South West UK RNA Club

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RNA Meeting 2015

Tuesday 2nd June 2015

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	TIME	SPEAKER	INSTITUTION	TALK TITLE
Welcome and Introduction: *Mark Lindsay*	9.15-9.20			
GENE EXPRESSION *Chair: Michael Ladomery*	9.20-9.35	Michael Ladomery	UWE Bristol	*The Scd6 protein xRAPB has properties different from RAP55 relating to early translation in Xenopus oocytes*
	9.35-9.50	Dipen Rajgor	King's College London	*Mammalian microtubule P-body dynamics are mediated by nesprin-1*
	9.50-10.05	Clare Pritchard	Cardiff University	*Cytoplasmic RNA regulation and cell motility*
	10.05-10.20	Yuhui Doi	Birmingham University	*Screening for novel UPF1 interacting factors in Schizosaccharomyces pombe*
	10.20-10.35	Subhendu Choudhury	Birmingham University	*Exon Junction Complex (EJC) protein components associate with transcription sites independently of splicing in Drosophila melanogaster*
Tea/Coffee				***Please visit our sponsors' stalls***
Pre-mRNA SPLICING *Chair: Lorna Harries*	11.15-11.30	Emma Woods	Cardiff University	*The role of CD44 variants in fibroblast differentiation and monocyte binding*
	11.30-11.45	Adam Midgley	Cardiff University	*Hyaluronidase-2 dependent regulation of CD44 splicing in anti-fibrotic versus pro-fibrotic cells*
	11.45-12.00	Ling Li	University of Bristol	*Control of epithelial splicing regulatory proteins (ESRPs) in epithelial-mesenchymal transitions*
	12.00-12.15	Keith Brown	University of Bristol	*Epigenetic inactivation of the epithelial splicing factor ESRP2 in Wilms' tumour*
	12.15-12.30	Elizabeth Bowler	UWE Bristol	*The effect of hypoxia on alternative splicing in prostate cancer cells*
	12.30-12.45	Eleanor Star	University of Bristol	*Finding anti-angiogenic molecules using VEGF-based splicing-sensitive fluorescent reporters*
	12.45-13.00	Benjamin Lee	University of Exeter	*Changes in splicing factor gene expression with age in mouse tissue*

Lunch				***Please visit our sponsors' stalls***
LONG NON-CODING RNAS *Chair: Sebastian Oltean*	14.00-15.00	Chris Ponting *Keynote Speaker*	University of Oxford	*Short steps along the long road to long non-coding RNA function*
	15.00.15.15	Adele Murrell	University of Bath	*GNG12-AS1 is a novel long non-coding RNA with a dual tumour suppressor function identified through differential siRNA targeting*
Tea/Coffee				***Please visit our sponsors' stalls***
TRANSCRIPTOMICS/miRNAs *Chair: Tim Bowen*	15.45-16.00	Luke Pilling	University of Exeter	*Whole blood gene expression associations with muscle strength in humans*
	16.00-16.15	Rocio Teresa Martinez-Nunez	University of Southampton	*RibomiR-seq in severe asthma: a novel look into disease signatures*
	16.15-16.30	Hadil Alahdal	University of Bristol	*Investigating miRNA-dependent genes in regulating neuronal function*
	16.30-16.45	Hannah Scott	University of Bristol	*Placenta-derived microRNAs may cause abnormal foetal development under gestational hypoxia*
	16.45-17.00	Ashleigh Bignell	University of Bristol	*Examining the potential role of placental exosome secretions in response to gestational hypoxia on foetal neurodevelopment*
Concluding remarks *Michael Ladomery & Sebastian Oltean*	17.00-17.05			

THE SCD6 PROTEIN xRAPB HAS PROPERTIES DIFFERENT FROM RAP55 RELATING TO EARLY TRANSLATION IN *XENOPUS* OOCYTES

Michael Ladomery* **** and John Sommerville*****

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Oocytes accumulate mRNAs in the form of maternal ribonucleoprotein (RNP) particles, the protein components of which determine the location and stability of individual mRNAs prior to translation. The Scd6 family of proteins, typified by RAP55, functions in a wide range of eukaryotes in repressing translation and relocating mRNPs to processing bodies and stress granules.

Here we describe in *Xenopus laevis a* variant of RAP55, *x*RAPB, a member of the LSM14B family of proteins found in many other organisms, which also contains conserved Lsm and FDF domains but differs in containing fewer RGG repeats.. *x*RAPB differs from *x*RAPA in other respects: it is expressed at high concentration earlier in oogenesis; it interacts specifically with the RNA helicase Xp54; it is first distributed to a subcortical layer and the Balbiani body; it is a component of mRNP particles with a different size distribution; its over-expression can lead to an increase in protein synthesis, whereas knock-down can lead to a decrease. Since Xp54 is a dominant repressor of translation, activation appears to be effected by the dislocation of Xp54 from *x*RAPB.

MAMMALIAN MICROTUBULE P-BODY DYNAMICS ARE MEDIATED BY NESPRIN-1

***Dipen Rajgor*[1], Jason A. Mellad[1], Daniel Soong[1], Jerome B. Rattner[2], Marvin J. Fritzler[2], and Catherine M. Shanahan[1]**

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Nesprins are a multi-isomeric family of spectrin-repeat (SR) proteins, predominantly known as nuclear envelope scaffolds. However, isoforms that function beyond the nuclear envelope remain poorly examined. Here, we characterize p50^{Nesp1}, a 50-kD isoform that localizes to processing bodies (PBs), where it acts as a microtubule-associated protein capable of linking mRNP complexes to microtubules. Overexpression of dominant-negative p50^{Nesp1} caused PB displacement from microtubules, resulting in reduced PB movement and cross talk with stress granules (SGs). These cells were unable to disassemble canonical SGs, leading to cell death and revealing PB–microtubule attachment is required for SG anti-apoptotic functions. Furthermore, p50^{Nesp1} was required for miRNA-mediated silencing and interacted with core miRISC silencers Ago2 and DDx6 in an RNA-dependent manner. These data identify p50^{Nesp1} as a multi-functional PB component and microtubule scaffold necessary for RNA granule dynamics and provides evidence for PB and SG micro-heterogeneity.

CYTOPLASMIC RNA REGULATION AND CELL MOTILITY

***Clare Pritchard*[1], *Kate Comber*[2], *Will Wood*[2] *and Sonia López de Quinto*[1]**

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2. University of Bristol, School of Cellular and Molecular Medicine, University Walk, Bristol BS8 1TD

Motile cells are highly polarised. One way in which this polarity may be achieved is through the localisation and local translation of mRNA by RNA-binding proteins (RBPs). In cultured cells, *β-actin* mRNA is localised and translationally-repressed at the leading edge of migratory fibroblasts by the RBP, Zipcode-binding protein 1 (ZBP1). Delocalisation of *β-actin* mRNA results in a loss of directional cell migration, suggesting that RNA localisation is required for proper cell motility. However, it is still unclear whether this type of cytoplasmic RNA regulation is relevant *in vivo*, in the 3D context of a living organism.

We have established an *in vivo* model system, using *Drosophila* embryonic macrophages, to study the role of RNA regulation in cell motility within the context of a living organism. Analysis of the localisation of Imp (*Drosophila* ZBP1 homologue) in *Drosophila* macrophages revealed that, contrary to published reports of cultured cells, Imp is not localised at the leading edge of migratory cells. Conversely, Imp was found enriched at the base of small protrusions which may correspond to either microtubule or actin extensions.

Overexpression of Imp in macrophages impaired both their developmental migration and directed migration to epithelial wounds. Our *in vivo* characterization revealed that haemocytes over-expressing Imp phenocopied the migratory defects observed upon reduction of β-integrin levels in *Drosophila* macrophages. Interestingly, we found that Imp binds the 3'UTR of *β-integrin* mRNA, and both proteins - Imp and b-integrin – co-localise in distinct cytoplasmic granules in *Drosophila* cultured haemocytes. Furthermore, we observed an increase in β-integrin protein levels upon Imp-RNAi treatment, suggesting that Imp represents a novel regulator of β-integrin expression.

SCREENING FOR NOVEL UPF1 INTERACTING FACTORS IN *SCHIZOSACCHAROMYCES POMBE*

***Yuhui Dou**, Jianming Wang and Saverio Brogna**

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UPF1 is an essential factor in nonsense-mediated mRNA decay (NMD), it also play key roles in other mRNA decay mechanisms. In recent years its nuclear roles are emerging. However, the role of UPF1 is still not fully understood. To have a better understanding of the role of UPF1, we used *Schizosaccharomyces pombe* as a model organism to screen for UPF1 interacting factors. A systematic genetic interaction screen was carried out to identify novel UPF1 interacting factors. A group of putative UPF1 interacting factors were identified in this study. And among these factors, AIR1 and PPN1 which have RNA metabolic roles and function in the nucleus were selected for further study. Through tetrad dissection and spot assay, the interaction between UPF1 and AIR1, UPF1 and PPN1 were further confirmed. And a C-terminal HA-tagged AIR1 strain was constructed for further investigations on physical interactions. This study discovered novel UPF1 interacting factors, which can improve our understanding on the role of UPF and also provided fundamental information and materials for further study.

EXON JUNCTION COMPLEX (EJC) PROTEIN COMPONENTS ASSOCIATE WITH TRANSCRIPTION SITES INDEPENDENTLY OF SPLICING IN *DROSOPHILA MELANOGASTER*

Subhendu Roy Choudhury* ***and Saverio Brogna***

School of Bioscience, University of Birmingham, Birmingham, UK

The results of number of studies across organisms show that pre-mRNA splicing, a strictly nuclear process, affects NMD, a process expected to be strictly cytoplasmic. It has been proposed that this link is mediated by the exon junction complex (EJC), a multiprotein complex deposited during splicing in the nucleus, which remains associated with the mRNA during export to the cytoplasm. Some observations are not consistent with this function attributed to the EJC. Additionally, all of the proteins that constitute the EJC are well conserved in Drosophila, yet these proteins are not required for NMD in this organism. To understand better the function of the EJC, we aimed to visualize its association with nascent RNA at the polytene chromosomes of Drosophila. Surprisingly, EJC recruitment is RNA-independent at both intron-containing and intron-less genes. Additionally, we found that not all of the EJC components are always present at transcription sites, suggesting that EJC assembly is not an obligatory step in mRNP biogenesis. Similar observations were made in S2 Drosophila cells using chromatin immunoprecepitation coupled to high throughput sequencing (ChIP-seq). Our data suggest that the core EJC components Y14 and Mago regulate transcription.

THE ROLE OF CD44 VARIANTS IN FIBROBLAST DIFFERENTIATION AND MONOCYTE BINDING

***E Woods*, A. Midgley, T Bowen, R Steadman**

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CD44 is a transmembrane surface receptor that is expressed as multiple variants. This variability in protein expression is due to the alternative splicing of 10 exons within the CD44 gene and a high level of post-transcriptional modifications.

Chronic kidney disease (CKD) is a progressive fibrotic disease that results in the destruction of kidney tissue and the loss of renal function. Transforming Growth Factor Beta (TGF-β1) has been widely implicated in fibrosis through its promotion of fibroblast to myofibroblast differentiation. Myofibroblasts form collagen rich scars and have a contractile phenotype due to the expression of α smooth muscle actin (αSMA) intracellular stress fibres. Central to the function of TGF-β1 is the assembly of a cell-surface protein complex consisting of CD44 (receptor for hyaluronan) and the epidermal growth factor receptor (EGFR). Without the formation of this complex, myofibroblasts cannot differentiate from fibroblasts. Furthermore, the inflammatory cytokine interleukin 1 beta (IL-1β) enhances monoctye binding to resident fibroblasts. The increase in monoctyes present in the surrounding tissue activates an inflammatory response, hence, contributing to the fibrotic progression. Fibroblasts stimulated with IL-1β have a re-organisation of Hyaluronan (HA) on cell membrane protrusions where CD44 co-localises with intercellular adhesion molecule 1 (ICAM-1).

This study aimed to identify which CD44 spliced variants are expressed in fibroblasts and to elucidate which of the variants are implicated in TGF-β1 induced fibroblast differentiation and Il1-β induced monocyte binding.

HYALURONIDASE-2 DEPENDENT REGULATION OF CD44 SPLICING IN ANTI-FIBROTIC *VERSUS* PRO-FIBROTIC CELLS.

***Adam Midgley*[1], Robert Steadman[1], Vincent Hascall[2], Timothy Bowen[1], Aled Phillips[1] & Soma Meran[1]**

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This work was funded by the UK Medical Research Council

Progressive fibrosis resulting in loss of organ function comprises a wide variety of chronic disorders including cardiac, kidney, lung and liver failure. Fibroblasts are the principal effector cells in fibrosis, and differentiate to their active pro-fibrotic phenotype (myofibroblasts) under the influence of the cytokine Transforming Growth Factor (TGF)-β1. Our recent studies indicate that the anti-fibrotic growth factor Bone Morphogenetic Protein-(BMP)-7 antagonises the effects of TGF-β1 in myofibroblasts and renders these cells resistant to differentiation. Hyal2 and a specific variant isoform of CD44, CD44v7/8 were noted to play critical roles in BMP7-driven resistance to myofibroblast differentiation. Hyal2 knockdown modulated the expression of CD44s and CD44v7/8, however the exact mechanism through which Hyal2 mediates CD44v7/8 expression and BMP7 anti-fibrotic effects were unclear. Here, we investigate the role of Hyal2 in the regulation of CD44 (standard and variant isoform) expression.

CD44 and its variant isoform expression levels differ depending on cellular treatment. Fibroblasts were incubated with TGF-β1 to induce myofibroblast differentiation, and compared to cells incubated with BMP7 or IL1β to induce the differentiation-resistant and immuno-associated phenotypes respectively. Hyal2 expression, distribution and cellular localisation were assessed using QPCR, immunocytochemistry and immunoblotting. ChIP and siRNA techniques were used to determine the functional significance of Hyal2 and splice regulators in the experimental systems. A panel of candidate splice regulators common to mesenchymal cell types which target CD44 were assessed for changes in expression: TRA2β, SLM2, SRSF2 and SRSF6.

CONTROL OF EPITHELIAL SPLICING REGULATORY PROTEINS (ESRPs) IN EPITHELIAL-MESENCHYMAL TRANSITIONS

[1]Ling Li and [1]Sebastian Oltean

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The epithelial-mesenchymal transition (EMT), one of the hallmarks of cancer, is a biological process that allows polarized epithelial cells to undergo multiple biochemical changes to become mobile mesenchymal cells(1). Epithelial splicing regulatory proteins 1 and 2 (ESRP1 and ESRP2) are cell-type-specific regulators of transcripts that switch splicing during the EMT(2). ESRPs drive splicing events of about 200 genes those give epithelial phenotype. One of the well-known genes regulated by tissue-specific alternative splicing is the fibroblast growth factor receptor 2 (FGFR2) (3) which has a switch between two mutually exclusive isoforms - FGFR2-III b and FGFR2-III c during the EMT (4). ESRP 1 and 2 were identified as regulators of FGFR2 alternative splicing, leading to the FGFR2 isoforms being switched to the epithelial cell-type-specific splicing program. Previous work established ESRPs as master regulators of EMT and underlie epithelial–mesenchymal transitions during tumour progression and fibrosis(2, 5). As FGFR2 splicing is a sensor of ESRPs activity and EMT, it may be used for understanding the regulation of ESRP in cancer and fibrosis. Using a splicing-sensitive fluorescent reporter based on inclusion/exclusion of FGFR2 exon IIIc we have performed a screen with the LOPAC library (Library of Pharmacologically Active Compounds). We have identified several compounds that are able to switch FGFR2 splicing and are now being validated as modulators of epithelial-mesenchymal transitions.

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EPIGENETIC INACTIVATION OF THE EPITHELIAL SPLICING FACTOR ESRP2 IN WILMS' TUMOUR

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Our genome-wide analysis of DNA methylation had previously identified ESRP2 as a gene that is frequently hypermethylated in Wilms' tumour (WT), with associated down-regulation of expression. ESRP2 encodes an RNA splicing factor that is essential for epithelial differentiation, a process that is deregulated in WT development.

We used pyrosequencing of the ESRP2 CpG island promoter region to analyse DNA methylation in two cohorts of WTs and found high levels of DNA methylation in both (70 and 72%) and also showed by QPCR that WTs had much lower expression of ESRP2 than normal kidney. We analysed DNA methylation in nephrogenic rests (NR), premalignant precursors of WT and found hypermethylation of ESRP2 in both the NR and WT. This suggests that ESRP2 becomes epigenetically inactivated early in WT development, prior to the acquisition of malignancy. A mechanistic link between DNA methylation and expression of ESRP2 was demonstrated by the induction of ESRP2 expression in two WT cell lines using the demethylating agent 5-aza-2'-deoxycytidine.

We found that transient overexpression of high levels of ESRP2 in WT cell lines induced massive apoptosis and was thus extremely growth suppressive. We therefore transfected the WT cell line Wit49 with an inducible ESRP2 construct, to produce a cell line with controllable ESRP2 expression. Induction of ESRP2 expression increased splicing of the ESRP2 target gene ENAH towards the epithelial spliceform and caused a decrease in colony-forming efficiency. Thus ESRP2 expression in a WT cell line induces epithelial-specific splicing and is growth suppressive.

This work was supported by Children with Cancer

THE EFFECT OF HYPOXIA ON ALTERNATIVE SPLICING IN PROSTATE CANCER CELLS

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Hypoxia is defined as the state in which the availability or delivery of oxygen is insufficient to meet tissue demand. It occurs particularly in aggressive, fast-growing tumours in which the rate of new blood vessel formation (angiogenesis) cannot match the growth rate of tumour cells. Cellular stresses such as hypoxia can cause cells to undergo apoptosis; however some tumour cells adapt to hypoxic conditions and evade apoptosis. Tumour hypoxia has been linked to poor prognosis and to greater resistance to existing cancer therapies. We hypothesize that one of the ways that tumour cells adapt to hypoxia is by changing the alternative splicing of key genes.

We confirmed a hypoxia-specific change in the alternative splicing of carbonic anhydrase IX (CAIX). CAIX is one of the best studied hypoxia markers, involved in maintaining an intracellular pH that favours tumour cell growth. A high throughput PCR analysis provided evidence of significant changes in the alternative splicing of several other cancer associated genes in PC3 prostate cancer cells. We also examined the effect of hypoxia on the expression of key splice factors and splice factor kinases. Ongoing work is aimed at determining the mechanisms through which hypoxia leads to changes in the alternative splicing of CAIX and of other cancer associated genes. We present our latest findings on this.

FINDING ANTI-ANGIOGENIC MOLECULES USING VEGF-BASED SPLICING-SENSITIVE FLUORESCENT REPORTERS

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Alternative splicing of the vascular endothelial growth factor (VEGF) terminal exon generates two protein families with differing functions. Pro-angiogenic $VEGF_{xxx}$ isoforms are produced via selection of the proximal 3' splice site of the terminal exon. Use of an alternative distal splice site creates the anti-angiogenic $VEGF_{xxx}$b proteins.

A bichromatic splicing-sensitive reporter was designed to mimic VEGF alternative splicing and used as a molecular tool to further investigate this alternative splicing event. VEGF's terminal exon and preceding intron were inserted into a minigene construct followed by the coding sequences for two fluorescent proteins. A different fluorescent protein is expressed depending on which 3' splice site of the exon is used during splicing. The fluorescent output can be used to follow splicing decisions *in vitro* and *in vivo*.

Following successful reporter validation in different cell lines and altering splicing using known modulators, small pilot screens were undertaken to search for novel regulators of the splicing decision that creates pro-/anti-angiogenic VEGF isoforms. A larger screen was performed using a library of 1280 small molecules (LOPAC), all compounds are pharmacologically active and have known targets. Alterations to reporter splicing were measured using a fluorescent plate reader to detect RFP and GFP expression. Compounds of interest were further validated using flow cytometry and assessed for effect on endogenous VEGF alternative splicing. *In vitro* angiogenesis assays were used to demonstrate anti-angiogeneic effect. Anti-angiogenic activity and the effect on tumour growth were investigated in several *in vivo* models.

CHANGES IN SPLICING FACTOR GENE EXPRESSION WITH AGE IN MOUSE TISSUES

***Benjamin Lee*[(1)], Kevin Flurkey[(2)], Florence Emond[(3)], John Watt[(4)], David Melzer[(1)], Lorna Harries[(1)].**

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Alternative mRNA splicing occurs in over 95% of human genes and is widely accepted to contribute to the plasticity and adaptability of the genome. Deviation from normal splicing is common in many human disorders, including several age-related diseases. Splice site choice is determined by binding of splicing regulatory factors. We recently demonstrated that these show deregulated expression during human ageing, both in vitro and in vivo.

We aimed to assess the tissue- and species-specificity of changes in splicing regulator expression in proliferative (spleen) and terminally differentiated (muscle) tissues from young and old mice of different strains with variable average lifespans.

In spleen, we noted changes with age in expression of splicing activators *Srsf2* (p=0.009) and *Srsf3* (p=0.015), also splicing inhibitors *Hnrnpd* (p=0.012) and *Hnrnph3* (p=0.015), broadly consistent with our observations in humans.

Changes in expression of splicing inhibitors *Hnrnpa2b1* (p=0.003), *Hnrnpk* (p=0.003), *Hnrnpm* (p=0.002) and *Hnrnpul2* (p=0.001) were noted in spleen when comparing long- and short-lived strains. Interestingly, changes in *Hnrnpa2b1* (p=0.008), *Hnrnpk* (p=0.028) and *Hnrnpm* (p=0.040) were present in the young animals of the longer-lived strains. We observed few alterations in splicing regulator expression, either with age or lifespan in muscle.

We conclude that changes in splicing regulator expression are similar in mouse spleen, to a lesser degree than in man. Our observation of differences in splicing inhibitor expression in young animals of long-lived strains compared with short-lived strains suggests that some of these factors may have roles in longevity.

KEYNOTE SPEAKER

SHORT STEPS ALONG THE LONG ROAD TO LONG NON-CODING RNA FUNCTION

***Chris P. Ponting*, W. Haerty, K. Roberts & T. Sirey**

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Thousands of human lncRNA loci have been documented yet only a handful have had their functions experimentally defined. A large-scale lncRNA knockout project will be required exploiting multiple targeting strategies to determine the full range of contributions that lncRNAs make to human biology and disease. In the meantime, either computational or experimental investigation of lncRNA loci can provide insights into lncRNA mechanism.

Our investigations of sequence conservation and constraint on lncRNA sequence have previously provided scant evidence for much of this sequence being functional. Nevertheless, we found that such patterns across multi-exonic lncRNA loci mirror those of protein coding genes, although to a lesser degree. Additionally we report strong evidence for the action of purifying selection to preserve exonic splicing enhancers within human multi-exonic lncRNAs and nucleotide composition in fruitfly lncRNAs. Our findings provide evidence for selection for more efficient rates of transcription and splicing within lncRNA loci.

Careful experimental investigations of single lncRNA loci are essential if we are to appreciate the breadth of lncRNA mechanisms. We have identified a mammalian-conserved lncRNA which acts as a microRNA sponge thereby increasing the transcript and protein abundance of mitochondrial oxidative phosphorylation subunits, and hence complex I enzymatic activity. Our experimental and computational approaches demonstrate that many lncRNAs are biologically consequential by regulating gene expression levels in *trans* either by regulating rates of transcription or levels of transcripts.

GNG12-AS1 IS A NOVEL LONG NON-CODING RNA WITH A DUAL TUMOUR SUPPRESSOR FUNCTION IDENTIFIED THROUGH DIFFERENTIAL siRNA TARGETING

Lovorka Stojic[1*], Malwina Niemczyk[1], Arturo Orjalo[2], Yoko Ito[1], Anna Elisabeth Maria Ruijter[1,3] and Adele Murrell

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The transcripts of long noncoding RNAs (lncRNAs) may have explicit functions to regulate gene expression in trans. The act of transcribing lncRNAs can modulate the expression of adjacent genes through transcriptional interference. *GNG12-AS1* is a lncRNA on chromosome 1p31, that originates from a bicistronic promoter of *GNG12* in an antisense orientation to overlaps the neigbouring imprinted tumour suppressor, *DIRAS3.* We used a small-interfering RNA (siRNA) strategy to differentially silence *GNG12-AS1* by transcriptional gene silencing (TGS) or post transcriptionally (PTGS) in order to identify its function. Targeting the 5' end of *GNG12-AS1* led to TGS mediated by Agonaute 2 and resulted in concomitant upregulation of *DIRAS3* in cis, whereas targeting the 3'end led to PTGS and no effect on *DIRAS3* transcription. However, depletion of *GNG12-AS1* transcripts resulted the upregulation of MET signaling, increased cell migration and actin cytoskeleton changes that were independent of its role in modulating imprinted *DIRAS3* expression. This dual function of GNG12-AS1 extends the tumour suppressor function of the locus . In addition this work sets a paradigm for using differential siRNA targeting to decouple the functions related to the process and products of lncRNA transcription.

WHOLE BLOOD GENE EXPRESSION ASSOCIATIONS WITH MUSCLE STRENGTH IN HUMANS

LC Pilling*, R Joehanes, MJ Peters, T Kacprowski, R Jansen, CHARGE Gene Expression Working Group, G Homuth, J van Meurs, J Murabito, D Melzer

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Introduction: Lower muscle strength in midlife predicts disability and mortality in later life. Several factors in blood, including growth differentiation factor 11 (GDF11), have been linked to muscle regeneration in animal models. We performed a comprehensive discovery analysis to identify gene transcripts associated with muscle strength in adults.

Methods: Meta-analysis of whole blood gene expression and hand-grip strength in four independent cohorts (n=7,781, ages: 20-104 years, weighted mean 56 years), adjusted for age, sex, height, weight, and leukocyte subtypes. Three cohorts used Illumina HumanHT-12 microarrays, one used Affymetrix Exon-array data, (overall 17,534 unique gene identifiers). Separate analyses were performed in subsets (older/younger than 60, male/female).

Results: Expression levels of 221 genes were associated with strength. Associated genes included *ALAS2* (rate limiting enzyme in heme synthesis), *PRF1* (perforin, a cytotoxic protein associated with inflammation), *IGF1R* and *IGF2BP2* (both insulin like growth factor related). We identified statistical enrichment for hemoglobin biosynthesis, innate immune activation genes and the stress response. Ten genes were only associated in younger individuals, four in males only and one in females only. For example *PIK3R2* (a negative regulator of PI3K/AKT growth pathway) was (negatively) associated with muscle strength in younger (<60 years) individuals but not older (>=60 years).

Conclusions: This first large-scale transcriptome wide array study in human adults confirmed associations with known pathways and provides new evidence for over half of the genes identified. There may be specific gene expression signatures in blood for muscle strength by age and gender.

RIBOMIR-SEQ IN SEVERE ASTHMA: A NOVEL LOOK INTO DISEASE SIGNATURES

Rocio Teresa Martinez-Nunez*, *Tilman Sanchez-Elsner

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Alternative splicing (AS) is an essential step in post-transcriptional regulation required for gene accurate expression. AS strongly links to translation, with mRNAs showing isoform-specific translation due to regulators such as microRNAs (miRs). To date, no genome-wide picture of AS effects in mRNA translation and its link to microRNAs in disease has been addressed. We chose to study asthma, a common inflammatory disease of the airways affecting ~300 million people globally. ~500,000 people in UK have severe asthma, experiencing persistent daily symptoms and frequent exacerbations despite standard therapy. Unfortunately, the mechanisms underlying asthma remain unknown. We performed polyribosome fractionation and high-throughput miR- and RNA-sequencing (ribomiR-seq) in primary bronchial epithelial cells from 8 severe asthmatics (SA) and 5 age-matched healthy donors. RibomiR-seq compares total RNA (Total, accounting for all mRNAs expressed), polyribosomal-bound mRNAs (Poly, specific mRNAs engaged in translation) and links them to microRNA presence in both the Total and Poly fractions. We divided the analysis into two groups: Total vs Total and Poly vs Poly mRNAs differentially expressed between healthy and SA. Our results can be summarized in three main points. Firstly, aggregate gene expression dysregulation differs when comparing Total-to-Total and Poly-to-Poly fractions. Secondly, dysregulated alternatively spliced isoforms show fraction-dependent differences and the information also differs to that one given by aggregate gene expression. Thirdly, small RNAseq/ miR analysis shows enrichment of microRNA-targeted mRNAs in Poly compared to Total mRNAs in disease. Our results show an unprecedented level of post-transcriptional gene regulation in asthma of broad implications in general biology and disease.

INVESTIGATING miRNA-DEPENDENT GENES IN REGULATING NEURONAL FUNCTION

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Neurogenesis in the embryonic and adult brain has critical roles in neural development and maintenance of brain function. Adult neural stem cells (NSCs) that are continuously generated in two regions, the subgranular zone (SGZ) and the subventricular zone (SVZ), have electrophysiological properties, connect with other cells and integrate into the existing neuronal circuitry (Zhao et al. 2008). Here we wish to investigate the effects of microRNAs on adult neurogenesis. MicroRNAs (miRNAs) are a class of small non-coding RNAs that act as post-transcriptional regulators. They are transcribed as precursor molecules that are subsequently processed into active ~21 nucleotide mature miRNA that. miR-21 is a miRNA that has been reported as an oncogene that can increase cell proliferation and reduce apoptotic cell death (Si et al. 2007). However, the role of mir-21 in the adult brain development is still unclear.

Using transgenic mice that either overexpress miR-21 or have loss-of-function in miR-21, we demonstrate that miR-21 over expression led to increased numbers of the dentate gyrus (DG) new-born neurons (DCX^+ cells) and mature neurons (NeuN+ cells) in the adult brain *in vivo*, while knockout of miR-21 showed an increase in the apoptotic marker cleaved caspase-3 expression. In vitro, neural progenitor cells from SVZ of miR-21 overexpressing mice showed enhanced neurosphere proliferation and increased number of neurons following differentiation ($Tuj1^+$ cells). Consistent with increased neurogenesis in the DG, miR-21 overexpressing mice exhibited enhanced learning in a Morris water maze task. Together, these results suggest that miR-21 plays a critical role in adult neurogenesis and hippocampus and has important effects on learning and memory.

PLACENTA-DERIVED microRNAS MAY CAUSE ABNORMAL FOETAL DEVELOPMENT UNDER GESTATIONAL HYPOXIA

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Abnormal oxygen levels during pregnancy occur in a range of pregnancy complications, including pre-eclampsia, as well as in response to smoking. Gestational hypoxia can be detrimental to foetal development and may lead to diseases later in life. Previous studies have shown that hypoxic insults may cause the placenta to secrete factors that give rise to abnormal brain development. We aimed to investigate if miRNAs are secreted by the placenta under hypoxic conditions and if these could cause developmental changes.

To this end, we analysed the concentration of small RNAs in conditioned culture media derived from both an *in vitro* model of the hypoxic placenta and an *in vivo* model of gestational hypoxia, which leads to growth restriction and abnormal brain development in the foetus. It was shown that levels of small RNAs and more specifically, levels of miRNAs, were increased after hypoxic insults. Application of the antioxidant MitoQ prior to the hypoxic exposure not only ameliorated the observed developmental defects but also reduced miRNA levels. NanoString nCounter analysis was performed to gain information regarding the identity of the miRNAs present in the media.

The results suggest that miRNAs are secreted by the placenta in response to a hypoxic insult and implicate these factors in causing the developmental defects observed in response to gestational hypoxia. The data points towards an important role of the placenta in mediating the effects of gestational hypoxia on foetal development and may provide a basis for the development of placenta-targeted drugs.

EXAMINING THE POTENTIAL ROLE OF PLACENTAL EXOSOME SECRETIONS IN RESPONSE TO GESTATIONAL HYPOXIA ON FOETAL NEURODEVELOPMENT

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Exosomes are a subclass of endosome-derived nano-vesicles (40-100nm) which function as important delivery systems for endogenously expressed, small non-coding RNAs (miRNAs). MiRNAs act as post-transcriptional regulators capable of modulating cellular processes, with aberrant levels being associated with the onset of pathological conditions. Our research focuses on the exposure of the placenta to oxidative stress during early stages of gestation, as a means to model obstetric complications during pregnancy. Current research has identified the secretion of placenta-derived exosomes into the maternal circulation in a hypoxic setting. However there is limited knowledge regarding the secretion of exosomes into the foetal domain and the effects these may elicit upon different aspects of foetal development.

We obtained conditioned media from both *in vitro* and *in vivo* models of gestational hypoxia and screened for the presence of purified exosomes via; Western blotting and NanoSight technology. The results indicated a higher level of exosome release towards the foetus from a hypoxic placenta compared to the normoxic control. In addition, through small RNA analysis, the purified exosome fraction obtained under hypoxic conditions was shown to contain higher concentrations of miRNA species compared to the normoxic control. Immunostaining of neuronal and astrocytic populations was conducted to explore the potential implications the exosome fractions had upon foetal neurodevelopment.

In summation miRNA-containing exosomes appear to act as important mediators in transferring signalling molecules between the hypoxic placenta and recipient foetal neuronal cells during critical stages of development, and could thus provide an insight into the aetiology of prevalent neurodevelopmental disorders.

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