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	TIME	SPEAKER	INSTITUTION	TALK TITLE
Welcome and Introduction: *Michael Ladomery & Sebastian Oltean*	9.55-10.00			
MicroRNAs I *Chair: Mark Lindsay*	10.00-10.15	Dr Kate Simpson	Cardiff University	Altered urinary microRNA profiles in diabetic nephropathy
	10.15-10.30	Dr Lucy Newbury	Cardiff University	Downregulated miR-192 and upregulated miR-141 are associated with non-recovery in Acute Kidney Injury
	10.30-10.45	Daniel Smith	Cardiff University	Direct electrochemical detection of urinary microRNAs via sulphonamide-bound antisense hybridisation
	10.45-11.00	Marina Batistuti	c/- Bath University (University of São Paulo)	Highly sensitive dual mode electrochemical platform for microRNA detection
Tea/Coffee	11.00-11.30			
Pre-mRNA SPLICING *Chair: Lorna Harries*	11.30-11.45	Francesca Carlisle	Cardiff University	Alternative splicing generates sarcoglycan isoforms with altered trafficking and protein interactions
	11.45-12.00	Elizabeth Bowler	University of the West of England	The effect of hypoxia on alternative splicing in prostate cancer cells
	12.00-12.15	Dr Megan Stevens	Bristol University	Manipulation of VEGF-A splicing using natural compounds as a potential therapeutic in diabetic nephropathy
	12.15-12.30	Ling Li	Bristol University	Modulation of alternative splicing regulators in epithelial-mesenchymal transitions during tumour progression and fibrosis

Lunch	12.30-13.30			
KEYNOTE LECTURE *Chair: Sebastian Oltean*	13.30-14.30	Prof Chris Smith	University of Cambridge	Understanding alternative splicing programmes and molecular mechanisms
	14.30-.14.45	Dr Eva Latorre	University of Exeter	Reduction of SASP response may rescue senescent cells by moderation of alternative splicing
MicroRNAs II *Chair: Tim Bowen*	14.45-15.00	Dr Rocio Martinez-Nuñez	University of Southampton	RibomiR-seq reveals that global microRNA binding fine-tunes the
	15.00-15.15	Melisa Lopez-Anton	Cardiff University	Regulation of mesothelial to mesenchymal transitions by miRNAs
	15.15-15.30	Ben Lee	University of Exeter	MicroRNAs 203, 664 and 708 are associated with strain lifespan in mouse spleen tissue
Tea/Coffee	15.30-16.00			
MISCELLANEOUS *Chair: Michael Ladomery*	16.00-16.15	Nicola Jeffery	University of Exeter	A more physiologically relevant set of culture conditions for EndoC BH1 human beta cells?
	16.15-16.30	Rosina Savisaar	University of Bath	Interactions with RNA-binding proteins constrain coding sequence evolution
	16.30-16.45	Dr Mark Pearson	University of Birmingham	LincRNAs mediate the inflammatory response in human OA joint tissues
	16.45-17.00	Dr Mirna Mourtada-Maarabouni	University of Keele	NEAT1, a long non-coding RNA, controls breast cancer cell survival and is up-regulated in breast cancer
Concluding remarks *Michael Ladomery & Sebastian Oltean*	17.00-17.10			

ALTERED URINARY microRNA PROFILES IN DIABETIC NEPHROPATHY

Kate Simpson[1], Cristina Beltrami[1], Mark Jesky[2], Christopher Carrington[1], Alexa Wonnacott[1], Lucy Newbury[1], Robert Jenkins[1], Simon Satchell[3], Paul Cockwell[2], Donald Fraser[1], Timothy Bowen[1]

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Aims: MicroRNAs (miRNAs) are endogenous, short, non-coding single-stranded RNA transcripts that regulate gene expression at the post-transcriptional level. The utility of urinary miRNAs as novel, non-invasive biomarkers for diabetic nephropathy (DN) was investigated in this study.

Methods: Urinary miRNAs from DN patients with established chronic kidney disease (n = 20) and from unaffected control subjects (n = 20) were quantified by Taqman Low Density Array analysis. Candidates were validated by qRT-PCR in two independent cohorts (early-stage DN (n = 99) and late-stage DN (n = 70)). Selected validated miRNAs were localized to nephron segments using laser capture microdissection of renal biopsy tissue, together with analysis of cell lines of renal origin cultured *in vitro*.

Results: Widespread differences in miRNA profiles were detected in urine from DN patients when compared to controls. For three key miRNAs, these statistically significant differences were then replicated in the individual urine samples from which the pools were composed. Further analyses of these transcripts in independent patient cohorts validated significant miRNA expression changes in disease. Following laser capture micro-dissection of biopsy tissue, expression of one target miRNA was seen in the renal glomerulus, proximal and distal tubules, while the other was localised to the glomerulus. Further *in vitro* analysis detected the latter miRNA primarily in the glomerular endothelial cell, and revealed transcript release in response to tumour necrosis factor-α.

Conclusions: Our data have revealed miRNA expression changes characteristic of DN. Furthermore, statistically significant DN-associated differences in the expression of key miRNAs were supported in large independent patient cohorts, and variation in one of these miRNAs was linked to release from glomerular endothelial cells.

DOWNREGULATED miR-192 AND UPREGULATED miR-141 ARE ASSOCIATED WITH NON-RECOVERY IN ACUTE KIDNEY INJURY

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Introduction: Acute kidney injury (AKI) affects 18% of UK hospital admissions, doubling length of stay and risk of death in hospital inpatients. Current biomarkers have limited ability to classify disease and stratify rapidly progressing patients. In this study, we analysed association of urinary miRNA expression with disease aetiology and progression in an AKI patient cohort. To investigate the mechanisms of candidate miRNA biomarkers in AKI, we developed a human proximal tubule epithelial cell (PTC) *in vitro* model using H_20_2 to simulate the oxidative stress seen in *in vivo* post-ischaemic injury.

Methods: Urine samples were collected from 30 stage III AKI patients over a 30-day period, with day 1 (D1) representing entry into the study. Expression of >750 miRNAs was analysed by Taqman Low Density Array (TLDA) in D1 recovery (n=6) and non-recovery (n=5) pooled urine samples. RT-qPCR detection of candidate miRNAs identified by TLDA analysis was then compared between our D1 AKI patient cohort and unaffected individuals (n=10), we also compared recovery with non-recovery, and non-immune with immune disease groups.

Results: Comparison of the D1 AKI cohort with control subjects revealed significant up-regulation of miR-21 (5-fold) and miR-141 (2-fold) expression, with corresponding down-regulation of miR-192 (70%) and miR-204 (40%). Decreased miR-192 (ROC Curve, AUC: 0.9667) and increased miR-141 (AUC: 0.8444) detection predicted non-recovery, while increased miR-21 was associated with underlying inflammatory glomerular disease (AUC: 0.88).

Conclusions: We have demonstrated association of key miRNA expression with AKI, inflammatory glomerular disease and non-recovery from disease. These data underline the potential of these transcripts as biomarkers, and we are currently validating these observations in larger, independent patient cohorts.

DIRECT ELECTROCHEMICAL DETECTION OF URINARY microRNAs VIA SULFONAMIDE-BOUND ANTISENSE HYBRIDISATION

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MicroRNAs (miRNAs) are ubiquitous endogenous, single-stranded noncoding RNA transcripts, most frequently of 19-25 nucleotides in length, that act as posttranscriptional regulators of gene expression by blocking protein translation and/or inducing messenger RNA degradation. It is currently estimated that miRNAs regulate the expression of at least 60 % of all human protein coding genes, and aberrant miRNA expression profiles have been observed in numerous pathological processes. There is therefore much current interest in miRNAs as novel biomarkers, and methods for sensitive and selective miRNA detection have significant potential for use as diagnostic biosensors.

Here we describe the development of a highly sensitive and specific, straightforward electrochemical biosensor assay for the detection and quantification of urinary miRNAs requiring minimal sample treatment. This was achieved using a fabricated probe in which single-stranded DNA oligonucleotides complementary to target miRNA sequences were bound to a glassy carbon electrode surface via sulfonamide linkages. This biosensor successfully discriminated between miR-21 sequences with a single nucleotide change, detected femtomolar miR-21 and selectively detected miR-16. Electrochemical analytical data for human urine samples were comparable to data obtained using the RT-qPCR urinary miRNA detection method that we have recently described.

To expedite commercialisation of our biosensor assay, we are currently adapting this technique for the use of disposable electrodes.

HIGHLY SENSITIVE DUAL MODE ELECTROCHEMICAL PLATFORM FOR microRNA DETECTION

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MicroRNAs are small non-coding RNAs that that play key roles in the regulation of gene expression of more than 60% of protein-coding genes. The level of miRNAs in blood can act like blueprints for the diagnosis and prognosis of a number of diseases, including cancer. However, these miRNAs are present in blood at very low levels, demanding novel detection techniques with very high sensitivity.

We report the development of a highly sensitive electrochemical platform for the detection of MicroRNAs using peptide nucleic acids (PNA) as probes on gold electrode surfaces to capture target miRNAs. A simple amplification strategy using gold nanoparticles (AuNPs) have been employed. Electrochemical impedance spectroscopy (EIS) was used to monitor the changes upon anybinding event, without redox markers. A complementary detection mode was developed using thiolated ferrocene. The increasing peaks of ferrocene was recorded using square wave voltammetry (SWV) with increasing miRNA concentration. Thus, giving a dual mode detection using the same chip, a limit of detection of 0.37 fM was achieved with clear distinction from mismatched miRNA sequences. This electrochemical platform could be easily expanded to other miRNA/DNA detection along with future development of a microarray platforms.

ALTERNATIVE SPLICING GENERATES SARCOGLYCAN ISOFORMS WITH ALTERED TRAFFICKING AND PROTEIN INTERACTIONS

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The sarcoglycans are a family of six transmembrane glycoproteins, some of which are widely expressed. In muscle and other tissues the sarcoglycans form a heterotetramer subcomplex of the dystrophin-associated glycoprotein complex; this helps stabilise the sarcolemma and is involved in signal transduction in muscle. Complex composition depends on which sarcoglycans are expressed in a given tissue. While mutations in four of the sarcoglycans (*SGCA, SGCB, SGCD* and *SGCG*) cause limb-girdle muscular dystrophy, mutations in *SGCE* encoding ε-sarcoglycan cause the neurological disorder myoclonus-dystonia syndrome (MDS). Tissue-specific splicing of *SGCE* mRNA generates a brain-specific isoform of ε-sarcoglycan which may contribute to the brain-specific MDS phenotype. We sought to identify whether other sarcoglycans produced alternatively spliced transcripts that could affect sarcoglycan complex behaviour by screening mini-libraries of skeletal muscle and cerebellum mRNA derived from *SGCA, SGCB, SGCD, SGCG* and *SGCZ*. Each gene had at least two alternatively spliced transcripts with some generating many more, though none appeared to be cerebellum-specific. Alternatively spliced *SGCB* and *SGCD* transcripts encoded putative non-functional isoforms, but *SGCA*, *SGCG* and *SGCZ* all had alternatively spliced transcripts encoding protein isoforms with altered protein interactions and/or cellular localisation. These isoforms provide insight into the assembly of sarcoglycan complexes, and could help inform the application of exon skipping as a treatment for the sarcoglycanopathies.

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 is particularly significant in aggressive, fast-growing tumours in which the rate of new blood vessel formation (angiogenesis) cannot match the growth rate of tumour cells. Tumour hypoxia has been linked to poor prognosis and to greater resistance to existing cancer therapies. Hypoxia can cause cells to undergo apoptosis; however tumour cells can evade hypoxia-induced apoptosis. We hypothesize that one of the ways that tumour cells adapt to hypoxia is by changing the alternative splicing of key genes.

CAIX is one of the best studied hypoxia markers, involved in maintaining an intracellular pH that favours tumour cell growth. We confirmed a hypoxia-specific change in the alternative splicing of *carbonic anhydrase IX* (*CA IX*) in response to 1% oxygen. A high throughput PCR analysis provided evidence of significant changes in the alternative splicing of several other cancer-associated genes including *FGFR1OP*, *APAF1* and *PUF60* in PC3 prostate cancer cells. We also examined the effect of hypoxia on the expression and localisation of SRSF1 and the splice factor kinases CLK1 and SRPK1. Chemical inhibitors of CLK1 (TG003) and SRPK1 (SPHINX) were used to assess the effect of inhibiting splice factor kinases on the alternative splicing of cancer-associated genes. We present evidence that CLK1 is involved in the modulation of alternative splicing in response to hypoxia.

MANIPULATION OF VEGF-A SPLICING USING NATURAL COMPOUNDS AS A POTENTIAL THERAPEUTIC IN DIABETIC NEPHROPATHY

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Introduction. Vascular endothelial growth factor (VEGF-A) is differentially spliced to give two functionally different isoform families, the pro-angiogenic VEGF-A_{xxx} and the anti-angiogenic VEGF-A_{xxx}b. The $_{xxx}$b isoforms, in particular $VEGF_{165}$b, has previously been shown to rescue / provide protection in several murine models of renal disease (Oltean et al. 2012; Oltean, Qiu et al. 2015). VEGF-A_{165}b also provides cyto-protection to podocytes in culture (Bevan et al. 2008).

This study aimed to investigate whether a blueberry and seabuckthorn extract, with the ability to switch VEGF-A splicing to promote VEGF-A_{165}b, is a potential therapeutic in mouse models of diabetic nephropathy.

Methods. DBA/2J mice injected with streptozotocin were used as a type I model of diabetic nephropathy. In study 1, mice were given the natural extract in their drinking water once blood glucose levels became elevated. In study 2, mice were given the natural extract once they had developed diabetic nephropathy (albuminuria 5 fold above baseline). Urinary albumin creatinine ratio (uACR) and blood glucose was measured weekly. Upon culling, the glomerular water permeability (L_pA/V_i) was assessed using an oncometric assay described by Salmon et al. (2006). Expression levels of the glomerular proteins nephrin, podocin and pecam were investigated by immunofluorescence and western blotting, and glomerular structure via PAS staining.

HUVEC tube formation in response to natural extract treatment was also assessed using a matrigel angiogenesis assay with pre-conditioned media from natural extract-treated podocytes.

Results. Study 1) the natural extract had no effect on blood glucose levels, however it did delay the onset of albuminuria compared with diabetic control mice. There was however no effect on glomerular L_pA/V_i at the time point of culling. Study 2) treatment of established albuminuria with the natural extract prevented further increases in albuminuria, compared to diabetic control mice. Natural extracted treated mice also had a lower glomerular L_pA/V_i. Diabetic control mice had a reduced glomerular expression of nephrin and reduced glomerular capillary circumference, which were rescued when treated with the natural extract.

HUVECs treated with conditioned media from podocytes treated with the extract showed reduced tube formation when plated onto matrigel. However, this was overcome upon addition of an antibody for VEGF-A_{165}b.

Conclusions. Certain unknown compounds within the blueberry and seabuckthorn extract are able to switch VEGF-A splicing to promote VEGF-A_{165}b expression in podocytes. The same extract plays a protective role in the prevention and treatment of diabetic nephropathy in the DBA/2J type I diabetic mouse model. This study highlights the therapeutic potential of switching VEGF-A splicing to promote VEGF-A_{165}b in diabetic nephropathy.

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MODULATION OF ALTERNATIVE SPLICING REGULATORS IN EPITHELIAL-MESENCHYMAL TRANSITIONS DURING TUMOUR PROGRESSION AND FIBROSIS.

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The epithelial-mesenchymal transition (EMT), known as one of the hallmarks of cancer, is an important process by which epithelial cells undergo multiple biochemical changes, show reduced intercellular adhesion and increased motility and invasiveness to become mesenchymal cells(1). EMT is regulated at different levels including alternative splicing. Epithelial splicing regulatory proteins 1 and 2 (ESRP1 and ESRP2) are established as master regulators of EMT during tumour progression and fibrosis (2, 3). ESRPs control splicing events of about 200 genes that give epithelial phenotype. Loss of ESRPs in EMT induces the phenotypic changes – epithelial to mesenchymal. One of the well-known genes regulated by ESRPs is the fibroblast growth factor receptor 2 (FGFR2) (4) which has a switch between two mutually exclusive isoforms - FGFR2-III b and FGFR2-III c during the EMT (5). As FGFR2 splicing is a sensor of ESRPs activity and EMT, we used a splicing-sensitive fluorescent reporter based on inclusion/exclusion of FGFR2 exon IIIc to understand the modulation of ESRPs and therefore EMT. Using this reporter we have performed a screen with the LOPAC library (Library of Pharmacologically Active Compounds) to identify compounds that induce exon IIIc skipping in FGFR2 and potentially block EMT. We have identified several compounds that are able to switch FGFR2 splicing, and currently being validated as modulators of EMT. The hit compounds show various activities on functional assays in prostate cancer PC3 cells. For example, in cell growth curve- one killed the cells and another slowed down the growth, and in migration assays one of them significantly decreases the cell migration rate.

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REDUCTION OF SASP RESPONSE MAY RESCUE SENESCENT CELLS BY MODERATION OF ALTERNATIVE SPLICING

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The load of senescent cells in rodents has recently been shown to be a major contributor to the ageing process. Gradual accumulation of senescent cells over the life-course is therefore likely to contribute to age-related disease and degeneration in humans. Although the precise underlying mechanisms are as yet unclear, it is known that senescent cells secrete a paracrine signal (senescence-associated secretory phenotype; SASP) which may enforce and/or maintain growth arrest in non-senescent cells. The SASP may also alter alternative splicing patterns by moderation of splicing factor expression.

We treated replicatively senescent human primary dermal fibroblast cells with a panel of known and novel compounds and assessed the effects on a series of cellular parameters including biochemical and molecular markers of senescence, splicing regulatory factor expression, alternative splicing of key senescence and proliferation genes, levels of SASP proteins, apoptosis (TUNEL) and proliferation (Ki67).

Treatment reduced several components of SASP (GM-CSF, IL10, IL6, IL8, IL2, TNF□, IFN□). These changes were accompanied by global changes in the expression of splicing regulatory factors and changes to the alternative splicing of genes involved in regulation of cellular proliferation (*TP53, RB1, CHEK1, CHEK2, CDKN1A, CDKN2A*). A concordant drop in the expression levels of molecular markers of cellular senescence (e.g *CDKN2A*) also occurred, accompanied by an increase in Ki67 staining in treated cells, but no evidence of increased apoptosis was observed.

Our results suggest that treatment modulates the paracrine signal mediating growth arrest. The mechanism of this may involve moderation of mRNA splicing pathways so as to push the alternative splicing patterns of key proliferative genes towards a more proliferative and less quiescent phenotype.

RIBOMIR-SEQ REVEALS THAT GLOBAL microRNA BINDING FINE-TUNES THE TRANSCRIPTOME BUT PROFOUNDLY ALTERS THE TRANSLATOME IN SEVERE ASTHMA

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Asthma is a chronic airways disease characterized by inflammation and tissue remodeling. Although asthma has been studied at the genome (DNA) and transcriptome (mRNA) levels, its global underlying molecular mechanisms remain poorly understood. Importantly, no studies have investigated global translation in asthma. Because transcription and translation correlate poorly, we hypothesized that, in asthma, changes in translation (polyribosome-bound mRNAs, Polysome) may reveal insight not evident when analyzing solely cytoplasmic mRNA (Total). We developed RibomiR-seq: high throughput sequencing of mRNA and small RNA in total- (Total) and polyribosome- (Polysome) sub-cellular fractions in human bronchoepithelial cells from severe asthmatics and healthy donors. We then analysed the expression levels of genes, isoforms and microRNAs on each fraction. Our data reveals that cytoplasmic mRNA analysis (883 dysregulated genes) misses ~ 80% of dysregulated translating mRNAs (1166 dysregulated genes) in asthma. Traditionally, mild/moderate asthma is associated to Th2-responses; in severe asthma, IL1- and Th1/Th17-related inflammatory pathways within Polysome genes were missed when only considering transcriptional expression. We found 1636 mRNA isoforms differentially engaged in translation of which only ~15% were revealed by cytoplasmic mRNA analysis. Thus, asthmatic airway cells fail in regulating both global alternative splicing and the binding of specific isoforms to polyribosomes, the latter mapping to epithelial remodeling pathways. Finally, our data show that microRNAs fine tune total mRNA levels but cause sharp changes in translation in severe asthma airway epithelium. Our results highlight a novel defect in human pathology affecting posttranscriptional gene regulation: a failure to coordinate genome-wide alternative splicing and translation in asthma.

REGULATION OF MESOTHELIAL TO MESENCHYMAL TRANSITION BY miRNAs

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Peritoneal dialysis (PD) is a renal replacement therapy for End Stage Kidney Disease. Peritoneal fibrosis is a problem for PD patients, and mesothelial cells may undergo mesothelial-to-mesenchymal transition (MMT) contributing to fibrosis and treatment failure. miRNAs are important regulators of fibrosis but their roles in peritoneal fibrosis are largely unknown. We performed a MMT process characterization in primary human mesothelial cells (HPMCs) in response to Transforming Growth Factor beta-1 (TGF-β1). Hybridization array showed mesothelial miR-21 and miR-31 expression up-regulation by TGF-β1 which was validated in different PD associated MMT models. HPMCs cultured *ex vivo* from PD patients exhibited phenotypic changes consistent with a progressive MMT process that correlated with increased miR-21 and miR-31 expression. Association of miRNA expression and MMT markers in 33 peritoneal biopsies from patients undergoing PD treatment and in PD effluent from 230 patients confirmed these results. *In silico* analysis combined 4 target prediction algorithms (Targetscan, miRanda, miRDB and Diana-microT) for miR-21 and integrated the resulting outcome with mRNA arrays comparing omentum vs PD effluent-derived HPMCs with epithealial (E) and non-epithelial (NE) phenotype. 13 possible miR-21 targets during the MMT process associated to PD therapy were identified and model scrutinized. Four of these were confirmed to be miR-21 targets. Functional gene analysis indicated that selected targets may be downstream modulators of Snail and cooperate driving peritoneal MMT. These data provide a detailed characterisation of mesothelial miRNA expression and responses to TGF-β1, and identify miR-21 and miR-31 as promising biomarkers for peritoneal fibrosis associated to PD therapy.

MicroRNAs 203, 664 AND 708 ARE ASSOCIATED WITH STRAIN LIFESPAN IN MOUSE SPLEEN TISSUE

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Advancing age confers increased risk of many chronic illnesses which negatively impact both quality of life and healthcare spending. It is known that a positive correlation exists between lifespan (attained age) and healthspan (proportion of life spent disease-free), so the study of lifespan may provide insight into improvement of healthspan.

Lifespan is a heritable trait, although twin studies have shown only 20-30% of the variation is attributable to genetics alone, so epigenetic and post-transcriptional mechanisms are likely to be important. This study focuses on the potential effect of microRNAs on longevity in a mammalian model organism. We have access to tissues from both young and old mice from 6 strains with different median lifespans, ranging from 623 days to 1005 days.

Expression of 521 microRNAs was measured in spleen samples from young mice of the longest- and shortest-lived strains, using Taqman® Array qRT-PCR. Expression of the 10 most highly associated microRNAs was then measured by manual qRT-PCR in both young and old animals from all 6 strains. Relationships of expression with strain longevity were tested with linear regression and interaction analyses.

We determined that levels of 3 microRNAs are robustly associated with strain lifespan; mmu-miR-203-3p (β-coefficient= -0.6447, p= 4.78E-11), mmu-miR-664-3p (β-coefficient= 0.5552, p= 5.12E-08) and mmu-miR-708-5p (β-coefficient= 0.4986, p= 1.61E-06). Furthermore, genes in key ageing/longevity pathways were seen to be enriched in binding sites for these 3 microRNAs.

Our data suggests these microRNAs may regulate pathways important in determination of lifespan, so potentially represent useful biomarkers of successful ageing.

A MORE PHYSIOLOGICALLY RELEVANT SET OF CULTURE CONDITIONS FOR ENDOC BH1 HUMAN BETA CELLS?

Nicola Jeffery[1], *Sarah Richardson*[2] *and Lorna W. Harries*[1]

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Recent studies in mouse models have found that apoptosis alone does not account for loss of beta cell mass in type 2 diabetes, and suggest a role for changes in the differentiation status induced by the diabetic milieu as a possible alternative. However, anatomical and physiological differences between rodents and man mean that these findings require investigation in humans.

Here, in advance of cellular insult designed to mimic the diabetic cellular microenvironment, we have acclimatised the human EndoC □H1 beta cell line to a normal human cellular microenvironment in vitro and measured the effects on the morphology of the cells, and on the transcriptional and immunofluorescence profile of islet progenitor and mature beta cell markers.

Immediate changes to morphology were observed with cells appearing rounder with increased variability in size variability and enhanced cell clustering in pseudo-islets. Characterisation by immunofluorescent microscopy demonstrated the cells contain and secrete insulin and express the mature beta cell markers NEUROD1 and PDX1.

We found differences in the expression of 5/30 genes when cultured in a human cellular microenvironment. Increases in the expression of *NEUROD1, GLUT2, PAX4* and *MYC* and a decrease in the alpha cell marker *ARX* were noted, but no differences in expression of mature beta cell markers *INS, PDX1* or *PAX6*. No differences were noted for *ARNT* and *DDIT3* genes associated with ER stress, but a significant increase in *HIF1A* expression was seen in cells grown in the human microenvironment, perhaps indicating increased sensitivity to hypoxic conditions.

This study presents a novel culture methodology for the EndoC BH1 cell line and describes the most comprehensive gene expression profile to date of genes involved in beta cell function and cell fate in this cell line. While further work needs to be done to fully characterise gene expression differences, preliminary findings are suggestive of the consolidation of the beta cell phenotype when cultured in human reagents.

INTERACTIONS WITH RNA-BINDING PROTEINS CONSTRAIN CODING SEQUENCE EVOLUTION

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To ensure correct gene expression, coding sequences (CDSs) must interact with RNA-binding proteins (RBPs), many of which bind sequence-specifically. In this study, we sought to quantify the constraint posed on CDS evolution by the need to preserve the relevant motifs. We examined how motifs that have been experimentally predicted to be recognized by particular RBPs distribute and evolve in human CDSs. We found the net effect of RBP-related constraints to be a reduction in the over-all rate of evolution at synonymous sites (d_S) of *ca.* 2.5% (alignment to macaque). This estimate, however, belies a more complex reality: while motifs putatively recognized by certain RBPs are enriched compared to random expectations, those recognized by many others are depleted, suggesting that selection to avoid sequences recognized by RBPs could also play a role in CDS evolution. Those motifs that are more strongly enriched over nucleotide-controlled null are also more conserved, supporting the functional relevance of our observations. Importantly, this correlation is significant only within the outer regions of exons and not at the centre. RBP-related constraints might therefore be strongest at exon ends. We predicted stronger constraint also in genes with larger introns, where splicing is expected to be more error-prone. Instead, we detected two classes of splice factors: one associated with motifs enriched more in genes with larger introns and one whose putative target motifs show the opposite tendency. Pre-mRNAs might therefore interact with different splice factors depending on their architecture.

LincRNAs MEDIATE THE INFLAMMATORY RESPONSE IN HUMAN OA JOINT TISSUES

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Purpose: Recent studies suggest that long intergenic non-coding RNAs (lincRNAs) may regulate the inflammatory response [1, 2]. This study identified lincRNAs associated with the inflammatory response in primary chondrocytes from osteoarthritis (OA) patients, determined their expression in human OA cartilage compared to non-OA cartilage, and examined their functional role in the inflammatory response.

Methods: OA cartilage was obtained from hip and knee OA patients following joint replacement surgery. Non-OA cartilage was obtained from post-mortem donors and neck of femur fracture patients. Primary OA chondrocytes were isolated by collagenase digestion. LincRNA expression analysis was performed by RNA sequencing (RNAseq) and qRT-PCR. Modulation of lincRNA expression was achieved using LNA RNA GapmeRs (Exiqon). Cytokine production was measured using a Bio-Plex assay (Bio-Rad).

Results: Following IL-1β stimulation of primary human hip OA chondrocytes 125 lincRNAs were differentially expressed ($q<0.05$) greater than 1.5-fold. The chondrocyte inflammation-associated lincRNAs (CILinc01 and CILinc02) were transiently induced in response to pro-inflammatory cytokines (TNFα, leptin, visfatin and IL-1β), but were differentially downregulated in both knee and hip OA cartilage compared to non-OA cartilage. Knockdown of CILinc01 expression in chondrocytes significantly ($p \leq 0.05$) enhanced the IL-1β-mediated secretion of IL-6 (1.9-fold), IL-8 (1.87-fold), G-CSF (2.89-fold), MIP-1β (1.91-fold) and TNFα (1.31-fold).

Conclusions: The inflammatory response in human OA chondrocytes is associated with widespread changes in the expression of lincRNAs. Expression of incRNA CILinc01 in hip and knee OA cartilage, and its role in modulating cytokine production during the chondrocyte inflammatory response suggests it may play an important role in mediating inflammation-driven cartilage degeneration in OA.

References

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NEAT1, A LONG NON-CODING RNA, CONTROLS BREAST CANCER CELL SURVIVAL AND IS UP-REGULATED IN BREAST CANCER

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Background:

Nuclear Enriched Abundant Transcript 1 (NEAT1) is a nuclear long-non coding RNA transcribed from the familial tumour syndrome multiple endocrine neoplasia (MEN) type 1 locus on chromosome 11. NEAT1 is reported to be overexpressed in prostate cancer and a direct transcriptional target of hypoxia-inducible factor in breast cancer cells. The aims of this study were to determine: i) the effects of silencing NEAT1 on breast cancer cell survival, ii) the effects of NEAT1 silencing on the expression of two genes located on the same chromosome, iii) the levels of NEAT1 in breast cancer samples.

Materials and Methods:

MCF7 and MDA-MB 231 breast cancer cells were transfected with NEAT1 antisense oligonucleotides (ASO), controls received scrambled oligonucleotide. In some experiments, cells were exposed to ultraviolet-C (UV-C) light post-transfection to induce apoptosis, and then culture viability and apoptosis were assessed. Commercial Breast Cancer cDNA Arrays were used to evaluate the levels of NEAT1 in breast cancer samples. NEAT1 expression was evaluated by qRT-PCR TaqMan® analysis, using relative standard curve method.

Results:

In MCF7 and MDA-MB-231 cells, silencing of NEAT1 reduced basal survival and the expression level of its neighbouring gene, MALAT1 (Metastasis-associated lung adenocarcinoma transcript 1). NEAT1 silencing enhanced UV- induced cell death and this response was associated with a significant increase in the expression levels of BAD (BCL2-Associated Agonist of Cell Death). NEAT1 levels were found to be significantly increased in breast cancer samples.

Conclusion:

Overall, the results suggest that NEAT1 regulates cell survival and the expression of neighbouring genes in oestrogen-receptor positive and triple negative breast cancer cells. The substantial increase in NEAT1 expression levels in breast cancer tissues suggests that NEAT1 may function as an oncogene.

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