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WEDNESDAY 23RD MAY 2012
10.00-17.00

ORGANISERS: Michael Ladomery & Sebastian Oltean

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PROGRAMME

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10.20-10.35	*Sarah Miles*		The effect of DNA lesions on RNA synthesis by the human mitochondrial RNA polymerase
10.35-10.50	*Alex Fellows*		Advancing age is associated with gene expression changes resembling mTOR inhibition: evidence from two populations
10.50-11.05	*Helen Whiteland*		Identification of potential biomarkers for the detection of aggressive prostate cancer
Coffee break			
11.30-11.45	*Melissa Gammons*		Targeting SRPK1 to control VEGF mediated tumour angiogenesis in metastatic melanoma, and ocular neovascularisation in AMD
11.45-12.00	*Keith Brown*		DNA methylation of the splicing factor ESRP2 in Wilms' tumour
12.00-12.15	*Richard Hulse*		Alternative splicing: the ‘key’ switch in the development of neuropathic pain
Lunch break			
13.25-13.40	*Zoe Jing-Wang*		Hyperglycaemia, IGFs and insulin affect alternative splicing of the insulin receptor in Du145 prostate cancer cells
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14.10-14.25	*David Robinson*		RNA analysis of DNA variants for abnormalities of splicing causative of human inherited disease
Break			
2.35-3.35	*David Elliott*		Identification of evolutionarily conserved exons as regulated targets for the splicing activator tra2β in development
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4.00-4.15	*Sonia López de Quinto*		Ribonucleoprotein complexes regulating mRNA localization and translation
4.15-4.30	*Robert Jenkins*		MicroRNA regulation of the cell cycle in acute kidney injury
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4.45-5.00	*James Heward*		Long intergenic non-coding RNAs and the regulation of the innate immune response

10.05-10.20

Transcriptome analysis using Next Generation Sequencing technologies

Christy Waterfall & Jane Coghill

Transcriptomics Facility, University of Bristol, Bristol

The University of Bristol Transcriptomics Facility houses cutting edge genomics instrumentation and expertise, in particular Next Generation Sequencing (NGS) and array-based technologies, which are available for researchers to use. The facility is currently involved in a variety of RNA-based research projects which are utilising NGS applications such as RNAseq, to carry out global and targeted transcriptome analyses**.** An overview of different local research projects utilising NGS approaches for transcriptome analysis will be presented.

10.20-10.35

The effect of DNA lesions on RNA synthesis by the human mitochondrial RNA polymerase

Sarah Miles & Nigel Savery

Department of Biochemistry, University of Bristol

The human mitochondrial genome is expressed by a single-subunit mitochondrial RNA polymerase (PORLMT), which requires two transcription factors for efficient function. The genome is located in the mitochondrial matrix and is subject to attack by reactive oxygen species produced as a by-product of oxidative phosphorylation. A common DNA lesion resulting from this attack is 8-oxoguanine, which can base pair ambiguously and therefore lead to mutations in DNA. This lesion can also cause a block to transcription, or be misread by RNA polymerases leading to errors in RNA transcripts, a process known as transcriptional mutagenesis. Whilst single- and multi-subunit RNA polymerases studied display efficient lesion bypass, nothing is known about the affect of DNA damage on transcription by PORLMT. I have reconstituted human mitochondrial transcription in vitro and will discuss the effect of 8-oxoguanine lesions on transcription in this system.

10.35-10.50

Advancing age is associated with gene expression changes resembling mTOR inhibition: evidence from two populations

Alex Fellows, Luigi Ferruchi, Andy Singleton, Dena Hernandez, David Melzer, Lorna Harries

Peninsula College of Medicine & Dentistry, Genetics Department, University of Exeter

Interventions which inhibit TOR activity (including rapamycin and caloric restriction) lead to downstream gene expression changes and increased lifespan in laboratory models. However, the role of mTOR signaling in human aging is unclear.

In the InCHIANTI population-study of ageing, we tested expression of mTOR-related transcripts across a wide age-range (30 to 104 yrs) in fresh blood leukocytes. Expression of 48% of genes were associated with age after correction for multiple testing (False Discovery Rate q value of <0.05). Genes involved in insulin signaling (*PTEN, PI3K, PDK1*), ribosomal biogenesis (*S6K*), lipid metabolism (*SREBP1*), cellular apoptosis (*SGK1*), angiogenesis (*VEGFB*), insulin production and sensitivity (*FOXO*), cellular stress response (*HIF1A*) and cytoskeletal remodeling (*PKC*) were inversely correlated with age, whereas genes relating to inhibition of ribosomal components (*4EBP1*) and inflammatory mediators (*STAT3*) were positively associated with age. We found similar results in an independent cohort (SAFHS).

We conclude that the expression of mTOR-related transcripts is associated with advancing age in humans. Changes seen are broadly similar to mTOR inhibition interventions associated with increased lifespan in animals. Work is needed to establish whether these changes are predictive of human longevity and whether further mTOR inhibition would be beneficial in older people.

10.50-11.05

Identification of potential biomarkers for the detection of aggressive prostate cancer

Helen Whiteland, Howard Kynaston, Spencer Jenkins, Murali Varma, Pradeep Bose, Neil Fenn, Paul Griffiths, Shareen Doak

College of Medicine, University of Swansea

Prostate cancer (PCa) patients who have organ-confined cancers pose a problem for treatment, as their cancer may not be life threatening as many tumours lay dormant without the need for major treatment. Current diagnostic tools are unable to distinguish between an aggressive and dormant tumour, resulting in unnecessary radical treatment in patients who likely have an indolent tumour. The aim of this study is to identify a panel of molecular biomarkers that detect those tumours that will progress to aggressive disease within transrectal ultrasound guided (TRUS) biopsies. Five types of prostate cell lines were used with increasing metastatic potential; RNA was extracted and applied onto oligoarrays where a total of 612 genes were screened. Six genes were subsequently selected for gene and protein expression analysis, which resulted in the gene STEAP2 being of potential interest due to its significant increase within the metastatic cell lines only. This was also confirmed within FFPE TRUS biopsy tissue samples where an over expression of STEAP2 was seen within the locally advanced PCa patients.

Using immunohistochemistry techniques, the expression of epithelial-to-mesenchyme transition markers within 216 archival patient tissue samples were also analysed. An increase in nuclear Snail expression and a loss of E-Cadherin was observed within the higher Gleason score patients and with increasing tumour T classification. A loss of E-Cadherin was also significantly associated with those patients who later progressed and died of the disease. An increase of STEAP2 and nuclear Snail expression together with a loss of E-Cadherin may possibly indicate those patients who will progress and die of the disease.

11.30-11.45

Targeting SRPK1 to control VEGF mediated tumour angiogenesis in metastatic melanoma, and ocular neovascularisation in AMD

Melissa Gammons[1A], Masatoshi Hagiwara[2], Andrew Dick[1B], David Bates[1A]

[A]Physiology and Pharmacology, [B]School of Clinical Sciences and School of Cellular and Molecular medicine, [1]University of Bristol, United Kingdom; [2]Department of Anatomy and Experimental Biology, Kyoto University, Kyoto, Japan.

Serine rich protein kinase 1 (SRPK1) has been identified as a target in controlling the splicing of vascular endothelial growth factor (VEGF). VEGF, a key regulator of tumour angiogenesis and ocular neovascularisation, is alternatively spliced to produce two families of isoforms, proangiogenic $VEGF_{xxx}$ during proximal splice site (PSS) selection and anti-angiogenic $VEGF_{xxx}b$ during distal splice site (DSS) selection. SRPK1 results in the phosphorylation and nuclear localization of alternative splice factor/splice factor 2 (ASF/SF2), a promoter of PSS selection, thus upregulating pro-angiogenic VEGF production. We wished to determine whether a small molecular inhibitor of SRPK1 (and to a lesser extent SRPK2), SRPIN340, or lentiviral transdution of SRPK1 shRNA is capable of switching the splicing of VEGF. In addition we wished to determine the effect of topical, rather than intravitreal injection, application of SRPIN340 in inhibiting choroidal neovascularisation (CNV).
Twelve C57/B6 mice were tested in a laser induced CNV model. Following laser photocoagulation (250mW; 75μm; 0.1ms; 810nm krypton red laser) mice received twenty topical eye drops (10μl) of 100, 10, 1 or 0.1μg/ml SRPIN340 or Saline. On day 14 mice were culled eyes dissected, choroids flatmounted and neovascular area stained with isoloectin.

SRPIN340 significantly reduced CNV area ($p<0.05$, students unpaired t-test) in treated eyes ($32343\pm5487\mu m^2$) compared to controls ($76849\pm14894\mu m^2$) with an EC_{50} of 3.2μg/ml. In addition both SRPIN340 and SRPK1 shRNA knockdown were capable of switching the splicing of VEGF to promote anti-angiogenic isoform production in metastatic melanoma cells, and SRPK1 knockdown significantly reduced tumour growth in vivo ($p<0.01$, two-way ANOVA). These results indicate that targeting SRPK1, which has been shown to switch splicing from pro to anti-angiogenic splice variants of VEGF, has the potential to prevent VEGF mediated CNV in the eye when given topically and VEGF mediated tumour angiogenesis in metastatic melanoma.

11.45-12.00

DNA methylation of the splicing factor ESRP2 in Wilms' tumour

Azef Zahed, Keith Brown

School of Cellular and Molecular Medicine, University of Bristol

The paediatric kidney cancer known as Wilms’ tumour is one of the most common solid tumours of childhood, and is thought to arise due to defective differentiation of the metanephric blastema in the developing kidney. Although several genetic loci have been identified as being involved in Wilms' tumour, the molecular pathogenesis of most cases is still unknown and we have therefore examined epigenetic changes as possible alterative mechanisms. Using methyl-CpG immunoprecipitated DNA from Wilms' tumour cell lines to probe promoter microarrays, we have identified genes that are epigenetically silenced by DNA methylation in Wilms' tumour. We have used systematic exclusion criteria to narrow the hypermethylated hit list, to identify genes that may have functional importance. One candidate gene is the splicing factor ESRP2 (epithelial splicing regulatory protein 2), which has been previously implicated epithelial-mesenchymal transition. Using pyrosequencing, we found that ESRP2 is hypermethylated in over 70% of Wilms' tumours, with a trend towards higher methylation in more advanced tumours. Moreover, we demonstrated that expression of ESRP2 is repressed in all Wilms' tumours compared to foetal kidney, suggesting additional epigenetic silencing mechanisms may also occur. We have also shown that ESRP2 downregulation corresponds with marked changes in the splicing of ESRP2 target genes such as ENAH and FGFR2, towards a more mesenchymal nature. These results suggest that inactivation of ESRP2 may be an important step in the development of Wilms' tumour and implicate developmental RNA splicing controls as potential biomarkers and therapeutic targets.

12.00-12.15

Alternative splicing: the ‘key’ switch in the development of neuropathic pain

Richard Hulse[1,2], Melissa Gammons[1,2,] Dave Bates[1,2], Lucy Donaldson[2]

[1]Microvascular Research laboratories, [2]School of Physiology and Pharmacology, University of Bristol, Bristol, BS8 1TD

Chronic pain is prevalent in a large percentage of the western world’s population and arises due to a variety of causes including physical trauma to nerves and disease or associated treatment. A number of agents have been tried to reduce pain, however many patients routinely do not receive benefit or they remain on these regimes for a short period of time due to intolerable side effects. Research has targeted specific channels or receptors. Recently, a further growth factor has been implicated in nociception, vascular endothelial growth factor (VEGF). Here we highlight that by targeting alternative splicing and specifically of the VEGF transcript, we can abolish the neuropathic pain phenotype.

Our studies have identified the serine-arginine rich protein SRSF1 in the cytoplasm of sensory dorsal root ganglia neurons. Post nerve injury, SRSF1 co-localises with hoescht a nuclei stain highlighting translocation. This arises due to serine phosphorylation, which leads to the preferential targeting of the proximal splice site (PSS), resulting in the expression of $VEGF_{xxx}a$ isoforms. Through quantitative PCR, an increase in the ratio of $VEGF_{165}a$:$VEGF_{total}$ was identified in the saphenous nerve samples taken from nerve injured animals compared to those from naïve animals. This preferential PSS expression was associated with the development of mechanical allodynia. Previous work has implicated SRPK1 in the preferential expression of PSS isoforms. In a further group of animals at the site of nerve injury an SRPK 1 inhibitor, SRPIN340, was administered. These animals did not develop mechanical allodynia compared to a nerve injured plus vehicle groups. In conjunction $VEGF_{165}a$ expression was reduced relative to total VEGF compared with that in the nerve injured plus vehicle group, with a reduction in the nuclei SRSF1. These data highlights that SRPK1 activation after nerve injury in sensory neurons contributes to the development of neuropathic pain and blockade of such a pathway may provide a suitable analgesic target.

13.25-13.40

Hyperglycaemia, IGFs and insulin affect alternative splicing of the insulin receptor in Du145 prostate cancer cells

Zoe Jing Wang[1], *Claire Perks*[1], *Emily Foulstone*[1], *Michael Ladomery*[2] *and Jeff Holly*[1]

[1]School of Clinical Sciences, University of Bristol
[2]Faculty of Health and Life Sciences, University of the West of England

The insulin-like growth factor (IGF) family and insulin has been strongly implicated in prostate cancer. Alternative splicing of the insulin receptor (IR) results in two isoforms: the full length IR-B and an IR-A isoform that lacks exon 11 and binds win ten times higher affinity to IGF-II in addition to insulin.

We compared the abundance of the IR and the ratio of the IR-A: IR-B isoforms in different prostate cancer cell lines and assessed if these were altered by the presence of the receptor ligands or altered glycaremic conditions.

VCaP, LNCaP and PC3 cells had comparable levels of IR that were higher than that found in DU145 cells. With DU145 cells the ratio of IR-A to IR-B significantly changed depending on whether the cells were grown in euglycaemic (5mM glucose) or hyperglycaemic (25mM glucose) conditions and following exposure to IGF-I, IGF-II or insulin. Hyperglycaemia increased the relative abundance of IR-B (From 40 to 70%) whereas exposure to IGF-I, IGF-II or insulin reduced the relative abundance of IR-B; even in hyperglycaemic conditions exposure to IGFs and insulin resulted in predominant expression of IR-A. This means that in conditions with chronic hyperinsulinemia or IGFs the vast majority of the insulin receptors would be the isoform that responds to IGF-II.

These data suggest that metabolic conditions that occur in diabetes may result in changes in the alternative splicing of the IR. This could favour a more mitogenic response and potentially enhance the growth and survival of malignant cells promoting carcinogenesis.

13.40-13.55

Gene expression and alternative splicing in human aging

Alice Holly, David Melzer, Lorna Harries

Peninsula College of Medicine & Dentistry, Genetics Department, University of Exeter

Aging is a major risk factor for chronic disease, but the factors that underpin why some people age well, and others succumb to certain diseases are to date unclear. Elucidating the biological mechanisms underlying human aging is therefore crucial to the understanding of age-related chronic disease.

Whole-genome expression profiling data from individuals within the InCHIANTI study was analysed. Each individual (n=733) was examined at year 0 (1998-2000) and every 3 years for a period of 9 years. At each examination extensive biochemical, physical and clinical studies were performed. RNA from whole blood was also extracted at each interval, and mRNA expression measured using the Illumina Human HT-12 microarray.

We identified statistically significant changes with age for 2% (295/16 571) of transcripts, with alterations predominantly related to splicing pathways. This led us to the hypothesis that alterations in core splicing machinery may result in changes to the amount or nature of mRNA transcripts.

To test our hypothesis we used TaqMan Low Density Array (TLDA) technology to measure the expression of alternative splice forms of genes which were within our top 250 age-associated genes from the microarray.

TLDA analysis validated the microarray results and revealed that the majority of selected genes expressed differences in the ratios of mRNA isoform expression with age.

We have identified changes in the balance of alternative mRNA isoforms with age, implicating disturbances to alternative splicing as a fundamental mechanism of aging. Further work is now necessary to define the molecular basis for this observation.

13.55-14.10

VEGF receptor inhibition further reduces water permeability in isolated glomeruli from mice over-expressing the splice isoform $VEGF_{165}b$ in kidneys

Megan Stevens, Andy Salmon, Dave Bates, Steve Harper, Sebastian Oltean

Microvascular Research Laboratory, Department of Physiology and Pharmacology, University of Bristol

Introduction

Several glomerular diseases are associated with increased expression of pro-permeability vascular endothelial growth factor (main isoform $VEGF_{165}$). The functionally different splice isoform, $VEGF_{165}b$, decreases glomerular water permeability (L_PA/V_i) in kidneys of mice over-expressing $VEGF_{165}b$ under a nephrin promoter (Qiu et al. JASN 2010;). However, the molecular mechanisms of this inhibition are unknown. We investigated whether chemical inhibition of VEGF receptors in isolated glomeruli from nephrin-$VEGF_{165}b$ mice has an additional effect on L_PA/V_i.

Methods

Glomeruli isolated from nephrin-$VEGF_{165}b$ mice and wild-type littermate controls then incubated in saline or 100nM PTK787 (tyrosine kinase receptor inhibitor). L_PA/V_i measured using a method previously described (Salmon et al. JPhys 2006). Western Blots were carried out for phospho-VEGFR2.

Results

Glomeruli from mice over-expressing $VEGF_{165}b$ showed a significant reduction in L_PA/V_i compared to WT mice ($p<0.01$). Upon incubation of WT glomeruli with PTK787, there is a significant reduction in L_PA/V_i compared with saline ($p<0.05$). A significant reduction in L_PA/V_i occurs when neph-$VEGF_{165}b$ glomeruli are incubated with PTK compared with saline ($p<0.05$). Western blot analysis of protein from glomeruli isolated from nephrin-$VEGF_{165}b$ mice showed reduced VEGFR2 phosphorylation when incubated for 1 hour in 100nM PTK787 compared to saline. In WT littermates, VEGFR2 phosphorylation is blocked by incubation of glomeruli with PTK787.

Conclusions

VEGF receptor inhibition in isolated glomeruli from nephrin-$VEGF_{165}b$ over-expressing mice decreases L_PA/V_i further suggesting $VEGF_{165}b$ doesn't block all VEGFR2 sites in the kidneys in this mouse model. This is supported by the decrease in VEGFR2 phosphorylation seen in western blot analysis when glomeruli are incubated with PTK787.

14.10-14.25

RNA analysis of DNA variants for abnormalities of splicing causative of human inherited disease

David Robinson[1], Matthew Lyon[1], Feng Lin[1] and Diana Baralle[2]

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[2]Wessex Clinical Genetics Service, The Princess Anne Hospital, Southampton.

The molecular identification of the genetic cause of most monogenic inherited diseases routinely relies upon analysis of DNA to identify point or other small mutations creating premature translational termination codons or significant amino acid changes. However a proportion of gene mutations in DNA are causative of defects at the level of mRNA splicing and these are not always easy to identify from DNA sequence data alone. Many DNA base changes that are currently viewed as missense mutations or unclassified variants may influence splicing.

To address this problem for the BRCA1, BRCA2 and FBN1 genes we have carried out (i) in silico analysis of DNA sequence changes with splice site prediction programs and (ii) direct analysis of RNA from patient blood. We found that some apparent missense mutations and some other unclassified mutations do have an affect upon splicing which can be identified by both methods of analysis, however splice site prediction programs do not always reflect the results of RNA analysis and in a diagnostic setting should be used with caution.

14.35–15.35

Identification of evolutionarily conserved exons as regulated targets for the splicing activator tra2β in development

David Elliott

Institute of Human Genetics, University of Newcastle

Alternative splicing amplifies the information content of the genome, creating multiple mRNA isoforms from single genes. We have been investigating a set of three groups of interacting proteins which control splicing: the core group are Tra2, STAR proteins and hnRNPG proteins. Our hypothesis is that these will control important splicing events in development. Recently the evolutionarily conserved splicing activator Tra2β (Sfrs10) was found to be essential for mouse embryogenesis, and our data links it also with male germ cell development. We found that Tra2β is up-regulated as the mitotic stem cell containing population of male germ cells differentiate into meiotic and post-meiotic cells. Using CLIP coupled to deep sequencing, we found that Tra2β binds a high frequency of exons and identified specific G/A rich motifs as frequent targets. Significantly, for the first time we have analysed the splicing effect of Sfrs10 depletion in vivo by generating a conditional neuronal-specific Sfrs10 knock-out mouse (Sfrs10(fl/fl); Nestin-Cre(tg/+)). This mouse has defects in brain development and allowed correlation of genuine physiologically Tra2β regulated exons. These belonged to a novel class which were longer than average size and importantly needed multiple cooperative Tra2β binding sites for efficient splicing activation, thus explaining the observed splicing defects in the knockout mice. Regulated exons included a cassette exon which produces a meiotic isoform of the Nasp histone chaperone that helps monitor DNA double-strand breaks. We also found a previously uncharacterised poison exon identifying a new pathway of feedback control between vertebrate Tra2 proteins. Both Nasp-T and the Tra2a poison exon are evolutionarily conserved, suggesting they might control fundamental developmental processes. Tra2β protein isoforms lacking the RRM were able to activate specific target exons indicating an additional functional role as a splicing co-activator. Significantly the N-terminal RS1 domain conserved between flies and humans was essential for the splicing activator function of Tra2β. Versions of Tra2β lacking this N-terminal RS1 domain potently repressed the same target exons activated by full-length Tra2β protein.

16.00-16.15

Ribonucleoprotein complexes regulating mRNA localization and translation

Sonia López de Quinto

School of Biosciences, University of Cardiff

It has been known for decades that RNA untranslated regions (5´ and 3´ UTRs) contain *cis*-acting sequences critical for RNA regulation. However, the pivotal role of 3′ UTR-dependent post-transcriptional control of gene expression has become apparent only recently, mainly due to the pervasive regulatory roles of 3´UTR-interacting small RNAs.

3´UTRs also associate with proteins to form highly dynamic ribonucleoprotein complexes (RNP) that define when and where mRNAs are expressed. This mode of gene expression regulation efficiently restricts protein synthesis in time and space and it represents a powerful mechanism for the establishment and maintenance of cell polarity. We use *Drosophila* as a genetically tractable model organism to study the global implications of this 3′ UTR-dependent mechanism of gene expression in different physiological contexts.

One example is the localization of *oskar* mRNA at the posterior pole of the *Drosophila* oocyte, where locally translated Oskar protein assembles the pole plasm containing the abdominal and germ line determinants of the future embryo. The specific enrichment of Oskar at the posterior pole is achieved by coordination of mRNA localization and translational control, such that unlocalized mRNA is translationally repressed and repression is overcome upon mRNA localization. We have characterized *in vivo* the role of several *oskar* mRNA regulatory proteins, such as PTB/hnRNP-I, which binds to multiple *oskar* 3´UTR regions and acts as a translational repressor. In order to define general principles governing RNP function, we are characterizing novel *oskar*-interacting proteins, as well as new RNA targets of known *oskar* regulatory proteins in the ovary.

16.15-16.30

MicroRNA regulation of the cell cycle in acute kidney injury

Robert Jenkins[1], *Luke Davies*[2], *Philip Taylor*[2], *Hideo Akiyama*[3], *Aled Phillips*[1], *Timothy Bowen*[1], *Donald Fraser*[1]

[1]Institute of Molecular and Experimental Medicine, School of Medicine, Cardiff University, Wales, UK, [2]Institute of Infection & Immunity, School of Medicine, Cardiff University, Wales, UK, [3]Toray Industries, Japan

Problem: Incomplete repair and subsequent fibrosis after acute kidney injury is a major contributor to chronic kidney disease. A recent study causally links epithelial cell G2/M cell cycle arrest to fibrosis, following acute ischaemic, toxic (Aristolochic acid, AA), and obstructive injuries (Yang L et al. Nat Med 2010; 16(5)535-). The mechanism of this cell cycle arrest is not determined.

Purpose: To investigate the role of microRNAs in cell cycle arrest in the context of AKI.

Design: An in vitro study of changes in microRNA expression in PTCs in the AA model of toxic renal injury, using Toray 3D-Gene™ microarrays, TaqMan RT-qPCR, flow cytometry, and western blots.

Findings: AA induced a profound G2/M cell cycle arrest and apoptosis in PTCs. Toray 3D-Gene™ microarrays composed of 1400 probes identified 7 significantly up-regulated microRNAs in AA treated PTCs. These changes were confirmed by qRT-PCR. In silico analysis of predicted microRNA targets showed enrichment for cell cycle regulators. Enforced expression of a subset of these microRNAs recapitulated the G2/M cell cycle arrest and apoptosis seen in AA treated PTCs. Over-expression of these microRNAs decreased expression of the E3 ubiquitin ligase MDM2, a key regulator of p53, resulting in increased p53 activity, and leading to the observed G2/M cell cycle arrest.

Conclusion: These data define a mechanism by which microRNAs control cell cycle in PTCs, of mechanistic importance in the recently described pro-fibrotic G2/M arrest seen following a range of pro-fibrotic acute renal injuries.

16.30-16.45

MicroRNAs and flowering in *Arabidopsis thaliana*

Kuiama Lewandowski, John Hancock, Ian Wilson

Centre for Research in Bioscience, University of the West of England

The transition from vegetative to reproductive growth is a major developmental step in the life cycle of a flowering plant. It is influenced by environmental cues which align development with favourable environmental conditions, as well as endogenous factors which control the ability of the meristem to respond to these stimuli. MicroRNAs (miRNAs) are a class of small (~21 nucleotide) noncoding regulatory RNA which regulate gene expression by complementary base pairing and subsequent cleavage of mRNA from protein coding genes, they have been shown to be involved in the regulation of meristem transition to reproductive growth. A 1Kb intergenic region centred on a putative miRNA was over expressed and found to delay flowering by 30% (10-15 days) with an increased leaf number in transgenic lines when compared to wild type *Arabidopsis thaliana* ecotype Columbia. Analysis of transgenic plants has found that the over-expression of the transgene may have caused a mis-regulation in the expression of photoreceptor and photoperiodic flowering related genes. These results suggest that a locus within this 1Kb region may be functioning as a novel regulator of flowering time, potentially acting as a miRNA.

16.45-17.00

Long intergenic non-coding RNAs and the regulation of the innate immune response

James Heward[1], Nick Ilott[2], Louise Donnelly[3], Mark Lindsay[1]

[1]Department of Pharmacy and Pharmacology, University of Bath; [2]dCGAT Programme and Functional Genomics Unit, University of Oxford; [3]National Heart and Lung Institute, Imperial College, London.

There is increasing evidence to show that long intergenic non-coding RNAs (lincRNAs) are novel regulators of a range of biological responses. In this report, we have investigated whether lincRNAs might also be involved in the innate immune response by examining their role during lipopolysaccharide (LPS) induced inflammatory responses in human monocytes and monocyte-derived-macrophages (MDM). Using next generation sequencing, we identified three lincRNAs (TCONS_00008694, TCONS_00012731 and TCON_00015565) that were up-regulated in MDMs following exposure to LPS. These increases were confirmed by qRT-PCR in both LPS-stimulated MDM and human monocytes. Subsequent studies in the human monocytic THP-1 cell line demonstrated that LPS induced a rapid time- and concentration-dependent increase in TCONS_00008694 and TCONS_0015565 (but not TCONS_00012731) that correlated with the release of CXCL8 and TNFα. The increase in TCONS_00008694, TCONS_0015565, CXCL8 and TNFα expression were attenuated in the presence of an inhibitor of IκB kinase 2 (IKK2), an upstream activator of nuclear factor-κB (NF-κB). To determine the function and mechanism of the lincRNAs action we investigated the effect of siRNA-mediated