RNA Club
SOUTH WEST UK

www.southwest.rna.org.uk

RNA Bristol 2022

Thursday 9th June 2022

Dorothy Hodgkin Building, Lecture Rooms A and B
University of Bristol, Whitson Street,
Bristol BS1 3NY

Meeting proudly supported by:

University of BRISTOL

University of Bristol – Faculty of Biomedical Sciences

2022 SPONSORS

cambridge bioscience

QIAGEN

Source BioScience

ThermoFisher SCIENTIFIC

	TIME	SPEAKER	INSTITUTION	TALK TITLE
Welcome and Introduction: *Michael Ladomery, Sebastian Oltean and James Uney*	9.00-9.10			
Session 1: Splicing *Chair: James Uney*	9.10-9.30	Htoo Aung Wai	University of Southampton	Uplifting rare genetic diseases diagnosis in post-genomic era using blood RNA splicing
	9.30-9.50	Monica Ayine	University of Exeter	Alternative splicing of the apoptosis gene *Bcl-x* in diabetic nephropathy
	9.50-10.10	Jingzia Zheng	University of Exeter	Investigation of properties and signalling pathways of compounds that switch *FGFR2* splicing and regulate EMT in prostate cancer cells
	10.10-10.30	Laura Bramwell	University of Exeter	Effects of trametinib on splicing factors in progeroid cells
Tea/Coffee	10.30-11.00			
Session 2: MicroRNAs *Chair: Sebastian Oltean*	11.00-11.20	Daniel Smith	University of Cardiff	Electrochemical detection of microRNA disease biomarkers
	11.20-11.40	Ryan Frankum	University of Exeter	Manipulation of microRNAs reverses senescence in aged human dermal fibroblasts
	11.40-12.00	Andriana Gialeli	University of Bristol	MicroRNA profiling of cerebrospinal fluid from preterm infants following perinatal brain injury
Sponsor talk 1	12.00-12.10	John Cousin	QIAGEN	TBA
Sponsor talk 2	12.10-12.20	Alexander Widger	THERMOFISHER	TBA

Lunch and networking	12.20-13.00			
Session 3: **KEYNOTE LECTURE** *Chair: Michael Ladomery*	13.00-14.00	Andre Furger	University of Oxford	mRNA in the context of the cellular space and stress
Molecular Biology	14.00-14.20	Alexander Moorhouse	University of Bristol	Transcriptional repression by WT1-BASP1
	14.20-14.40	Keith Vance	University of Bath	Chromatin interaction maps identify Wnt responsive *cis*-regulatory elements coordinating *Paupar-Pax6* expression in neuronal cells
	14.40-15.00	Mona Suleyman	University of Bath	Exosome-like vesicles secreted by the parasitic nematodes *Strongyloides* target and manipulate host genes
	15.00-15.20	David McQuarrie	University of Birmingham	ELAV forms a saturable complex on extended RNA, but can nucleate
Tea/Coffee	15.20-15.40			
Session 4: **Transcriptomics** *Chair: Keith Vance*	15.40-16.00	Dominika Lastik	University of Bath	Using nanopore sequencing to identify transcript variation between different life cycle stages of the parasitic nematode
	16.00-16.20	Zhuofan Mou	University of Exeter	Gene expression analysis reveals a five gene signature for progression-free survival in prostate cancer
	16.20-16.40	Maki Asami	University of Bath	Human embryonic genome activation initiates at the one-cell stage
	16.40-17.00	Andrew Douglas	University of Southampton	Blood RNA-seq in diagnostic genomic medicine
Concluding remarks and winning PhD talk prize: *Michael Ladomery &* *Sebastian Oltean*	17.00-17.10			

Uplifting rare genetic diseases diagnosis in post-genomic era using blood RNA splicing

Htoo A Wai[1], Jenny Lord[1] , Matthew Constable[1], Cosima Drewes[1], Ian C Davies[1], Eliska Svobodova[5], Esther Dempsey[6], Anand Sagger[6], Tessa Homfray[6], Sahar Mansour[6], Sofia Douzgou[7], Kate Barr[8], David Hunt[4], Matthew Lyon[2], Adam Gunning[3], Hugh Kelly[1], Penelope Cibin[1], Elenor G Seaby[1], Kerry Spiers-Fitzgerald[1], Jed Lye[1], Sian Ellard[3], N Simon Thomas[2], David J Bunyan[2], Spicing and disease working group, Andrew G L Douglas[1,4], Diana Baralle[1,4]

1. Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK.
2. Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, UK.
3. Exeter Genomics Laboratory, Royal Devon & Exeter NHS Foundation Trust, Exeter, UK.
4. Wessex Clinical Genetics Service, University Hospital Southampton NHS Foundation Trust, Southampton, UK.
5. Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic
6. Department of Clinical Genetics, St George's University of London, London, UK.
7. Manchester Centre for Genomic Medicine, St Mary's Hospital, Manchester University NHS Foundation Trust, Health Innovation Manchester, Manchester, UK
8. Bristol Regional Clinical Genetics Service, St Michael's Hospital, Bristol, UK

Faster pace and cheaper high throughput sequencing technology allows the clinicians to sequence the patient's genome to increase the diagnostics rate of diseases. However, it also generates a vast number of variants of uncertain significance (VUS). VUS represents 42 percent of total variants on the ClinVar database. RNA analysis into VUSs analysis increase the diagnostic rate upto 36% through differential gene expression, splicing and allelic specific expression depending on disease type and tissue source. RNA splicing analysis alone can increase the VUSs diagnostic rate by 33-35% (Cummings *et al.*, 2017; Wai *et al.*, 2020). It was predicted up to 62% of VUSs can affect the splicing. Blood provides a rich source of RNA to detect aberrant splicing. Abnormal splicing caused by VUS are detected using RT-PCR and RNA-seq methods. While RNA-seq can provide the whole transcriptomic views on gene expression level and splicing, RT-PCR is more sensitive for the genes which are not well expressed in blood. In general, implementing RNA splicing analysis in routine genetic testing can uplift the diagnostic rate of rare genetic diseases and open up therapeutic opportunities to correct the abnormal splicing.

Alternative splicing of the apoptosis gene *Bcl-x* in diabetic nephropathy

Monica Lamici Ayine, Kate Bosson, Pedro Marqueti, Megan Stevens, Sebastian Oltean.

Institute of Biomedical and Clinical Sciences, College of Medicine & Health, University of Exeter Medical School, Exeter, UK

An alternative splicing (AS) event occurring in exon 2 of the Bcl-x gene produces pro-apoptotic Bcl-xS and the anti-apoptotic Bcl-xL. The aim of this study is to investigate the regulation of this AS event in kidney cells grown in a diabetic environment as well as the correlation of Bcl-xS/Bcl-xL splicing ratio with various degrees of severity in DN.

Immortalised Human Embryonic Kidney cells (HEK293) exposed to three different high glucose environment for 48 hours. Splice isoforms were analysed using RT-PCR followed by a bioanalyser quantification. Urine samples were obtained from type I and type II diabetic patients enrolled on the Biomarker Enterprise to Attack DKD (BEAt-DKD) and VIBE (Exeter) studies with varying stages of renal disease. RNA was extracted from the urinary sediment for RT-PCR analysis of Bcl-x splice isoforms

A dose-dependent IL-6 treatment caused an increase in the Bcl-xS/Bcl-xL ratio, suggesting an influence of IL-6 on Bcl-x splicing. RT-PCR followed by bioanalyser analysis showed an upregulation of the Bcl-xS/Bcl-xL ratio when HEK293 cells treated under diabetic conditions. There is an increase in the pro-apoptotic Bcl-xS isoform as a result of a combined effect of excessive glucose and IL-6 on the Bcl-x splicing event. Furthermore, pilot data from a small cohort of patients with varying stages of nephropathy indicated that an increase in the pro-apoptotic Bcl-xS in RNA extracted from urinary cells correlated with a decline in GFR, implicating Bcl-x AS as a potential biomarker for DN severity.

Investigation of properties and signalling pathways of compounds that switch FGFR2 splicing and regulate EMT in prostate cancer cells

[1]Jinxia Zheng, [1]Holly Russell, [1]Ling Li, and [1]Sebastian Oltean

[1] University of Exeter Medical School, St Luke's Campus, Exeter, EX1 2LU

Introduction:

Research in the area of hallmarks of cancer has opened the possibility of designing new therapies based on modulating these cancer properties. A process that has not yet been targeted therapeutically in prostate cancer (PCa) is the epithelial-mesenchymal transitions (EMT), which is the reversible interchanges between an epithelial phenotype and a mesenchymal. Previously, in a repositioning screen using a reporter based on FGFR2 splicing, we have found three new chemicals that modulate EMT in PCa (named LLSOs). We present here the functional effect of these chemicals in vitro in different PCa cell lines and mechanism of these compounds to induce MET and switch FGFR2 splicing.

Materials and methods:

Various properties of different PCa cell lines treated with chemicals were assessed by apoptosis assay and Boyden chamber assay. Mechanisms were investigated on EMT markers and FGFR2 splicing by inhibiting specific steps of possible pathways.

Results and Discussion:

The compounds affected differently various properties of cancer cells but all of them inhibit the migration of PCa cells, in line with modulating EMT. We further present mechanistic insights into the LLSOs compounds. Knowing the molecular heterogeneity of prostate cancer, we probed this mechanism in several cell lines – PC3, LNCaP and DU145.

LLSO1 (NNC-55-0396 dihydrochloride, a highly selective T-type calcium channel blocker) - usually signals through calcineurin; however, this seems not to be involved in the MET effect in PC3 cells.

LLSO2 (Nemadipine, an L-type Ca-channel antagonist) - in DU145 it is inducing MET through NFAT; however, in PC3 and LNCaP cells other pathways may be involved.

LLSO3 (Naltrexone, an opioid antagonist) - in PC3 cells, JNK pathway may be involved in the MET effect, but not AKT.

Conclusions:

We have found some of the signalling pathways used by LLSO compounds to regulate EMT and switch FGFR2 splicing in PCa cells. We hope that dissecting these mechanisms will point out to common molecules (e.g. kinases) that could be targeted efficiently to inhibit tumour growth.

Effects of trametinib on splicing factors in progeroid cells

Laura R. Bramwell[1]* & *Lorna W. Harries*[1]

[1]RNA-Mediated Mechanisms of Disease group, College of Medicine and Health, Institute of Biomedical and Clinical Science, University of Exeter

Background/Aims: Cellular senescence is an important mechanism in cellular ageing[1], and is driven in part by dysregulation of splicing factors.[2] Trametinib has previously been demonstrated to rescue splicing factor expression and senescence in wild-type cells.[2] Here, we assess if trametinib can alter splicing factor expression and senescence phenotypes in three types of progeroid cells.

Method: Dermal fibroblasts from Werner syndrome (WS), Cockayne syndrome (CS) and Hutchinson-Gilford progeroid syndrome (HGPS) patients (Coriell Institute) were treated with 10μM trametinib or control for 24h. Senescence phenotypes (SAB staining, markers for proliferation, DNA damage and apoptosis) and splicing factor expression were then assessed relative to wild-type dermal fibroblasts.

Results: Trametinib-treated progeroid cells demonstrated altered expression of several splicing factors. Reduction of senescent cell load was also observed for treated HGPS and CS, but not WS cells (HGPS: 60% change; $p=0.021$, CS: 31% change; $p=0.0417$). Proliferation was lowered with treatment in all three progerias, whilst the apoptosis marker *CASP7* was increased in CS (62% change, $p<0.001$), decreased in WS (194% change, $p=0.043$), and unchanged in HGPS.

Conclusions: Splicing factor expression in progeroid fibroblasts resembled that of senescent wild-type cells. Trametinib caused broadly similar changes in splicing factor expression to those previously associated with rescue of senescence in wild-type cells. Treatment was also able to rescue some, but not all, senescence phenotypes in CS and HGPS, but not WS cells. These data suggest that progeroid cells share some features of senescence with aged wild type cells, and that trametinib may be useful as a senotherapeutic.

(1) Terzi, M. Y.; Izmirli, M.; Gogebakan, B. The Cell Fate: Senescence or Quiescence. *Mol. Biol. Rep.* **2016**, *43* (11), 1213–1220. https://doi.org/10.1007/s11033-016-4065-0.

(2) Latorre, E.; Birar, V. C.; Sheerin, A. N.; Jeynes, J. C. C.; Hooper, A.; Dawe, H. R.; Melzer, D.; Cox, L. S.; Faragher, R. G. A. A.; Ostler, E. L.; Harries, L. W. Small Molecule Modulation of Splicing Factor Expression Is Associated with Rescue from Cellular Senescence. *BMC Cell Biol.* **2017**, *18* (1), 1–15. https://doi.org/10.1186/s12860-017-0147-7.

Electrochemical detection of microRNA disease biomarkers

Daniel A. Smith[1,2]*, Kate Simpson,[1] Lucy J. Newbury,[1] Elliot Jones,[3]
John-Mark Seymour,[3] Donald J. Fraser[1], James E. Redman[2], Timothy Bowen[1]

[1] Wales Kidney Research Unit, School of Medicine, Cardiff University, Cardiff CF14 4XN, UK
[2] School of Chemistry, Cardiff University, Cardiff CF10 3AT, UK
[3] Haydale Ltd., Clos Fferws Parc Hendre, Tycroes, Ammanford SA18 3BL, UK
*email: SmithD16@cardiff.ac.uk

Abstract
We have developed an RT-qPCR-based method for microRNA (miRNA) quantification in urine and peritoneal dialysis effluent [1,2], and recently identified expression profiles to identify bacterial peritonitis [2], diabetic kidney disease [3] and to predict delayed graft function following renal transplantation [4]. In parallel, we are investigating the use of electrochemical detection to expedite quantification of these miRNAs in a variety of biological matrices.

We established proof of principle using glassy carbon electrode-based biosensors, which detected urinary microRNAs with increased sensitivity compared to RT-qPCR [5]. We then developed disposable screen-printed carbon electrode (SPCE)-based miRNA sensors with comparable performance that are potentially adaptable to the clinical laboratory/point of care environment. These sensors were then used to analyse patient and control urine samples, and detected a diabetic kidney disease-associated miR-192 decrease we identified previously using RT-qPCR [3,6].

However, the above electrochemical methodologies are not amenable to high-throughput and/or point of care analysis due to complexities in sensor fabrication [5,6]. We are therefore currently developing more suitable protocols to immobilise DNA oligonucleotides of complementary sequence to our target miRNAs at the SPCE surface for miRNA hybridisation and quantification. The use of amine-modified SPCEs facilitates solution-based DNA attachment to the electrode surface under aqueous conditions. This new methodology accelerates biosensor fabrication and has generated promising new data for miRNA detection in peritoneal dialysis effluent that will be discussed in this presentation.

References
[1] C. Beltrami, A. Clayton, L.J. Newbury, P. Corish, R.H. Jenkins, A.O. Phillips, D.J. Fraser, T. Bowen, Noncoding RNA, **1**, (2015), 151-166
[2] A. C. Brook, R. H. Jenkins, A. Clayton, A. Kift-Morgan, A-C. Raby, A. P. Shephard, B. Mariotti, S. M. Cuff, F. Bazzoni, T. Bowen, D. J. Fraser, M. Eberl, Scientific Reports, 9 (2019) 10136.
[3] C. Beltrami, K.A. Simpson, M. Jesky, A. Wonnacott, C.P. Carrington, P.A. Holmans, L.J. Newbury, R.H. Jenkins, T. Ashdown, C.M. Dayan, P. Corish, S.C. Satchell, P. Cockwell, D.J. Fraser, T. Bowen, American Journal of Pathology 188 (2018) 1982-1992
[4] U. Khalid, L.J. Newbury, K. Simpson, R.H. Jenkins, T. Bowen, L. Bates, N.S. Sheerin, R. Chavez, D.J. Fraser, Scientific Reports 9 (2019) 3584
[5] D.A. Smith, L.J. Newbury, G. Drago, T. Bowen, J.E. Redman, Sensors and Actuators B: Chemical 253 (2017) 335-341
[6] D. A. Smith, K. Simpson, M. Lo-Cicero, L. J. Newbury, P. Nicholas, D. J. Fraser, N. Caiger, J. E. Redman, T. Bowen, RSC Advances 11 (2021) 18832-18839

Acknowledgements
This work is supported by funding and materials from Kidney Research UK, Cardiff University, Haydale Ltd., Wales Kidney Research Unit and Wellcome Trust.

Manipulation of micro-RNAs reverses senescence in aged human dermal fibroblasts

Ryan Frankum and Lorna W. Harries

Institute of Biomedical and Clinical Science, University of Exeter Medical School, Barrack Road, Exeter, EX2 5DW, UK

Cellular senescence underpins ageing and common chronic diseases, it is driven, at least in part, by dysregulated expression of splicing factors (SF) and altered patterns of alternative splicing. Age-related alteration in SF levels arises from aberrant expression of the FOXO1 and ETV6 genes. Here we set out to evaluate the mechanistic links between ETV6/FOXO1 target genes and cellular senescence.

We evaluated the expression of 20 noncoding RNAs which are targets of FOXO1/ETV6 in early and late passage human primary dermal fibroblasts. Targets demonstrating altered expression in aged cells were then manipulated using miRNA mimics or antagomiRs followed by assessment of effects on cellular senescence kinetics.

We identified that 3 FOXO1/ETV6-regulated miRNAs demonstrated elevated expression in aged cells, and 1 demonstrated reduced expression. Targeted manipulation of 2 miRs with elevated expression in late passage cells brought about alterations in the relative gene expression of SRSF3, SRSF6 and NOVA1 splicing factors ($p = 0.003, 0.029, 0.006$ and $p = 0.011, 0.016, 0.011$ for treatments 1 and 2 respectively, a 13.65% and 18.84% drop in senescent cell load ($p = 0.020$ and 0.008 respectively), a drop in the relative gene expression of the pro-inflammatory SASP component IL6 from 1.008 to 0.265 and 0.330 ($p = 0.0002$ and 0.020 respectively). These effects were seen without re-entry to cell cycle.

Our data indicates that these miRs may act as potential points of intervention between FOXO1/ETV6 and splicing factor expression for the attenuation of senescent cell load in vitro and potentially in vivo.

MicroRNA profiling of cerebrospinal fluid from preterm infants following prenatal brain injury

Gialeli A [1,2] , Spaull R[2], Plösch T [1] , Uney J [2] , , Heep A [12] , Cordero-Llana Ó [2]

[1]Perinatal Neurobiology lab, Fakultät für Medizin und Gesundheitswissenschaften, Carl von Ossietzky Universität Oldenburg, Oldenburg, Germany

[2]Regenerative Medicine lab, Bristol Medical School, University of Bristol, Bristol, United Kingdom

Background: Preterm infants are at high risk of suffering from brain injury due to intraventricular haemorrhage (IVH) which causes the accumulation of cerebrospinal fluid (CSF). Studying the CSF that accumulates in IVH allows the molecular signalling and intracellular communication that contributes to pathogenesis to be elucidated. Extracellular vesicles (EVs) have been isolated from CSF and shown to mediate intercellular communication via selective enrichment in proteins and microRNAs.
The aim of this study was to explore the potential interactions between signalling molecules, such as miRNAs, in the CSF of preterm infants with neural progenitor cells (NPCs) in the pre-term developing brain. We hypothesized the changes in miRNA content in the IVH-CSF could occur as a protective mechanism or contribute to the pathogenesis seen in the developing brain.

Methods: The levels of 2,083 miRNAs in fifteen CSF samples across the duration of CSF sampling, from ten infants with IVH, were profiled using miRNA whole transcriptome assays. Gene ontology (GO) analysis was performed by MiEAA tool. Immunostaining was performed in human foetal NPCs treated with either IVH-CSF or EVs extracted from IVH-CSF and were analysed by fluorescent microscopy.

Results: 587 miRNAs were differentially expressed in the CSF extracted after at least two months following injury, compared to CSF extracted within the first month of injury. GO analysis showed that these miRNAs are regulating JAK/STAT pathway. IVH-CSF regulated the JAK/STAT pathway in human foetal NPCs and promoted their differentiation towards astrocytes. EVs extracted from IVH-CSF partially mediated this astroglial switch.

Conclusions: Our results provide novel insights into potential mechanisms of CSF-NPCs interactions during brain development following perinatal brain injury.

Gialeli A [1,2] , Spaull R[2], Plösch T [1] , Uney J [2] , Heep A [12] , Cordero-Llana Ó [2]

Transcriptional repression by WT1-BASP1

Alexander J. Moorhouse, Amy E. Loats, Phoebe Crawley, Marina Johnson, Kathryn F Medler and Stefan G. E. Roberts*

Cell and Molecular Medicine, University of Bristol

Abstract

The transcriptional corepressor BASP1 requires lipidation for its activity and previous studies demonstrate that it performs multiple chromatin modifications. Here we determine the role of BASP1 lipidation in the placement and removal of several histone modifications. We find that the removal of active chromatin marks by BASP1 requires the N-terminal myristoylation of BASP1. In contrast, the placement of the repressive histone mark by BASP1, H3K27me3, does not. Our findings reveal that BASP1 has both lipid-dependent and lipid-independent functions in transcriptional repression. RNA-seq and ATAC-seq analysis finds that BASP1 regulates a substantial number of genes and induces extensive changes in chromatin accessibility.

Chromatin interaction maps identify Wnt responsive *cis*-regulatory elements coordinating *Paupar-Pax6* expression in neuronal cells

Ioanna Pavlaki, Michael Shapiro, Giuseppina Pisignano, Stephanie M. E. Jones, Jelena Telenius, Silvia Muñoz-Descalzo, Robert J. Williams, Jim R. Hughes and Keith W. Vance

Central nervous system-expressed long non-coding RNAs (lncRNAs) are often located in the genome close to protein coding genes involved in transcriptional control. Such lncRNA-protein coding gene pairs are frequently temporally and spatially co-expressed in the nervous system and are predicted to act together to regulate neuronal development and function. Although some of these lncRNAs also bind and modulate the activity of the encoded transcription factors, the regulatory mechanisms controlling co-expression of neighbouring lncRNA-protein coding genes remain unclear. Here, we used high resolution NG Capture-C to map the *cis*-regulatory interaction landscape of the key neuro-developmental *Paupar-Pax6* lncRNA-mRNA locus. The results define chromatin architecture changes associated with high *Paupar-Pax6* expression in neurons and identify both promoter selective as well as shared *cis*-regulatory-promoter interactions involved in regulating *Paupar-Pax6* co-expression. We discovered that the TCF7L2 transcription factor, a regulator of chromatin architecture and major effector of the Wnt signalling pathway, binds to a subset of these candidate *cis*-regulatory elements to coordinate *Paupar* and *Pax6* co-expression. We describe distinct roles for *Paupar* in *Pax6* expression control and show that the *Paupar* DNA locus contains a TCF7L2 bound transcriptional silencer whilst the *Paupar* transcript can act as an activator of *Pax6*. Our work provides important insights into the chromatin interactions, signalling pathways and transcription factors controlling co-expression of adjacent lncRNAs and protein coding genes in the brain.

Exosome-like vesicles secreted by the parasitic nematodes *Strongyloides* target and manipulate host genes

Mona Suleiman[1], Asuka Kounosu[2], Mehmet Dayi[2], Akemi Yoshida[2], Taisei Kikuchi[2] and Vicky L. Hunt[1]

Author address(es):

[1] Department of Biology and Biochemistry, University of Bath, Bath, BA2 7AY UK

[2] Parasitology, Department of Infectious Dieses, Faculty of Medicine, University of Miyazaki, Miyazaki, 889-1692 Japan

Abstract: Parasitic nematodes are estimated to infect more than 1.5 billion people globally, causing a substantial disease burden. Understanding how these parasites infect their host is an important step towards developing novel ways of controlling and treating nematode infections. Recently, it has been discovered that nematodes secrete small RNAs (sRNAs) within exosome-like vesicles (ELVs) into their host. The ELVs can directly target components of the host immune response including host genes and proteins, thereby improving the survival of the parasite. Here, we have investigated the role of ELVs in the parasitic nematodes *Strongyloides ratti* and *S. venezuelensis*, the gastrointestinal parasites of humans and animals. Our results have shown that both *Strongyloides* species secrete ELVs that are taken up by host cells and which are enriched with sRNAs that target host genes. The ELVs are enriched with a cocktail of different classes of sRNAs, including putative short interfering RNAs (siRNAs) and microRNAs (miRNAs). We hypothesise that these sRNAs manipulate host gene expression, particularly immune-related genes important in the anti-nematode immune response.

ELAV forms a saturable complex on extended RNA, but can nucleate

David McQuarrie[1] and Matthias Soller[1]

[1]School of Biosciences, University of Birmingham, Birmingham, UK, B15 2TT

The RNA binding protein (RBP) ELAV (Embryonic Lethal Abnormal Visual system) is a gene-specific regulator of alternative pre-mRNA processing in *Drosophila* neurons. ELAV/Hu family proteins bind to short U-rich motifs which are found in most pre-mRNAs, making it unclear how ELAV and other RBPs achieve gene specificity. ELAV/Hu proteins have been shown to multimerise and ELAV forms a dodecameric complex on ewg RNA. However, it is unclear how ELAV recognises degenerate sequence motifs and whether multimerisation is a mechanism for gene specificity. Here we show that ELAV forms a saturable complex with extended RNA and has the ability to nucleate from a single binding element. We reveal that the *ewg* binding site forms a stemloop secondary structure that is unwound upon ELAV binding starting at three distal AU_4 motifs. Further, we indicate that ELAV poly(A) sites are enriched in stemloops, and that these correlate with known AU_4 motifs. Through binding assays, we probe the *ewg* site and show that ELAV requires consecutive U-rich motifs for binding. Furthermore, through cross-species analysis and binding assays we describe a minimal binding element for ELAV target recognition. Our findings address a crucial gap in how ELAV/Hu family RBPs recognise degenerate sequences for gene-specific mRNA processing and could provide a gene-specific template for improved therapeutic targets in disease.

Using nanopore sequencing to identify transcript variation between different life cycle stages of the parasitic nematode *Strongyloides ratti*

Dominika Lastik, Ben Murcott, Vicky Hunt

Author address(es): Department of Biology and Biochemistry, University of Bath, BA27AY

Abstract (250 words max):
The parasitic nematode *Strongyloides* has a unique lifecycle that alternates between genetically identical parasitic and free-living generations, a great model to study parasitism. Identification and characterisation of lifecycle specific gene expression is important for understanding the fundamental principles of parasitic mechanisms at a molecular and genetic level. mRNAs are differentially expressed in the lifecycle stages, implying an underlining mechanism of post-transcriptional gene regulation. However, most of our knowledge about mRNA transcripts in parasites is incomplete as it relies on short-read sequencing, which is often missing information about alternative splicing, 5'UTRs and 3'UTRs, which are associated with an important role in gene regulation. Using Oxford Nanopore long-read sequencing, we have identified full-length transcripts for the gastrointestinal parasite *Strongyloides ratti* for five lifecycle stages. Together, we have obtained 60 million reads with a minimum of 76% of reads representing full-length transcripts per sample. The sequenced lifecycle stages of *S. ratti* include genetically identical parasitic and free-living adults, both with at least two million full-length transcripts per replicate. We have identified more than 10,000 novel transcript variants and 4000 putative novel genes across the five lifecycle stages. In at least **80%** and almost **50%** of full-length mRNA we have established the 3'UTR or a 5'UTR sequences, respectively. To better understand the mechanisms of gene regulation in parasitism, we investigate how alternatively spliced transcripts and UTRs vary between lifecycle stages to help us recognise the role of differentially expressed transcript variants and UTRs in parasitism and how long-read sequencing can improve current genome annotations.

Gene expression analysis reveals a 5 gene signature for progression-free survival in prostate cancer

Zhuofan Mou[1], Jack Spencer[2], Bridget Knight[3], Joseph John[1,4], Paul McCullagh[5], John S McGrath[1,4], Lorna W. Harries[1*]

[1]Institute of Biomedical and Clinical Sciences, University of Exeter Medical School, Devon, UK
[2]Translational Research Exchange @ Exeter, Living Systems Institute, University of Exeter, Exeter, U.K.
[3]NIHR Exeter Clinical Research Facility, Royal Devon and Exeter NHS Foundation Trust, Royal Devon and Exeter Hospital, Barrack Road, Exeter, UK
[4]Exeter Surgical Health Services Research Unit, Royal Devon and Exeter NHS Foundation Trust, Exeter, United Kingdom.
[5]Department of Pathology, Royal Devon and Exeter NHS Foundation Trust, Exeter, UK

Prostate cancer (PCa) is the second most common male cancer worldwide but effective biomarkers for the presence or progression-risk of disease are currently elusive. In a series of 9 matched histologically-confirmed PCa and benign samples, we carried out an integrated transcriptome-wide gene expression analysis, including differential gene expression analysis and weighted gene co-expression network analysis (WGCNA), which identified a set of potential gene markers highly associated with tumour status (malignant vs. benign). We then used these genes to establish a minimal progression-free survival (PFS) associated gene signature (GS) (*PCBP1*, *PABPN1*, *PTPRF*, *DANCR* and *MYC*) using least absolute shrinkage and selection operator (LASSO) and stepwise multivariate Cox regression analyses from The Cancer Genome Atlas prostate adenocarcinoma (TCGA-PRAD) dataset. Our signature was able to predict PFS over 1-, 3-, and 5-years in the TCGA-PRAD dataset, with area under the curve (AUC) of 0.64 to 0.78, and our signature remained as a prognostic factor independent of age, Gleason score, and pathological T and N stages. A nomogram combining signature and Gleason score was constructed which demonstrated improved predictive capability for PFS (AUC: 0.71-0.85), and was superior to the CPG model alone in predicting PFS. In conclusion, we have identified and validated a novel five-gene signature and established a nomogram that effectively predicted PFS in patients with PCa. The findings may improve current prognosis tools for PFS and contribute to clinical decision-making in PCa treatment.

Keywords: prostate cancer (PCa), gene expression, transcriptomics, predictive model

Human embryonic genome activation initiates at the one-cell stage

Maki Asami[1], Brian YH Lam[2], Marcella K Ma[2], Kara Rainbow[2], Stefanie Braun[3], Matthew D VerMilyea[3], Giles S H Yeo[2], Anthony C F Perry[1]

[1]Laboratory of Mammalian Molecular Embryology, Department of Biology and Biochemistry, University of Bath, Bath BA2 7AY, England

[2]MRC Metabolic Diseases Unit, Wellcome-MRC Institute of Metabolic Science, Addenbrooke's Hospital, University of Cambridge, Cambridge CB2 0QQ, England

[3]Ovation Fertility Austin, Embryology and Andrology Laboratories, Austin, TX 78731, USA

Abstract

Fertilizing spermatozoa and metaphase II (mII) oocytes are transcriptionally quiescent. The first transcription in newly formed embryos is known as embryonic genome activation (EGA), but its onset, timing, and profile are poorly understood. In human embryos, EGA is held to have occurred by the eight-cell stage, up to ~68 h (~3 days) after fertilization, but this model is likely incomplete: its exact timing and profile are unclear. To address this, we profiled gene expression at depth in human metaphase II oocytes and bipronuclear (2PN) one-cell embryos. High-resolution single-cell RNA sequencing revealed previously inaccessible oocyte-to-embryo gene expression changes. This confirmed transcript depletion following fertilization (maternal RNA degradation) but also uncovered low-magnitude upregulation of hundreds of transcripts. Upregulated transcripts are spliced, and their gene expression analysis predicted embryonic processes including cell-cycle progression and chromosome maintenance as well as transcriptional activators that included cancer-associated gene regulators. Transcription was disrupted in abnormal monopronuclear (1PN) and tripronuclear (3PN) one-cell embryos. These findings indicate that human embryonic transcription initiates at the one-cell stage, sooner than previously thought. The pattern of gene upregulation promises to illuminate processes involved at the onset of human development, with implications for epigenetic inheritance, stem-cell-derived embryos, and cancer.

Blood RNA-seq in diagnostic genomic medicine

Andrew G L Douglas[1,2], Htoo A Wai[1], Carolina Jaramillo Oquendo[1], Jenny Lord[1], David Hunt[3], N. Simon Thomas[4], Diana Baralle[1,3]

1. Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK
2. Oxford Centre for Genomic Medicine, Oxford University Hospitals NHS Foundation Trust, Oxford, UK
3. Wessex Clinical Genetics Service, University Hospital Southampton NHS Foundation Trust, Southampton, UK
4. Wessex Regional Genetics Laboratory, Salisbury District Hospital, Salisbury, UK

Background/Objectives:
RNA sequencing (RNA-seq) can identify changes in splicing not readily apparent through DNA analysis, allowing better functional interpretation of genetic variants of uncertain significance (VUSs). Blood is the most readily available clinical tissue for RNA analysis. However, the degree to which blood RNA-seq provides adequate coverage for analysing splicing in different genes has not been well studied. Here, we have investigated the sensitivity of RNA-seq for the detection of splicing and other abnormalities.
Methods:
RNA was extracted from blood in 56 patients with likely genetic disorders, 33 with candidate VUSs. RNA-seq was performed in three batches at 70M 150-bp paired-end reads per sample with STAR alignment to GRCh38. Transcripts per million (TPM) values were calculated for all genes using stranded raw read counts. Splice junction and intronic reads were counted, filtered and annotated for skewed usage. UK Genomic Medicine Service (GMS) PanelApp gene panels were used to filter outputs.
Results:
72% of GMS genes had TPM>1 and 65% of observed annotated junctions had sufficient average coverage to detect alternative splicing occurring at a 0.5 usage level (55% at 0.25 usage, 42% at 0.1 usage). Genes with identified splicing abnormalities were not consistently down-regulated when compared across samples within the same RNA-seq batch. The RNA effects of previously identified chromosomal microdeletions were generally visible as contiguous regions of decreased gene expression. Skewed X-inactivation was identified in one case.
Conclusions:
Blood RNA-seq can detect not only splicing abnormalities but also chromosomal microdeletions and skewed X-inactivation.