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**RNA Cardiff 2023**

***Thursday 22nd June 2023***

**Lecture Theatre 1,**

**University Hospital of Wales (UHW),**

**Heath, Cardiff, CF14 4XN**

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|  | **TIME** | **SPEAKER** | **INSTITUTION** | **TALK TITLE** |
| ***Welcome and Introduction:***  *Michael Ladomery, Sebastian Oltean and Tim Bowen* | 9.20-9.30 | Tim Bowen | Cardiff University |  |
| **Session 1: RNAs in cancer, from biomarkers targets to treatment targets**  *Chair: Lorna Harries* | 9.30-9.50 | Therese Dix-Peek | University of Bath | Discordance between immunohistochemistry and intrinsic subtyping in black South African women with breast cancer |
|  | 9.50-10.10 | Duygu Duzgun | University of Exeter | The roles of SRPK1 in tumour chemoresistance |
|  | 10.10-10.30 | Zhuofan Mou | University of Exeter | Comprehensive analysis of alternative splicing events across multiple studies identifies prognostic signatures in prostate cancer |
|  | 10.30-10.50 | Bethany Clark | University of the West of England | Muscleblind-like 3, a potential target for tumour metastasis |
| ***Tea/Coffee*** | 10.50-11.20 |  |  |  |
| **Session 2: RNA in senescence and development**  *Chair: Sebastian Oltean* | 11.20-11.40 | Fiona Messer | University of Cardiff | Identification and analysis of key transcription factor genes in differential spermatogenesis in *Drosophila pseudoobscura*, a sperm heteromorphic species |
|  | 11.40-12.00 | Abdulla Almoalem | University of Cardiff | A novel non-neuronal role of Alan Shepard (Shep) in *Drosophila* body axis patterning |
|  | 12.00-12.20 | Ryan Frankum | University of Exeter | Targeting downstream intermediates of FOXO1 and ETV6 attenuates senescence |
| Sponsor talk 1 | 12.20-12.30 | Alex Widger | ThermoFisher Scientific |  |
| Sponsor talk 2 | 12.30-12.40 | Jose Martins | Bio-Rad |  |
| ***Lunch and networking*** | 12.40-13.30 |  |  |  |
| **Session 3: Single cell sequencing and bioinformatics**  **KEYNOTE LECTURE**  *Chair: Michael Ladomery* | 13.30-14.30 | Katherine Bull | University of Oxford | Single cell RNA sequencing and spatial transcriptomic analysis in mouse models of systemic lupus erythematosus |
|  | 14.30-14.50 | Stephanie Hanna | University of Cardiff | Using scRNAseq to characterise T cell phenotypic changes and clonal expansions in skin, draining lymph nodes and peripheral blood in response to intradermal injection of diabetes autoantigen in humans |
|  | 14.50-15.10 | Bnar Talabani | University of Cardiff | Single cell RNA sequencing of myeloid cells in murine models of acute kidney injury and chronic kidney disease, identifies a mechanistic target |
|  | 15.10-15.30 | Tanya Smith | University of Cardiff | Single-nucleus RNA sequencing identifies sex-specific Proximal Tubular Cell differentiation pathways in the developing kidney |
| ***Tea/Coffee*** | 15.30-16.00 |  |  |  |
| **Session 4: RNA biology**  *Chair: Tim Bowen* | 16.00-16.20 | Birong Zhang | University of Cardiff | A bioinformatics tool for comprehensively characterizing circular RNAs in liver disease |
|  | 16.20-16.40 | Hanna Dixon | University of Birmingham | UPF1 is required for productive pre-mRNA splicing of ribosomal protein genes |
| **Chair:** | 16.40-17.00 | Jodie Evans | University of Exeter | HNRNPD splicing in new models of diabetic kidney disease |
|  | 17.00-17.20 | Giuseppina Pisignano | University of Bath | Transcription of *GNG12-AS1* lncRNA formats a chromatin loop to modulate the expression of the adjacent imprinted gene *DIRAS3* |
|  | 17.20-17.40 | Samantha Gould | University of Exeter | A tissue-engineered retinal barrier model to study Diabetic Retinopathy (DR). |
| ***Concluding remarks and winning PhD talk prize:***  *Michael Ladomery &*  *Sebastian Oltean* | 17.40-17.50 |  |  |  |

**Discordance between immunohistochemistry and intrinsic subtyping in black South African women with breast cancer**

Thérèse Dix-Peek

Department of Life Sciences, University of Bath

PURPOSE:

Breast cancer is a heterogeneous disease with different gene expression profiles, treatment options and outcomes. In South Africa, tumors are classified using immunohistochemistry (IHC), while high-income countries utilise multiparameter genomic assays. These different diagnostic test have implications for tumour classification and treatment.

METHODS:

In a cohort of 378 breast cancer patients from the South African Breast Cancer and HIV outcomes (SABCHO) study, we investigated the concordance between tumor samples classified by IHC and the PAM50 gene assay.

RESULTS:

IHC classified patients as oestrogen receptor-positive (77.5%), progesterone receptor-positive (70.6%), and epidermal growth factor receptor 2 (HER2)-positive (32.3%). These results, together with Ki67, were used as surrogates for intrinsic subtyping, and showed 6.9% IHC-A-clinical, 72.7% IHC-B-clinical, 5.3% IHC-HER2-clinical and 15.1% triple negative breast cancer (TNBC). Typing using the PAM50 gave 19.3% luminal-A, 32.5% luminal-B, 23.5% HER2-enriched and 24.6% basal-like. The basal-like and TNBC had the highest concordance, while the luminal-A and IHC-A group had the lowest concordance. By altering the cutoff for Ki67, and realigning the HER2/ER/PR-positive patients to IHC-HER2, we improved concordance with the intrinsic subtypes.

CONCLUSION:

We suggest that the Ki67 be changed to a cutoff of 20-25% in our population to better reflect the luminal subtype classifications. This change would inform treatment options for breast cancer patients in settings where genomic assays are unaffordable.

**The role of SRPK1 in tumour chemoresistance**

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Resistance to chemotherapeutic drugs is a major setback in cancer therapy, which leads to a high proportion of relapses and poor survival outcomes in cancer patients. Chemoresistance is frequently elicited by abnormal pre-mRNA alternative splicing, regulated by crucial kinases such as the serine-arginine protein kinase 1 (SRPK1). While SRPK1 has been implicated recently in chemoresistance in several tumours, the molecular mechanisms of this process are not known. In the present study, we aim to investigate in depth the role of SRPK1(and other kinases from the same family) in chemoresistance across multiple cancers. We first generated two cisplatin (CDDP)-resistant cell lines (breast cancer CDDP-4R and colon cancer HCT-116-4R) and a docetaxel (DTX)-resistant (prostate cancer PC-3-5R) cell line by continuous exposure (over a period of 8 months) of PC-3, MDA-MB-231 and HCT-116 cells to sub-lethal, stepwise increasing concentrations of drugs. Subsequently, we evaluated how sensitivity to chemotherapeutic drugs measured by the MTT assay changes with SRPK1 inhibition using SPHINX31 in the three cell lines. Combining SPHINX31 with chemotherapeutic drugs significantly sensitizes the parental (sensitive) cell lines but less prominently the resistant cell lines. Immunoblotting showed that the level of SRPK1 in the parental (sensitive) MDA-MB-231 cells increased when treated with CDDP alone as well as in combination with SPHINX31. In resistant MDA-MB-231 cells, CDDP did not increase SRPK1 expression on its own but did so when combined with SPHINX31. In the parental (sensitive) HCT-116 colon cancer cells, both CDDP on its own and in combination with SPHINX31 decreased the expression of SRPK1, while in the resistant line, both treatments increased the expression. Finally, in the parental (sensitive) PC-3 prostate cancer cells, treatment with DTX alone or in combination with SPHINX31 decreased SRPK1 expression, while in the resistant PC-3 line, DTX on its own did not affect SRPK1 expression; however, when combined with SPHINX31, expression of SRPK1 decreased. This indicates that SRPK1 protein levels might be differentially regulated in various resistant cell lines and support the hypothesis that SRPK1 is involved in chemoresistance. Potentially, our future studies will reveal the role of SRPK1 in the development of chemoresistance in cancer cells and suggest a potential therapeutic avenue for overcoming chemotherapy resistance.

**Comprehensive analysis of alternative splicing events across multiple studies identifies prognostic signatures in prostate cancer**

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*Background:* Alternative splicing (AS) plays a key role in transcriptomic diversity, influencing prostate cancer (PCa) development and progression. However, a comprehensive study exploring AS events and their prognosis in PCa across multiple cohorts remains unfulfilled.

*Methods:* Using AS techniques, we explored PCa-associated differentially expressed alternative splicing (DEAS) events across an Affymetrix human Clariom D array and two RNA-seq (TCGA and PRJEB2449) datasets. Common DEAS events from the RNA-seq sets facilitated screening for prognostic events. Employing Kaplan-Meier analysis, Cox regression, and LASSO regression, an AS event-based signature predicting 3-, 5-, and 8-year biochemical recurrence-free survival (BCRFS) was developed and validated internally and externally (GSE107299). A network model examined potential splicing regulatory, and a nomogram was formulated for 3-, 5-, and 8-year BCRFS prediction in PCa patients.

*Results:* One retained intron (RI) event in *ZWINT* was consistent across the three cohorts. Of the 141 overlapping differentially-regulated splicing events in RNA-seq datasets, predominantly RIs, 21 were strongly correlated with BCRFS. A minimal splicing event-based signature was established with six events (in genes: *CYP4F12*, *NFATC4*, *PIGO*, *CYP3A5*, *ALS2CL*, *FXYD3*). These risk events, negatively correlating with BCRFS, assigned risk scores to PCa patients, effectively distinguishing high-risk individuals who experienced worse BCRFS. The AS event signature outperformed conventional clinicopathological factors in predicting 5-year BCRFS, also showing strong performance in the external validation. The nomogram exhibited robust predictive power.

*Conclusion:* Prediction of clinical progression remains elusive in PCa. This research uncovers novel splicing events associated with BCRFS, augmenting existing prognostic tools, thus potentially refining clinical decision-making.

**Muscleblind-like 3, a potential target for tumour metastasis**

Bethany Clark, Michael Ladomery

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MBNL3 (Muscleblind-like 3) is an oncofetal splice factor associated with the progression of tumorigenesis in hepatocellular carcinoma, ovarian cancer, pancreatic cancer and non-small cell lung cancer. MBNL3 expression was knocked down in PANC-1 cells using a translation blocking morpholino (MBNL3.204). The effect of MBNL3 knockdown on PANC-1 migration was assessed in both 2D and 3D models, including scratch, transwell migration and invasion assay. MTT assay and live cell imaging were used to determine cell proliferation and apoptosis. RNA sequencing was also used to identify any changes in spicing following the knockdown of MBNL3. The reduced expression of MBNL3 was found reduce PANC-1 cell ability to migrate, furthermore MBNL3 knockdown cells had an increased expression of E-cadherin and decreased expression of N-cadherin, cell adhesion molecules that modulate cell migration and tumour invasiveness. MBNL3 knockdown was found to have no effect on cell viability and proliferation. RNA sequencing identified multiple splice differences between PANC-1 with MBNL3 knocked down. Alternative splicing was seen in long non-coding RNA PXN-AS1, previously identified to be splice regulated by MBNL3 in hepatocellular carcinoma, as well as several genes associated with apoptosis, APAF and CASP8. Taken together, these novel results highlight the role of MBNL3 in migration, but not in cell proliferation. This suggests that the MBNL3 has a pivotal role in tumour metastasis rather than tumour growth, offering a potential therapeutic target to reduce metastasis.

**Identification and analysis of key transcription factor genes in differential spermatogenesis in *Drosophila pseudoobscura*, a sperm heteromorphic species**

Fiona Messer, Emma Moth, Saurabh Chaudhary, Helen White-Cooper

Helen White-Cooper Lab, Molecular Biosciences, School of Biosciences, Cardiff University

*Drosophila pseudoobscura* produce three distinct sperm morphs: a long fertilising morph, the eusperm, and short and medium non-fertilising morphs, parasperm 1 and 2. Parasperm protect the eusperm from female-derived spermicides in the female reproductive tract.

*Drosophila* spermatogenesis follows a well-characterised pattern of differentiation, mitosis, meiosis, elongation and individualisation. Transcription of genes whose products are required during meiosis and post-meiosis predominantly occurs during the pre-meiotic primary spermatocyte stage.

Prior to this work, little was known regarding the specific molecular and developmental processes that produce multiple sperm morphs in *D. pseudoobscura*, although it was known primary spermatocytes within a single cyst all generate the same sperm morph. We hypothesised that transcriptional variation would be present between sub-sets of primary spermatocyte cysts, and that this would underpin sperm morph differentiation. RNA-seq analysis of manually isolated individual spermatocyte cysts, followed by cluster analysis, confirmed transcriptional differences between three classes of cysts, prior to the onset of meiosis.

Among putative transcriptional regulators, we validated, by *in situ* hybridisation, differential expression of some components of the testis meiotic arrest complex (tMAC), consistent with variant tMAC composition between cysts. We also validated differential expression of the tMAC regulator *kumgang (kmg)*. A transgenic line expressing Kmg-GFP fusion revealed that the Kmg protein is high in some late spermatocyte cysts, but low or absent from others and we propose that this contributes to morph differentiation. Ongoing ChIP-seq analysis will reveal how *kmg* association with chromatin correlates with differences in gene expression between spermatocyte classes.

**A novel non-neuronal role of Alan Shepard (Shep) in *Drosophila* body axis patterning**

Abdulla Almoalem and Sonia Lopez De Quinto

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Cells within a multicellular organism carry the same genetic information and acquire their cell-specific uniqueness through the expression of subsets of genes in a regulated manner. Development heavily relies on the regulation of gene expression patterns in the different cell types within an organism. In fruit flies, post-transcriptional regulation in the developing egg is fundamental for establishing early body axis patterning, specifying cell fate during embryogenesis, and ultimately proper development of the adult fly body. *Drosophila* oogenesis serves as a powerful model system to study asymmetrical localization and translational control of maternally loaded mRNAs in the oocyte, such as *oskar* and *gurken* mRNA. The spatiotemporal regulation of these mRNAs in the oocyte is crucial for proper embryonic patterning and adult body axis formation.

In *Drosophila*, the gene *alan shepard* (*shep*) encodes a highly conserved RNA-binding protein, which has primarily been studied in the nervous system. We are investigating the role of Shep during oogenesis as little, if anything, is known about it in this context. Our *in vivo* characterization revealed that during mid-oogenesis Shep localises to the oocyte cortex with enrichment at the posterior and dorsal-anterior corner, where *oskar* and *gurken* mRNAs are restricted to define posterior and dorsal fate, respectively. Using a combination of genetic manipulation techniques, biochemical assays, and microscopy, we found that Shep interacts with *oskar* and *gurken* mRNA *in vivo* and *in vitro*. Interestingly, overexpression of Shep resulted in a significant reduction of *oskar* and *gurken* levels, which compromises body axis formation. Our results reveal a novel non-neuronal role of *shep* in *Drosophila* body axis patterning that has not been previously described.

**Targeting downstream intermediates of FOXO1 and ETV6 attenuates senescence**

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Senescent cells accumulate as we age and are known drivers of age-related diseases. Dysregulated expression of splicing factor genes is a newly identified provocation for senescence. This effect is mediated by inappropriate activation of FOXO1 and ETV6 genes, because of constitutive and unresolved signalling through ERK and AKT signalling pathways. FOXO1 and ETV6 are transcriptional regulators that regulate expression of splicing factors via intermediates, which includes a module of miRNAs.

ETV6/FOXO1-regulated miRNAs demonstrating differential expression in senescence were identified by gene expression analysis, which were evaluated as splicing factor and senescence regulators by targeted overexpression or gene knockdown approaches in senescent primary human cells.

4 miRNAs downstream targets of FOXO1 and ETV6, demonstrated differential expression in late passage (PD = 63) primary normal human dermal fibroblasts (nHDF). Following manipulation of miR levels, expression of *SRSF3*, *SRSF6* and *NOVA1* splicing factors was restored and a reduction in senescent cell load observed (20.0% (SEM = 1.467), 18.7% (SEM = 0.985), 23.1% (SEM = 0.978) and 22.2% (SEM = 1.591) for mir-D AntagomiR, miR-E AntagomiR, miR-I AntagomiR and miR-K mimic respectively compared with 59.0% (SEM = 1.426) in the control. Treated cells also exhibited a reduction in DNA damage burden. Effects on cell cycle were not restored, and no changes in apoptosis were observed.

These data suggest miR-D, miR-E, miR-I and miR-K may represent intermediate regulators of *SRSF3*, *SRSF6* and *NOVA1* splicing factors in senescence, and targeted restoration of their levels may prove a fruitful future senotherapeutic avenue.

**Using scRNAseq to characterise T cell phenotypic changes and clonal expansions** **in skin, draining lymph nodes and peripheral blood in response to intradermal injection of diabetes autoantigen in humans**

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Background: To develop immunotherapies for type 1 diabetes it is vital to monitor autoantigen-specific T cells that drive beta cell destruction. These are very rare in the blood and cannot be extracted directly from the human pancreas.

Methods: We used i.d. injection of GAD-Alum to attract GAD-specific T cells to accessible sampling sites in the skin and skin-draining lymph nodes (LNs), and extracted them using skin blisters and fine needle aspiration. scRNAseq was used to identify T cell phenotypes and clonal expansions. TCRs were expressed in a cell based system to confirm antigen specificity. *In vitro* GAD restimulation was used to identify GAD-reactive CD4+ T cells in peripheral blood.

Results: GAD-specific cells were enriched in the post injection LNs and blisters (~30% of clonally expanded cells were GAD-specific). Clonal cells were mainly CD8+, with small expansions of Tregs and CD4+ T cells. Post injection the DEG indicated increased antigen recognition and activation of interferon and cytokine signalling. In peripheral blood, following *in vivo* injection of GAD, the predominant phenotype of GAD-reactive CD4+ T cells changed from IL22+IL32+FAS+ to PDCD1+IFNG+. Re-expressed TCRs recognised soluble epitopes from GAD, suitable for use in antigen specific immunotherapy.

Conclusion: Phenotyping of antigen-specific T cells in the LN, blister and peripheral blood using scRNAseq identified novel biomarkers of autoreactive cells to monitor immunotherapy effectiveness and tolerance induction.

**Single cell RNA sequencing of myeloid cells in murine models of acute kidney injury and chronic kidney disease, identifies a mechanistic target**

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**Introduction.** Acute Kidney Injury (AKI) is a well-recognised risk factor for chronic kidney disease (CKD), but the mechanism remains unknown. It is proposed that bone marrow derived macrophages can determine outcome following AKI. However, resident macrophages in the kidney are thought to play little part in this. Single Cell RNA Sequencing (scRNAseq) provides unparalleled opportunities to uncover heterogeneity in macrophage responses and provide new mechanistic understanding in AKI and CKD. We have performed scRNASeq of myeloid cells at specific timepoints mimicking human disease pathology in models of AKI and renal fibrosis.

**Methods.** Aristolochic acid was used to induce renal injury and fibrosis in male C57BL/6 mice. Kidneys were harvested from three mice at each time point mimicking important disease states in AKI and CKD. Using a cell sorting strategy, CD45+ cells were isolated from whole kidneys and libraries prepared on the 10X Genomics platform. ScRNASeq was performed using the Illumina NextSeq 550 System. Genome mapping was conducted using Cellranger and zUMIs and downstream expression analysis of myeloid cells, was carried out using the R package, Seurat. Following this analysis, transcription factor MAF was identified as a mechanistic target and a knockout mouse model was subsequently used to assess the degree of renal fibrosis in mice deficient in MAF in myeloid cells, versus wild-type mice.

**Results.** 21,734 CD45+ Cells were sequenced in total. Analysis of gene expression profiles delineated transcriptomic profiles in distinct sub-clusters of macrophages specific to the recovery and non-recovery state, following injury. Resident macrophages demonstrated dynamic transcriptomic signatures in response to injury and inflammation. MAF is a transcription factor that is thought to aid macrophage maturation and differentiation to an anti-inflammatory phenotye. Knock-out experiments of transcription MAF in myeloid cells in mice, demonstrated a higher degree of renal fibrosis in the renal cortex.

**Conclusion.** ScRNASeq has enabled unbiased profiling of gene expression in AKI-CKD at single cell resolution. We have identified heterogenous macrophage clusters, important in recovery and non recovery, following renal injury. We have demonstrated that MAF, a transcription factor expressed predominantly by resident macrophages, may play a vital role in the development of renal fibrosis following renal insult.

**Single-nucleus RNA sequencing identifies sex-specific Proximal Tubular Cell differentiation pathways in the developing kidney**

Tanya Smith1,2, Yueh-An Lu1,2,3, Sumukh Deshpande1, Chia-Te Liao1,4, Bnar Talabani1,2,4, Robert Andrews1, Timothy Bowen1,2, Philip R. Taylor1,4,5, Donald Fraser1,2

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**Background**: The cellular states exhibited by renal proximal tubular cells (PTCs) during dedifferentiation, proliferation, and reacquisition of a fully differentiated phenotype have been poorly characterised. This critical knowledge gap is likely to be of importance for PTC proliferation, physiological growth of the kidney, as well as in recovery from kidney injury. Furthermore, PTC’s exhibit sex differences in propensity for recovery, but sex-specific differences in PTC phenotype are not well characterised.

**Aims:** Characterisation of kidney transcriptomes at the single cell level in male and female mouse kidneys.

**Methods**: Kidneys were harvested from naïve female and male mice at 1, 2, 4, and 12 weeks of age (n=2 per group, 16 mice in total). Libraries were prepared on the 10x platform, and single nuclear RNA sequencing (snRNAseq) was completed using the Illumina NextSeq 550 System.

# **Results**: Unbiased clustering analysis was performed on 68,775 nuclei. High levels of proliferation were evident at early time points in some clusters (eg. PTCs) but were absent in others (eg. Podocytes). PTC’s demonstrated unique sex-specific expression profiles at 4 and 12 weeks. Mapping of PTC differentiation pathways using techniques including trajectory and RNA Velocity analyses delineated increasing PTC specialization and sex-specific phenotype specification. Trajectory analysis has identified the differentiation pathway for tubular cells.

**Discussion**: For the first time, we have characterised sex-specific phenotypical subtypes of PTCs in the growing kidney and differentiation pathways leading to these phenotypes. This new appreciation may enable us to develop ways to protect patients from kidney disease and more effectively treat them.

**A bioinformatics tool for comprehensively characterizing circular RNAs in liver disease**

Birong Zhang, You Zhou

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Circular RNAs (circRNAs), a type of covalently closed looped noncoding RNAs, have recently emerged as crucial regulators of gene expression and cellular functions. However, little is known about their expression profiles and molecular roles of circRNAs in liver diseases. Here, we developed a new bioinformatics pipeline to comprehensively characterize the expression landscape of circRNAs using publicly available bulk RNA sequencing data. We performed a deep data mining of RNA sequencing data derived from 644 liver biopsies across 6 independent cohorts. Using 3 different algorithms, we identified ~4,000 common circRNAs and ~40 mitochondria-encoded circRNAs. Among them, 157 circRNAs exhibited differential expression in liver samples with nonalcoholic steatohepatitis compared to controls, and several circRNA-miRNA-mRNA networks were found to be enriched in the disease.The pipeline further revealed a few differentially expressed circRNAs that were associated with fractions of immune cells. Overall, our findings highlight the utility of this bioinformatics pipeline for characterizing circRNAs and uncovering potential mechanisms involving circRNAs in immune cells.

**UPF1 is required for productive pre-mRNA splicing of ribosomal protein genes**

Hannah L. Dixon and Saverio Brogna

School of Biosciences, University of Birmingham

The RNA helicase UPF1 is commonly discussed for its cytoplasmic roles in nonsense mediated mRNA decay (NMD). However, recent evidence shows that it also functions in the nucleus where it associates with nascent transcripts. Analyses of RNA-seq datasets show that several mRNAs are alternatively processed in UPF1 depleted cells. This effect is most apparent at ribosomal protein and related genes where there is increased usage of intronic 5’ splice sites leading to the production of putative NMD-sensitive mRNA isoforms. It is currently understood that such isoforms are at low levels in wild-type cells because they are destroyed by NMD in the cytoplasm. On the contrary we have evidence that these switches in splicing are apparent in nuclear RNA and that this correlates with UPF1 binding to the nascent RNA. This switching phenotype is not observed in cells depleted of other NMD factors. Furthermore, we found increased build-up of Pol II downstream of the transcription start site at ribosomal protein genes upon knocking down UPF1. Cumulatively, our data reveals that UPF1 is likely to be a direct player in the regulation of transcription and nuclear processing, particularly at ribosomal protein genes.

**HNRNPD splicing in new models of diabetic kidney disease**

Jodie Evans, Lorna Harries

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Dysfunctional metabolism causes complications of diabetes such as chronic kidney disease (CKD), however therapeutic targets are currently limited. Dysregulation of the multifunctional RNA-binding protein HNRNPD has been associated with cell identity changes under diabetic conditions in other tissues. The differential functionality of HNRNPD may be driven by alternative splicing. We aim here to develop new models and assess isoform expression of *HNRNPD*.

We developed 2D cell culture models involving human renal cells (HEK293, HK-2 and HRPTEC) to assess the effect of diabetes related stresses and *HNRNPD* splicing on cellular proliferation, viability, morphology, and *HNRNPD* splicing following exposure to diabetomimetic conditions. These conditions include palmitic acid (0.5mM), high glucose (25mM) and a combination of the two treatments.

Models have successfully been developed without the use of animal derived biomaterial. HK-2 cells have been treated with diabetomimetic stimuli and isoform expression analysed. With improved understanding of the isoform expression in response to diabetomimetic stimuli, therapeutic targeting using splice switching oligonucleotides may be able to aid in management of this disease.

**Transcription of *GNG12-AS1* lncRNA formats a chromatin loop to modulate the expression of the adjacent imprinted gene *DIRAS3***

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Imprinted genes are expressed in a parent-of-origin dependent manner and are mainly organized in clusters that frequently embed long non-coding RNAs (lncRNAs). The maternal imprinted gene *DIRAS3* is commonly silenced in breast and ovarian cancers, and its transcription is modulated by the overlapping *GNG12-AS1* lncRNA. Depletion of *GNG12-AS1* by siRNAs targeting its transcription start site (TSS) leads to the upregulation of *DIRAS3*. Conversely, the imprinting state of *DIRAS3* influences *GNG12-AS1* splicing, and when *DIRAS3* is upregulated after treatment, *GNG12-AS1* expression levels are reduced. However, the mechanism underpinning the mutual relationship between *GNG12-AS1* and *DIRAS3* remains unclear.

Several studies have demonstrated that lncRNAs affect chromatin architecture, which has a significant impact on gene expression. Given the distance between the promoters of *GNG12-AS1* and *DIRAS3* and the presence of putative insulator/enhancer regions surrounding *DIRAS3*, we employed the powerful genome-wide interaction assay, called Capture Hi-C, to investigate how *DIRAS3* and *GNG12-AS1* are topologically organised. We found that *GNG12-AS1* orchestrates three-dimensionally the local chromatin by the formation of a genomic loop that is missing when its TSS is removed, leading to the concomitant re-expression of *DIRAS3*. Progressive deletions of regulatory elements linked to structural domains, corroborate the *GNG12-AS1* and *DIRAS3* anti-correlation and further highlight the pivotal role of chromatin conformation in regulating their expression. We also identified a critical topologically associated domain (TAD) boundary that, when removed or inverted, augments the *DIRAS3* expression despite the presence of *GNG12-AS1*. Our findingssuggest that the inhibitory effects exerted by *GNG12-AS1* on *DIRAS3* could be mediated by the recruitment at the locus of the CTCF protein and the formation of a confining TAD.

Altogether, our results will advance our comprehension of a complex mechanism like imprinting and elucidate how it gets impaired during cancer by changes in chromosomal architecture associated with lncRNA transcription.

Keywords: lncRNA; imprinting; chromatin architecture; transcriptional interference; tumor suppressor

**A tissue-engineered retinal barrier model to study Diabetic Retinopathy (DR)**

Samantha Gould

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Dysregulation of splicing drives cellular senescence and therefore age-related disease. DR is a common diabetic complication that leads to vision loss. Hyperglycaemic conditions dysregulate the retinal microenvironment, driving DR onset. Therapeutic targets are currently limited.

*In vitro* models of the retinal barrier (RB) are valuable tools for the study of DR. A reproducible, tissue-engineered RB construct may be utilised as a tool to identify targets and validate therapeutics for the treatment of DR such as antisense oligonucleotides (ASOs) designed to target the master regulator gene HNRNPD.

Retinal pigmented epithelial cells (RPEs)and retinal microvascular endothelial cells (RECs) were cultured in newly derived media, and validated by means of proliferation and morphology. To form the structure of the RB and permit cellular crosstalk, both cell types were incorporated into a collagen matrix. HNRNPD isoform expression was determined in RECs, RPEs, and models by RT-qPCR when challenged with diabetomimetic stressors.

RECs and RPEs remained proliferative and maintained their morphology. Histological evaluation revealed the formation of endothelial tube networks within the collagen matrix. Diabetic stressors altered HNRNPD isoform expression within REC, RPE and models. Combined glucose and palmitic acid treatment led to the inclusion of a poison exon and a shift in isoform expression in REC cells, identified by an increase in exon 2 exclusion (P = 0.0054).

HNRNPD isoform expression was altered in cells and models after treatment with diabetic stressors. Therefore, this model could shed light on the therapeutic potential of ASOs to manipulate HNRNPD isoform expression to treat diabetic retinopathy.