into the ~28 nt mature length. Previous studies have suggested that this maturation process involves several different nucleases, including the endonuclease Zucchini (Zuc), the exonuclease Trimmer (Tri), and the slicer activity of PIWI proteins. However, it remains unclear how these nucleases cooperate or compete with each other in the 3'-end formation of piRNAs. To answer this question, we generated Trimmer knock out (Tri-KO) BmN4 silkworm cells and analyzed their piRNA profiles. The overall length of piRNAs was markedly extended at their 3' ends in the Tri-KO cells, indicating that Tri is critical in piRNA maturation in silkworms. Interestingly, a group of piRNAs that were particularly elongated in Tri-KO cells showed a clear bias of uracil at the position immediately downstream of the extended 3' end (+1U). This is consistent with the idea that those extended pre-piRNAs are generated by Zuc, which is thought to cleave preferentially before U in vivo in Drosophila and mice. In contrast, another group of piRNAs whose length was less sensitive to the absence of Tri lacked the +1U bias but instead tended to have antisense piRNAs in the downstream region, which is indicative of PIWI protein-mediated cleavage. Notably, the putative cleavage sites by Zuc or PIWI protein coincided at the position of 32-42 nt from the 5' end of mature piRNAs. We envision that, in silkworm piRNA biogenesis, Zuc or PIWI protein produces 32-42 nt pre-piRNAs, depending on the context of the downstream region, and then their 3' ends are trimmed by Tri to the mature length.

POSTER NUMBER: 3017

microRNAs form distinct silencing complexes to regulate their target mRNAs differently

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Animal germ cells possess a specific post-transcriptional regulatory context allowing the storage of maternal transcripts in the oocyte until their translation at a specific point in early development. As key regulators of gene expression, miRNAs repress translation mainly through mRNA destabilization. Thus, germline miRNAs likely use distinct ways to regulate their targets. Here we use *C. elegans* to compare miRNA function within germline and somatic tissues. We show that the same miRNA displays tissue-specific gene regulatory mechanisms. While translational repression occurs in both tissues, targeted mRNAs are instead stabilized in the germline. Comparative analyses of miRNA silencing complexes (miRISC) demonstrate that their composition differ from germline to soma.

We show that germline miRNA targets preferentially localize to perinuclear regions adjacent to P granules and their repression is dependent on the core P granule component GLH-1. Together, our findings reveal the existence of different miRISC in animals that affect targeted mRNAs distinctively.

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POSTER NUMBER: 3018

MicroRNAs 93-5p, 106b-5p, 17-5p, and 140-5p target the expression of early growth response protein 2 in Schwann cells

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Early growth response protein 2 (EGR2) is an essential transcription factor for peripheral nerve myelination. Schwann cells, the peripheral myelin-forming glial cells, express high levels of EGR2 during postnatal myelination. In contrast, Schwann cells exhibit low EGR2 expression during Wallerian degeneration after injury. In this study, we screened 10 potential microRNAs (miRNA) (miRNA 20a-5p, miRNA 137-5p, miRNA140-5p, miRNA148b-3p, miRNA150-5p, miRNA 17-5p, miRNA 93-5p, miRNA 20b-5p, miRNA 106b-5p, miRNA 152-3p) that potentially target EGR2 using miRNA algorithms and identified that miRNAs 106b-5p, 140-5p, 93-5p, and 17-5p target EGR2 in Schwann cells. These miRNAs directly target EGR2 by binding to the 3'-UTR to suppress EGR2 mRNA levels. Additionally, the levels of miRNA 93-5p, 106b-5p, 17-5p and 140-5p were decreased in the sciatic nerves during postnatal development, while these miRNAs were increased on day 1 after sciatic nerve injury. Taken together, these findings suggest that the expression of EGR2 during postnatal development and Wallerian degeneration could be regulated by the inverse expression of miRNAs 106b-5p, 140-5p, 93-5p, and 17-5p, which target EGR2.

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POSTER NUMBER: 3019

microRNAs regulating mitochondrial dynamics in human neurogenesis

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Mitochondria are involved in diverse metabolic processes, mainly energy production and biomolecule synthesis. Depending on the metabolic status at specific cell states, mitochondria change their morphology and distribution. These changes are observed during neuronal development. Here, we present a microRNA that regulates the mitochondrial dynamics during human neurogenesis. The microRNA (miR-neuro) we investigated in neuronal development is increased in its expression along with the process of neuronal differentiation. We have observed that neuronal development is impaired when miR-neuro is inhibited in neural progenitor cells (NPCs) and in differentiating neurons. Since mitochondria mature during neuronal development, we have hypothesized that mitochondrial dysfunction will be induced by altering the level of miR-neuro. Hence, miR-neuro was inhibited to investigate the mechanism of neurogenesis failure. The resulting changes in mitochondrial morphology, mitochondrial membrane potential, and protein levels of oxidative phosphorylation complexes will be presented. Our findings show the significance of miR-neuro in the metabolic reprogramming of neural progenitor cells to neurons.

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POSTER NUMBER: 3020

Highly recurrent U1 small nuclear RNA hot spot mutation drives alternative splicing in sonic hedgehog medulloblastoma

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Background: Medulloblastoma is the most common malignant pediatric brain tumor which comprises four distinct subgroups and harbors few recurrent somatic mutations as well as other pediatric cancers. The genetics underlying medulloblastoma pathogenesis are still unclear.
Methods: To delineate non-coding somatic mutations, we analyzed whole genome sequencing (WGS) from 341 medulloblastoma cases. U1 spliceosomal small nuclear RNA (snRNA) mutations were further analyzed in an additional 179 sonic hedgehog (Shh) medulloblastoma cases using allele specific PCR. Alternative splicing was analyzed using LeafCutter software.
Results: We identified hotspot mutations of the U1 snRNA in ~50% of Shh-medulloblastomas, which were not present across other subgroups. The U1 snRNA mutation occurs in the 5' splice site binding region (r.3a>g), and snRNA mutant tumors have significantly disrupted RNA splicing with an excess of 5' cryptic splicing events. The sequences of alternative 5' sites in cases with U1 snRNA mutation demonstrate an enrichment for a dominant C nucleotide at the 6th base. Cryptic cassette exons induced by mutated U1 snRNA in both *PTCH1* and *GLI2* generate new stop codons. Consequently, both alternative isoforms translate incomplete mRNAs, which reduce PTCH1 function and increase GLI2 function. As well as Shh pathway genes, U1 snRNA mutations affected several oncogene and tumor suppressor genes such as *CCND2* and *PAX5*. The mutation was assessed in 2,442 cases of WGS across 36 other tumor types, and the mutations were highly specific to Shh-medulloblastomas, with only one case found in pancreatic cancer.
Conclusion: Our results provide the first evidence of a hot spot mutation in U1 snRNA, implicating splice site targeting mutations in medulloblastoma pathogenesis and reveal a novel mechanism of aberrant splicing in human cancer. As U1 mutation is a hot spot and gain-of-function type mutation, it represents an exciting target for novel therapy.

POSTER NUMBER: 3021

Mir-17~92 Confers Differential Vulnerability of Motor Neuron Subtypes to ALS-associated Degeneration

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Selective motor neuron (MN) degeneration is the hallmark of amyotrophic lateral sclerosis (ALS). Among degenerated MNs, lateral motor column motor neurons (LMC-MNs) innervating limbs are one of the most vulnerable types. Previously, we reported that deletion of *mir-17~92* in MNs leads to selective apoptosis of LMC-MNs in embryos by promoting PTEN nuclear import. Here, we further revealed that a reduction in *mir-17~92* expression, with concomitant nuclear PTEN accumulation, is manifested in spinal MNs before disease onset in *SOD1G93A* ALS mice. Using a novel double-transgenic reporter system in embryonic stem cells (ESCs), we uncovered down-regulation of *mir-17~92* and increased nuclear PTEN in ALS-linked degenerating LMC-MNs, whereas non-LMC-MN subtypes remained relatively unaffected. This hallmark dysregulation axis of *mir-17~92*/nPTEN is recapitulated in human ALS iPSC-derived MN system. Finally, we demonstrate that overexpression of *mir-17~92* can significantly rescue human *SOD1L144F* iPSC derived MNs and improve motor deficits as well as survival in *SOD1G93A* mouse model. These findings envisage *mir-17~92* to be a potential prognosis marker for MN degeneration and a promising candidate for therapeutic target in ALS.

POSTER NUMBER: 3022

The role of small non-coding regulatory RNAs in *t*-resveratrol biosynthesis in *Vitis amurensis* Rupr. cells

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Grape growing in Primorsky region of Russia, *Vitis amurensis* Rupr., is interesting in terms of biotechnology because of its capability to produce a pharmaceutically valuable 3,5,4′-trihydroxy-*trans*-stilbene (*t*-resveratrol). Studies concerning *t*-resveratrol biosynthesis revealed stilbene synthase (STS) enzyme to be responsible for catalyzing the reaction of the t-resveratrol formation. STS enzyme is represented as a multigene family, counting up to 48 *STS* members in the genome of *Vitis vinifera*, and the certain isofrms can significantly vary in their expression. Among the other exogenous stimuli, treatment of V. amurensis cells with salicylic acid (SA) and prolonged exposure to ultraviolet C (UV-C) were shown to significantly increase the rate of *t*-resveratrol content and certain *VaSTS* expression.
The current study is focused on the role of small non-coding regulatory RNAs (sncRNAs) in regulation of *t*-resveratrol biosynthesis. In course of our investigation, the levels of sncRNAs expression were analyzed by high-throughput sequencing and potential target genes were predicted in RNA samples derived from *V. amurensis* cells treated with SA and UV-C. Our data implies *VaCAMTA*4, *VaRPP*13, *VaCHI*3, *VaWRKY*53 to be regulated by sncRNAs.

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POSTER NUMBER: 3023

Deciphering long dsRNA fate in mammalian cells

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In *A. thaliana*, *C. elegans* and *Drosophila*, long dsRNA accumulation during virus infection, transposon activation or transgene overexpression, elicits a potent RNAi response: long dsRNA is processed into siRNAs by Dicer proteins, before being loaded into Ago proteins which assemble into RISC to slice complementary RNA or methylate DNA [1]. In mammals dsRNA accumulation is also detected by type I interferon (IFN) dsRNA binding proteins (dsRBP), leading to translational inhibition and apoptosis [2]. However, in certain cell lines [3] or when the type I IFN pathway is inactivated [4], specific RNAi can be revealed, although it is unclear how both pathways interact or which proteins engage dsRNA into RNAi. We aim to unravel these processes using state-of-the-art biochemical, cell biology and genetic approaches.

First, using stable lines expressing fluorescently-tagged dsRBPs, we show that Dicer co-factors PACT and TRBP, as well as type I IFN dsRBP PKR, rapidly congregate in dsRNA-rich foci upon long dsRNA stress. MS analysis of the foci reveals global recruitment of endogenous RNA silencing- and type I IFN factors, suggesting coordinated recognition and processing of long dsRNA by both pathways. Second, we confirm that inactivating PKR in novel GFP-sensor 293T cells, uncovers a specific RNAi response on transgenes and endogenous targets, as shown previously [4]. This response is characterized by production of phased siRNAs, loading into Ago-complexes and complementary mRNA degradation, respectively in a Dicer- and Ago2-dependent manner. Interestingly, we observe two modes of Dicer processing depending on the dsRNA template: full- and end processing, confirming earlier *in vitro* results [5]. Finally, we show that TRBP knock-out abolishes processing and loading of specific dsRNA templates, suggesting an important role of TRBP, but not PACT, in mammalian RNAi.

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POSTER NUMBER: 3043

Molecular Mechanisms of Target Recognition by the microRNA-Guided *Drosophila* Argonaute1 Protein

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In flies, gene silencing is directed by different classes of small RNAs that guide distinct members of the Argonaute protein family to specific RNA targets: small interfering RNAs (siRNAs) are loaded into Argonaute2 (Ago2), whereas microRNAs are loaded into Argonaute1 (Ago1). The biochemical properties of *Drosophila* Ago2—a protein specialized for antiviral defense and found only in arthropods—have been extensively studied. Ago2 divides its RNA guide into functionally distinct domains. Ago2 pre-organizes the siRNA seed region, guide (g) nucleotides g2-g8, into an A-form helix, thereby prepaying the entropic penalty paid upon target binding. Once bound to target, complementarity beyond the seed region, especially pairing up to nucleotide g11, determines whether or not Ago2 cuts the target. In flies, Ago2 dissociates so slowly from extensively complementary target RNAs that nearly all binding events end with target cleavage; fly Ago2 is paired to a target RNA solely through the seed sequence of the small RNA guide binds 80 times less tightly.
Here, we report a detailed characterization of the rules for stable target binding by *Drosophila* Ago1, the homolog of the mammalian miRNA-guided Ago2 protein. Like mammalian Ago2, fly Ago1 binds its target via both its seed sequence and four additional nucleotides, usually positions g13-g16, that form 3' supplementary base pairs with the target. Equilibrium binding competition assays show that central mismatches (g8g9, g10g11) have no detectable effect on binding, consistent with miRNAs being centrally unpaired with their targets. In contrast, mismatches at position g4 and g5 of the seed reduced binding 150-fold. Interestingly, combining dinucleotide mismatches at g4g5 and g15g16 reduced binding 1400-fold, suggesting that fly-Ago1 requires extensive complementarity to compensate for a lack of seed pairing. We will present data suggesting that fly Ago1-RISC and Ago2-RISC bind their targets by distinct molecular mechanisms.

POSTER NUMBER: 3024

Delivery of stabilized microRNA mimics into the mouse brain: lessons from models of neurodegenerative and metabolic diseases

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MicroRNAs (miRs) are important regulators of gene expression and neuronal functions. The growing field of RNA neurobiology dictates development and improvement of effective and reliable *in vivo* techniques to address the function of particular miR molecules within the brain. Our approach implements application of minimally LNA-modified double-stranded miR-mimics to miR-depleted neuronal populations. Since other approaches require a nuclease cleavage of precursors by Dicer, our technique allows restoration of the functions of specific miRs on the background of globally inactivated miR maturation pathway. Moreover, this approach allows a delivery of a group of candidate miR mimics at a time. This opens a possibility of reductionist approach to decrease the number of specific miRs in the mixture and finally identify the molecules causative for a studied phenotype. We have successfully implemented the approach of continuous infusion of miR into the brain while studying PI3K-mTOR pathway over-activation-dependent weight gain caused by miR pathway depletion in the hypothalamic neuronal populations. Indeed delivery of candidate miRs targeting this pathway to the arcuate hypothalamic nucleus resulted in dosage-dependent attenuation of the hyperphagic obesity. In our other recent study, we have demonstrated that the expression of Dicer, is down-regulated in aged mouse midbrain similar to its depletion in dopaminergic neurons from Parkinson's disease patients. Using a laser-assisted microdissection and qPCR profiling, we identified a predominant decrease of miR expression in aged dopaminergic neurons. Further, using genetic and pharmacological approaches, we have shown that miR-mediated regulation provides an essential protection mechanism from neurodegeneration. Injection of putatively neuroprotective miRs to the midbrains normalized motor deficits in mice with dopamine neuron-restricted Dicer depletion. In conclusion, delivery of stable oligonucleotides into the brain in combination with advanced genetic tools proves to be an effective way to study the functions of non-coding RNA in specific neuronal populations.

POSTER NUMBER: 3025

Optimization of siRNA delivery for the management of pest insects

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RNA interference (RNAi) constitutes a key component of the insect antiviral immune system. As such, this post-transcriptional gene silencing process has been widely studied within this animal group. RNAi can also be triggered through the external administration of artificial dsRNA constructs. In this way, targeted downregulation of endogenous genes can be achieved. Due to its high degree of sequence specificity, this technique shows great promise for future application in insect management approaches. However, several major issues remain to be resolved. The most pertinent problem is the current inability of several economically important insect species to induce a systemic RNAi response after feeding with dsRNA's. This could be caused by a reduced cellular uptake of dsRNA from the gut lumen. In the desert locust *Schistocerca gregaria*, the presence of dsRNA degrading enzymes in the gut is thought to lead to premature degradation, preventing the induction of an RNAi response. Moreover, while *S. gregaria* shows an excellent RNAi response to injection with long dsRNAs, it remains refractory to injection with siRNAs. To circumvent these obstacles and improve efficiency of dsRNA-induced toxicity in insects, we propose the use of delivery systems that protect the dsRNA and facilitate its intracellular uptake. To this extent, siRNAs were complexed with a carrier. Stability of the carrier:siRNA complex was first assessed in an *in vitro* gut environment. Next, complexes were injected into the body cavity of the locust to ascertain cellular uptake. Finally, complexes were fed to the insect to determine their ability to induce an *in vivo* RNAi response through this uptake route. While siRNA remained complexed with the carrier in an *in vitro* gut environment, complexes were unable to induce an RNAi response when fed to *S. gregaria*. Intriguingly, injection with the carrier:siRNA complex did lead to a transcript reduction, indicating that this system could facilitate the cellular uptake of siRNA in insects. This discovery could have significant implications for the development of future pest management strategies against several insect species.

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POSTER NUMBER: 3027

Convergent and Divergent Evolution of microRNAs

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MicroRNAs (miRNAs) are endogenously expressed small noncoding RNAs. Metazoan miRNAs and their target genes often interplay to compose complex regulatory networks. Given the complexity of these networks, it is intriguing to investigate how miRNAs originate and evolve. Our longstanding interests is to decipher the evolutionary principles of miRNAs and their target sites. Previously, my collaborators proposed a birth-and-death model of miRNA evolution which suggests most newly emerged miRNAs are evolutionarily transient and have a high birth rate followed by a high death rate. We also found that once a new miRNA survives its initial stage, many adaptive mutations are required to drive that miRNA to develop function. Furthermore, we found variation in miRNA target sites are associated with increased gene expression variation in human populations . Nevertheless, it yet remains unclear what factors affect the survival and function development of a new miRNA or target site. Recently, we found that the genomic clustering helps new miRNAs survive and develop functions in animals. We put forward a “functional coadaptation” model which describes how natural selection has driven miRNAs in the same cluster to target the same or functionally related genes although those miRNAs have independent origins. Our results advance our understanding of the mechanisms and evolutionary driving force of miRNA clustering, and expands our view of the regulatory functions of clustered miRNAs. We also show that miRNA duplication accelerates the recruitment of functional new targets even if the paralogous miRNAs maintain the same seeds. Collectively, our new discoveries indicate that (1) clustering drives miRNAs of different origins to evolve convergently towards similar function and that (2) functional diversification following miRNA duplications influences the evolution of new target sites.

POSTER NUMBER: 3026

RNA helicase AQR cooperates with the DROSHA-DGCR8 complex to promote primary microRNA processing

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MicroRNAs (miRNAs) are ~22nt small RNA regulators involved in many important physiological processes. To achieve such regulatory function, primary miRNA (pri-miRNA) transcripts need to be firstly cropped by microprocessor complex, composed of DROSHA and its cofactor DGCR8, into ~70 nt precursor miRNA (pre-miRNA) in nucleus. To systematically identify new factors involved in pri-miRNA processing, we inserted two pri-miRNA cassettes into the 3'UTR of GFP and made two stable cell lines, in which the GFP intensity is negatively correlated with the microprocessor activity. By combining loss-of-function screening, GFP sorting and deep sequencing, we unexpectedly identified 45 additional protein coding genes that can significantly boost GFP signal upon deletion, among which DROSHA and DGCR8 reproducibly stand out, indicating the robustness of our screening strategy. We next focused on one of the top hits AQR for further analysis because it can directly associated with DGCR8. Consistently, CLIP-seq mapping also revealed that AQR and DGCR8 co-occupied a large number of pri-miRNAs. Surprisingly, we found that AQR preferably binds the G-rich sequence in the upper stem of pri-miRNA, and such binding may facilitate RNA structure remodeling to promote DROSHA cleavage. Together, these data suggest that AQR may function as a cofactor of the DROSHA-DGCR8 complex to promote primary microRNA processing in vivo.

POSTER NUMBER: 3028

A Novel Bioinformatics Pipeline for miRNA-Seq Annotation

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The importance of microRNAs (miRNA) as regulatory molecules in key biological pathways has led to experiments generating vast quantities of data, driving the development and expansion of bioinformatics capability in the area. Expression analysis of miRNA-Seq data differs from that of standard RNA-Seq in fundamental ways. One key difference is the relatively short length of miRNA-Seq reads, affecting the trimming, adapter clipping, and contaminant filtering of reads. As a result, this increases the likelihood of spurious alignments when mapping reads to a reference and the generation of false-positive data in downstream applications. Our miRNA-Seq pipeline uses a mix of published bioinformatics and RNA-Seq tools with customised scripts and techniques to effectively pre-process miRNA-Seq data, map reads to a curated reference set, and identify significant levels of miRNA in experimental samples. There are two primary considerations our pipeline addresses. Firstly, aggressive adapter removal was applied to prepare the 80GB of raw data for analysis. A reduction of adapter content by a further 10% compared to standard RNA-Seq procedures was observed. Additional custom alignment filtering then allowed selection of uniquely mapped perfect alignments only for downstream analysis. Secondly, the available references of known miRNAs contain a significant amount of sequence redundancy with many host-specific miRNA IDs referring to the same miRNA sequence, increasing the complexity of miRNA-Seq data annotation. MiRBase,the primary miRNA sequence repository, contains about 40% redundant sequence, which must be collapsed to represent the relevant taxonomic IDs for the study in question. We illustrate how our resource for miRNA-Seq analysis produces robust, reproducible results that will improve and advance the field of miRNA bioinformatics.

POSTER NUMBER: 3029

miRNA Isoform Quantification Method Development and Expression Analysis in Schizophrenia

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Amplification and reverse transcription biases in small RNA sequencing (sRNA-seq) have been understudied. Method comparisons of miRNA isoform (isomiR) quantifications have also been limited. We sought to determine methods that may improve miRNA and isomiR quantifications and then used our optimized methods to evaluate isomiR expression in schizophrenia (SZ). We hypothesized that using unique molecular identifiers (UMI) to remove duplicate reads would improve miRNA quantification. We also predicted that commonly used methods would differ for isomiR detection accuracy and consistency. Further, we hypothesized that the overall expression and diversity of isomiRs might be altered in SZ because miRNA biogenesis has previously been shown to be altered. We utilized synthetic small RNAs and total RNA from a brain sample to evaluate bias among a variety of library preparation methods for sRNA-seq. We determined that the use of UMIs does improve quantification accuracy and consistency, and that template switching methods are particularly prone to inaccurate isomiR detection. Using our optimized methods, we then compared isomiR expression in 56 postmortem neurotypical brain samples with 30 SZ brain samples. No difference was observed in overall isomiR abundance or diversity. However differential expression analysis, in which we controlled for confounding factors, demonstrated that several isomiRs and canonical miRNAs showed differences between cases and controls. We are now validating these findings with qPCR, comparing the overall expression of different types of isomiRs between the diagnostic groups, and determining the regulatory implications of the identified differentially expressed miRNAs and isomiRs.

POSTER NUMBER: 3030

sRNAs dynamics during epigenetic memory formation in embryos of Norway spruce

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Epigenetic memory in Norway spruce permanently affects the timing of vitally important adaptive traits, such as dormancy, bud burst and bud set in a predictable and reproducible manner. Epigenetic memory is established in response to the temperature conditions prevailing during embryogenesis.
We used NGS sequencing and computational *in silico* methods to analyze dynamics of sRNAs, to identify and profile conserved and novel miRNAs among small RNAs in embryogenic tissues of Norway spruce at three epitype inducing (EpI) temperatures (18, 23 and 28C). We detected three predominant classes of sRNAs related to a length of 24 nt, followed by a 21-22nt class and a third 31 nt group of sRNAs.
This 31 nt group of sRNA is close to absent in vegetative tissues. In embryos we identified around 1600 31sRNA families containing large amounts of isoforms differing by terminal nts. Most of our defined 31sRNAs families were conserved among most of gymnosperm species. More than half of these may originate from the non-coding part of transcriptome, with more than 30% putatively from tRNAs and some from enzyme-coding transcripts. High redundancy of 31sRNAs putative origin was apparent. Transcriptomic analysis showed that around 1100 31sRNA families were differentially expressed in spruce embryos at different EpI temperatures.
We defined more than 2100 different miRNAs within the prevailing length 21-22 nt. Profiling of these putative miRNAs allowed identification of 1053 highly expressed miRNAs, including 523 conserved and 530 novels. 654 of these miRNAs were found to be differentially expressed (DEM) depending on EpI temperature. For most DEMs, we defined their putative mRNA targets. Notably, 124 DE miRNAs targeted 203 differentially expressed epigenetic regulators.
We have demonstrated that developing Norway spruce embryos possess a more complex sRNA composition than that reported for somatic tissues. A variety of the sRNAs showed distinct EpI temperature dependent expression patterns. Putative EpI miRNAs target spruce genes with a wide range of functions, including genes known to be involved in epigenetic regulation, which in turn could provide a feedback process leading to the formation of epigenetic marks. We suggest that TIR, NBS and LRR domain containing proteins could fulfill more general functions for signal transduction from external environmental stimuli and conversion them into molecular response. Unknown mechanisms provide fine-tuning of the sRNA pool content participating both developmental regulation and epigenetic memory formation in Norway spruce embryos.

POSTER NUMBER: 3031

Bifunctional role of miR-124 during neuronal reprogramming of human fibroblasts

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Brain-enriched miRNAs, miR-9/9* and miR-124, function as potent cell fate effectors that when ectopically expressed in human adult fibroblasts, initiate dramatic remodeling of transcriptome and chromatin landscape, resulting in the direct reprogramming into neurons. This process of miRNA-mediated direct conversion of human non-neuronal somatic cells into neurons recapitulates numerous genetic switches involved in the acquisition of neuronal fate. As such, homologous chromatin modifiers switch from a non-neuronal to neuronal counterpart, similarly to mammalian neurogenesis, including subunits within the BAF chromatin remodeling complex, BAF53a to BAF53b, and BAF45a to BAF45b/c, epigenetic modifiers, DNMT3b to DNMT3a, as well as splicing regulators, PTBP1 to PTBP2. Several of these switches are directly mediated by microRNAs, including the switching of PTB proteins for neuron-specific alternative splicing. At the onset of neurogenesis, miR-124 functions to selectively repress PTBP1 for the upregulation of PTBP2, as it allows for the de-repression of PTBP2 by PTBP1. Although miR-124-mediated downregulation of PTBP1 is required for PTBP2 expression in neurons, PTBP2 is also a target of miR-124 and therefore suggests that additional mechanism is at play for the selective repression of PTBP1 and preferential upregulation of PTBP2 in neurons in the presence of miR-124. Here, we show that miR-124 plays a bifunctional role in repressing non-neuronal PTBP1 while promoting neuronal PTBP2 expression during the direct conversion of human adult fibroblasts into neurons. We demonstrate that miR-124 selectively synergizes with neuronal RNA-binding proteins, such as ELAVL3, at PTBP2 3'UTR and not PTBP1 3'UTR for its differential PTB output.

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Identifying essential *mir-35* targeting sites in *C. elegans*

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In *C. elegans, mir-35* family members have been shown to be essential for the development of embryos [1]. Complete knockout of the *mir-35* family (*mir-35-41* cluster and *mir-42*) causes fully penetrant embryonic or L1 larval lethality [1]. Phenotypic characterization of a temperature sensitive deletion strain of *mir-35-41* (*gk262* or *nDf50*) at permissive temperature revealed that the *mir-35* family plays various roles throughout *C. elegans* development [2, 3]. The lethal phenotype of *mir-35* family mutants is still poorly understood though genetic data suggests that derepression of more than one target gene underlies the lethality. Among the mRNA transcripts that have been identified as direct targets of the *mir-35* family, most play roles in non-lethal phenotypes. Interestingly, disruption of the *mir-35* seed matching sequence in the *nhl-2* 3'UTR resulted in a dominant lethal phenotype [5], implying that *nhl-2* is one of the essential targets of the *mir-35* family.
We are taking a CRISPR-Cas9 screening approach to identify other sites at which edited alleles are negatively selected, indicating that they are also essential for *mir-35* binding and target repression. Starting with a list of candidate binding sites predicted by Targetscan, we designed a guide RNA to target each of the predicted *mir-35* binding sites. To increase the throughput of this large-scale CRISPR-Cas9 screening, we decided to introduce the gRNAs to the animals in a multiplexed way without losing the efficiency of each individual gRNA. After a series of optimizations, multiplexing of 10 gRNAs in one pool proved to be ideal for screening. Transformed animals were selected based on a co-CRISPR marker, and the genomic DNA was collected and subjected to site-specific amplifications. The frequency and position of editing at each site will be analyzed through deep sequencing of these amplicons. Sites in which CRISPR-Cas9 editing of the seed match is not observed will be categorized as potential essential *mir-35* binding sites and analyzed in later confirmation studies. This approach will not only allow us to identify essential *mir-35* binding sites but also lay the foundation for future multiplexed CRISPR-Cas9 screen for other classes of essential negative regulatory elements in *C. elegans*.

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Neuronal exosomal miRNA mediates genetic regulation of astroglial functions

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Astroglia are essential and critical components of functional synapses in the central nervous system (CNS), playing active and diverse roles in modulating neuronal/synaptic functions. How these important astroglial functions are regulated, especially by neuronal signals, remains largely unknown. Exosomes, a major type of extracellular vesicles (EVs) that are originated from endosomes, have recently emerged as a new intercellular communication process. By generating cell-type specific intraluminal vesicles (ILVs)/exosome reporter (CD63-GFP$^{f/f}$) mice, here we show that CD63-GFP^{+} exosomes are widely secreted from both neurons and glial cells *in situ* in the CNS. Neuronal exosomes are able to travel extensively after initial secretion and can be taken up into astrocytes *in vivo*. Interestingly, neuronal exosomes contain an abundant and selective subset of microRNAs (miRs) that is distinct from the total miR profile of neurons. These miRs, especially the neuron-specific miR-124-3p, are able to transfer into astrocytes via secreted neuronal exosomes *in vitro* and *in vivo*. Exosomally transferred neuronal miR-124-3p subsequently up-regulates the predominant astrocyte-specific glutamate transporter GLT1. The blockade of exosome secretion significantly abolishes miR-124-3p transfer into astrocytes and astroglial glutamate uptake. Interestingly, exosomally transferred miR-124-3p is able to reduce expression of GLT1-binding miRs (miR-132 and miR-218) to suppress their inhibitory effects on GLT1 protein expression, partially through miR-124-3p's down-regulation of the miR-132 promoting transcriptional factor Creb1. In summary, our findings identified a new mechanism how a exosomally transferred miR regulates endogenously transcribed miRs, which unveils a previously undescribed neuronal exosomal miR-mediated genetic regulation of astrocyte functions.

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Analysis of the tear MicroRNA levels in sjögren's syndrome

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Purpose: We investigated the expression of microRNAs (miRNAs) in the tears of patients with sjögren's syndrome (SS) compared to the tears of healthy controls. And we also examined the correlation between miRNAs expression and ocular parameters.
Methods: Eighteen tear samples were collected from SS patients and eight from normal subjects at the Hanyang University Hospital. Clinical ophthalmologic assessments included Schirmer I test, tear film breakup time (tBUT) and ocular staining score (OSS). The expression of 43 different miRNAs were measured by real-time polymerase chain reaction. Differentially expressed miRNAs were stratified for fold regulation (FR) larger than ±2 and for significance of $P < 0.05$.
Results: For this study, 18 patients with SS (mean age 47.22 ± 11.56 years) and 8 controls (mean age 42.5 ± 11.78 years) were included. We found four miRNAs with significantly different expression in SS patients compared to controls. Expression levels of miR-16-5p (FR=2.34, P=0.009) in patients with SS was significantly higher than that in controls. Expression levels of miR-30b-5p (FR=−2.14, P=0.023), miR-30c-5p (FR=−3.50, P=0.043), miR-203a-3p (FR=−2.28, P=0.031) in patients with SS were significantly lower than that in controls. All four miRNAs were not significantly correlated with OSS scores.
Discussion: Our findings indicate that miR-16-5p, miR-30c-5p, miR-203a-3p, miR-30b-5p are deregulated in SS. These miRNAs may play a role in the pathogenesis of SS and serve as biomarkers for diagnosis of SS.

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Deciphering the neural epitranscriptome during mammalian brain development

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Proper development of the nervous system is critical for its function, and deficits in neurodevelopment have been implicated in many brain disorders. A precise and predictable developmental schedule requires highly coordinated gene expression control that orchestrate the dynamics of mammalian cortical neurogenesis. Recent discoveries of widespread mRNA chemical modifications raise the question of whether this mechanism plays post-transcriptional regulatory role in cortical neurogenesis. A selective RNA degradation mechanism has received recent attention in development and function of the nervous system. Although N^6-methyladenosine (m^6A), installed by the Mettl3/Mettl14 methyltransferase complex, is the most prevalent internal mRNA modification, the function of m^6A methylation in selective RNA degradation during the brain development is still elusive. Here we used the *Mettl14* conditional knockout mouse as a model to examine m^6A function in embryonic cortical neurogenesis in vivo. *Mettl14* deletion in the embryonic mouse brain resulted in diminished m^6A content, altered cell cycle progression of neural stem cells, and impaired temporal progression of the cortical development. To gain insight into the potential molecular mechanism underlying m^6A dependent regulation, we performed m^6A-seq from mouse developing forebrain and identified high confident m^6A peaks on gene transcripts related to transcription factors, cell cycle and neuron differentiation. Mechanistically, m^6A-tagging marks specific set of mRNAs including critical transcription factors for stem cell properties and neural differentiation, and promotes selective RNA degradation mediated by the CCR4-NOT complex. Together, our results reveal crucial epitranscriptomic control of mammalian cortical neurogenesis and novel insight into mechanisms underlying this highly coordinated developmental program.

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A Pandas complex adapted for piRNA-guided transposon silencing

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The repression of transposons by the Piwi-interacting RNA (piRNA) pathway is essential to protect germ cells. In Drosophila ovaries, Panoramix enforces transcriptional repression via the binding to the target-engaged Piwi-piRNA complex, though the precise mechanisms by which this occur remain elusive. Here we show that a germline specific paralogue of a nuclear export factor, dNxf2, functions together with Panoramix and dNxt1 as a ternary complex to suppress transposon expression. Biochemical and functional analysis demonstrated an essential role of the UBA domain of dNxf2 in Panoramix association and transposon silencing. Moreover, the RRM domain of dNxf2 plays an essential role in binding to transposon transcripts. Therefore, we propose that dNxf2 functions as Pandas (Panoramix-dNxf2 dependent TAP/p15 silencing) complex, which counteracts the canonical RNA exporting machinery (TAP/p15) and restricts transposons to nuclear peripheries.

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Exploring Active RNAi in Mitochondria to Reveal Epistatic Translational Control of mtDNA-Encoded Cytochrome C Oxidase Subunits

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Small interfering RNAs (siRNAs) have been widely used to post-transcriptionally silence gene expression in higher

eukaryotic cells, but it has remained unclear whether the RNAi pathway is also active within the mitochondria and the lack of such tool prevents direct perturbation of mitochondrial DNA (mtDNA)-encoded genes. However, recent studies have documented the presence of nuclear DNA (nDNA)-encoded microRNAs (miRNAs) in the mitochondria, suggesting the ability of small RNAs to enter the mitochondria. We now use a newly developed Click-in strategy to demonstrate efficient entrance of exogenous siRNAs into the matrix of mitochondria and their ability to specifically target individual mtDNA-encoded transcripts. Similar to miRNAs, these siRNAs function in an Ago2-dependent, but GW182-independent manner. Using this new tool, we investigate the direct contribution of mtDNA-encoded gene products to the coordinated assembly of respiratory chain complexes, unexpectedly revealing sequential translational control of Cytochrome c oxidase subunit I (COXI) by COXII and both COXI and COXII by COXIII. These findings demonstrate a distinct active RNAi system in the mitochondria and extend the concept of mitochondrial translational plasticity previously established with imported nDNA-encoded subunits to mtDNA-encoded components to achieve epistasis of a key respiratory chain complex.