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# RNA Exeter 2024

***Thursday 20th June 2024***

**Baring Court 114 and 03,**
**St Luke's Campus, University of Exeter**
**Exeter, EX1 2LU**

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| | TIME | SPEAKER | INSTITUTION | TALK TITLE |
|---|---|---|---|---|
| ***Welcome and Introduction*** | 9.25-9.30 | Seb Oltean & Michael Ladomery | | |
| **Session 1:**<br>*Chair: Michael Ladomery* | 9.30-9.50 | Zhuofan Mou | Univ of Exeter | Integrating single-cell and bulk RNA sequencing to assess expression profiles and prognostic potential of epithelial gene markers in prostate cancer using machine learning and meta-analysis |
| | 9.50-10.10 | Duygu Duzgun | Univ of Exeter | SRPK1: a novel mechanism for tumour chemoresistance |
| | 10.10-10.30 | Dana Jackson | Cardiff University | Elucidating putative RNA binding protein interactions and RNAi knockdown phenotypes in *Drosophila melanogaster* sperm development. |
| | 10.30-10.50 | Dan Smith | Cardiff University | Identifying urinary microRNA biomarkers for IgA nephropathy |
| ***Tea/Coffee*** | 10.50-11.20 | | | |
| **Session 2:**<br>*Chair: Tim Bowen* | 11.20-11.40 | Bethany Clark | Univ of West of England | Muscleblind-like 3, a potential target for tumour metastasis |
| | 11.40-12.00 | Stephanie Jones | Univ of Bath | Investigating functions for syntenic long non-coding RNAs during zebrafish melanocyte development and human melanoma |
| | 12:00-12:20 | Anthony Klokkaris | Univ of Exeter | MicroRNAs expression profiling in Parkinson's disease brains |
| Sponsor talk 1 | 12.20-12.30 | Elizabeth Bowler | BioRad | Single Cell Analysis using Droplet Digital PCR (ddPCR) |
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| ***Lunch and networking*** | 12.40-13.40 | | | |
| **Session 3:**<br><br>**KEYNOTE LECTURE**<br>***Chair: Seb Oltean*** | **13.40-14.40** | **Prof Dave Bates** | **Univ of Nottingham** | **Control of alternative splicing by SR protein kinases - therapeutic potential** |
| | 14.40-15.00 | Sophie Hughes | Cardiff University | Urinary MicroRNA Changes in Living Kidney Donors |
| | 15.00-15.20 | Elliott Ruf | Cardiff University | Characterisation of iPSC-derived Renal Proximal Tubule Epithelial Cells Using Single Cell RNA Sequencing |
| ***Tea/Coffee*** | 15.20-15.50 | | | |
| **Session 4:**<br><br>*Chair: Mark Lindsay* | 15.50-16.10 | Rosie Bamford | Univ of Exeter | An atlas of expressed transcripts in the prenatal and postnatal human cortex |
| | 16.10-16.30 | Stuart Canon | Univ of Exeter | Insights into downstream translation initiation from 33,000 start-loss variants |
| | 16.30-16.50 | Robyn Murdoch | Univ of<br>West of England | Can environmental RNA characterise the physiological status of the European eel? |
| | 16.50-17.10 | Fiona Messer | Cardiff University | scRNAseq of *Drosophila pseudoobscura* testis shows development of multiple sperm morphs |
| ***Winning talks prizes:*** | 17.10-17.20 | *Lorna Harries & Keith Vance* | | |
| ***Concluding remarks:*** | 17.20-17.30 | *Michael Ladomery &*<br>*Seb Oltean* | | |

# Integrating single-cell and bulk RNA sequencing to assess expression profiles and prognostic potential of epithelial gene markers in prostate cancer using machine learning and meta-analysis

Zhuofan Mou[1] & Lorna W. Harries[1*]

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*Background*

Prostate cancer (PCa) is the second most common cancer in men worldwide. Its heterogeneity complicates diagnosis, prognosis, and treatment, with current clinicopathological parameters often insufficient for accurate prognostication and risk stratification. Epithelial cells are crucial in PCa development, but the potential of epithelial cell marker genes (ECMGs) to improve clinical outcomes in PCa patients is underexplored. This study aimed to assess cell type specific differences using single-cell RNA sequencing (scRNA-seq) data and evaluate the prognostic potential of epithelial biomarkers in PCa patients.

*Methods*

We analysed microarray, bulk, and scRNA-seq data across nine PCa cohorts. Based on our previously identified PCa markers, we conducted a meta-analysis to assess expression changes between tumour and normal prostate samples. We developed an ECMG-based risk signature to predict biochemical recurrence-free survival (BCRFS) in PCa patients using ten major machine learning models. This signature was compared with existing PCa signatures and evaluated for its relationship with the tumour microenvironment, immune cell infiltration, and drug response. Potential non-invasive biomarkers were explored using blood samples.

*Results*

The meta-analysis identified 33 upregulated genes in prostate tumours, selecting 11 as key components of the ECMG signature (*LAMTOR2*, *RAB25*, *FBP1*, *TMED3*, *FASN*, *PHB2*, *FKBP4*, *ERBB3*, *PSMG4*, *CACNA1D*, and *APEX1*). This signature effectively predicted BCRFS and stratified patients into distinct risk categories post-treatment, with high-risk patients exhibiting worse outcomes. The ECMG signature outperformed various published PCa signatures and traditional clinicopathological parameters. High-risk patients identified by the ECMG signature showed increased estimated immune and stromal cells. The signature risk score positively correlated with monocytes and activated NK cells and negatively with resting CD4 memory T cells. Blood-based data revealed that *FBP1*, *ERBB3*, *CACNA1D*, and *APEX1* exhibited significant expression changes following external beam radiotherapy in localised patients and effectively stratified castration-resistant patients for overall survival.

*Conclusions*

Our study identified a set of ECMGs consistently upregulated in PCa tumours, offering significant prognostic value for BCRFS in PCa patients. The ECMG-derived signature enhances BCRFS prediction and enables more accurate risk stratification, potentially improving patient management and outcomes.

# SRPK1: A NOVEL MECHANISM FOR TUMOUR CHEMORESISTANCE

**Duygu DUZGUN, Sebastian OLTEAN**

**Department of Clinical and Biomedical Sciences, University of Exeter Medical School, EX1 2LU, Exeter, UK**

**Introduction:** 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 (AS), 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 aimed to investigate whether chemotherapeutic drugs affect the expression and function of SRPK1 in various cancer cells, as well as the mechanism through which SRPK1 is involved in chemoresistance.

**Material and Method:** A cell culture technique was used to establish the drug-resistant cell lines from their parental cells. Molecular and cellular biological techniques including MTT assay, immunofluorescence, western blotting, siRNA knockdown, immunoprecipitation, and RT-PCR were used to characterize in the drug-resistant cell lines.

**Result and Discussion:** We selected two cisplatin (CDDP)-resistant cell lines (breast cancer MDA-MB-231$^R$ and colon cancer HCT-116$^R$) and a docetaxel (DTX)-resistant (prostate cancer PC-3$^R$) cell line by continuous exposure (over a period of 6 months) of PC-3, MDA-MB-231 and HCT-116 cells to sub-lethal, stepwise increasing concentrations of drugs. As indicated by IC50 value, drug-resistant cells were about five to seventeen times more resistant to drugs than parental lines. Immunoblotting showed that while there was a decrease in SRPK1 protein level in CDDP-treated HCT-116 cells, SRPK1 expression was upregulated by the DTX in PC-3 cells. Interestingly, SRPK1 expression was not affected by the treatment in all resistant cell lines, but was generally higher in MDA-MB-231$^R$ and PC-3$^R$ than their parental lines, and lower in HCT-116$^R$ than HCT-116. Also, IC50 values significantly decreased in PC-3$^R$ and MDA-MB-231$^R$ cells with knockdown of SRPK1, while its downregulation showed markedly increase in IC50 in HCT-116$^R$, implying that the expression level of SRPK1 may be major determinant of chemotherapeutic drugs responsiveness. To confirm the resistance status of the derived cell lines, immunostaining of phosphorylated H2AX (pH2AX) showed that while CDDP dramatically increased pH2AX in MDA-MB-231 and HCT-116 cells, but not in MDA-MB-231$^R$ and HCT-116$^R$, it was potently switched on by the addition of SPHINX31 in MDA-MB-231$^R$, but not HCT-116$^R$. In parallel study, we also performed immunostaining analysis in PC-3 cells to assess the role of class-III- β tubulin in DTX-resistance. We found that class-III β tubulin was overexpressed in PC-3$^R$ cells and siRNA knockdown of SRPK1 showed that a dramatic decrease in class-III β tubulin, which may be a target for overcoming resistance to DTX. As marker of apoptosis, CDDP-induced and DTX-induced activation of caspase-8, caspase-3 and cleavage of PARP was marked in PC-3 and MDA-MB-231 cells, but not in PC-3$^R$ and MDA-MB-231$^R$ ones. Additionally, combination treatment increased cleavage of caspase -8, caspase-3 and PARP in PC-3$^R$ and MDA-MB-231$^R$ cells compared with DTX, CDDP and SPHINX31 alone conditions; the opposite effects were observed in SRPK1-downregulated HCT-116$^R$ cells. These results suggested that inhibiting SRPK1 expression restored the sensitivity of drug-resistant cells and the anti-apoptotic effect of SRPK1 are related to its expression levels. We further studied the splicing of two members of the BCL2L1 (Bcl-x). For Bcl-x genes, the long isoform (Bcl-xL) possesses the anti-apoptotic activity while the short isoform (Bcl-xS) function as pro-apoptotic factor. By RT-PCR, we found that the relative abundance of Bcl-xS was increased by docetaxel in PC-3 but decreased in PC-3$^R$ in a dose-dependent manner. Our results suggest that SRPK1-mediated AS of Bcl-X is involved in promoting DTX resistance in PC-3$^R$ cells. Collectively, these results indicated that aberrant SRPK1 expression induces CDDP and DTX resistance by inhibiting apoptosis in various cancer cells.

**Conclusion:** Our research could reveal a key role of SRPK1 in the development of drug resistance in various cancer cells, suggesting a potential therapeutic avenue for overcoming chemotherapy resistance.

# Elucidating putative RNA binding protein interactions and RNAi knockdown phenotypes in *Drosophila melanogaster* sperm development.

**Dana Jackson**, Sonia Lopez de Quinto and Helen White-Cooper;
Cardiff School of Biosciences, Cardiff University, UK.

In *Drosophila* sperm development, most transcriptional activity occurs in pre-meiotic primary spermatocytes. However, a small subset of genes also undergo post-meiotic transcription in the mid-to-late elongating spermatids – at a point preceding the histone-transition protein-protamine switch. These genes express a number of asymmetrically-localised mRNAs that accumulate towards the distal tail-ends of growing spermatid cyst bundles; arranging in unusual localisation patterns of shooting speckled “comets” or U-shaped acorn “cups”. Analysis of fluorescently tagged reporter constructs have revealed that this mRNA localisation precedes the formation of distinct protein gradients.

In addition to these localised mRNAs, we have also identified several RNA binding proteins (RBPs) enriched at the spermatid tail-ends. How and why do these non-uniform, subcellular mRNA distributions arise? What role do these RBPs play in regulating the active localisation, anchoring and translation of comet and cup mRNAs?

We have used RNA-affinity pull-down assays to extract out bound fractions of comet and cup mRNAs with a pool of endogenous RBPs, including the highly-conserved IGF-II mRNA-binding protein (Imp). This revealed differential binding between 4 candidate RBPs and 11 comet and cup mRNAs *in vitro* – with binding affinities varying depending on the mRNA of interest. A follow-up Cl-AP experiment, modified to precipitate whole testis-specific multi-RBP:RNA complexes, has since been undertaken to re-verify Imp binding interactions. Comparative proteomics indicates that interactors of Imp are involved in RNA binding, actin and cytoskeletal interactions, translation, and splicing.

We have also performed RNAi screening to investigate the functional roles of Imp in the spermatids. We found that a knockdown of *imp* leads to a variable spectrum of abnormal testis phenotypes, including mislocalisation of mRNA transcripts, loss of localised RNA and fluorescent protein reporter signals, and considerable disruption to spermatid elongation. By conducting HCR RNA-FISH and Lightsheet Fluorescence Microscopy, we have determined that this RNAi-induced dysregulation is detectable at an earlier point of mRNA production and localisation.

# Identifying urinary microRNA biomarkers for IgA nephropathy

Smith, D.A., Hughes, S., Simpson, K. A., Watkins, J., Bergin, M., Fraser, D. J., Bowen, T.

## Introduction

Immunoglobulin A nephropathy (IgAN) is the most common form of primary glomerulonephritis worldwide, particularly in Europe and Asia.[1, 2] Kidney Research UK estimates that around 30% of those with IgA nephropathy will progress to kidney failure, requiring transplant or dialysis.[3, 4] No current biomarker test predicts the likelihood of this progression accurately. Indeed, the current reference metric, proteinuria, fails to detect up to 50% of progressive cases,[3, 5] and IgAN diagnosis requires invasive biopsy.[6, 7] Novel biomarkers of IgA progression are therefore needed to focus treatment strategies and improve clinical outcomes.

MicroRNAs are short, non-coding transcripts with significant biomarker potential. Recent studies of IgAN identified a microRNA panel that predicted disease progression in biopsy samples.[8, 9] Nevertheless, these analyses used tissue from invasive kidney biopsy procedures. In this study, we investigated whether urinary microRNAs have the same potential.

## Materials and methods

We used Taqman low density array RT-qPCR technology to profile 754 microRNAs in urine samples from 20 control individuals and a discovery cohort of 38 people with IgAN: 20 with stable disease phenotype (loss of estimated glomerular filtration rate (eGFR) <2 mL/min/yr) and 18 progressor phenotype (>2 mL/min/yr). Using lasso analysis, we compared urinary microRNA profiles for i) people with IgAN and unaffected controls to identify diagnostic biomarkers, and ii) stable disease phenotype vs. progressive IgAN to identify biomarkers of progression. We then analysed these findings in a replication cohort of 153 patients and 30 new controls.

## Results

Our replication analyses identified 2 diagnostic miRNA biomarkers for IgAN, and 5 biomarkers of progression. Further statistical analysis is underway to identify optimal combinations of microRNA and clinical/biochemical biomarkers to maximise translational impact.

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

Bethany Clark[1], Franziska Görtler[2], Sushma-Nagaraja Grellscheid[2], Giuseppina Pisignano[3], Nikolas Nikolaou[3], Rocco D'Antuono[4] and Michael Ladomery[1]

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MBNL3 (muscleblind like 3) is a splice factor know for the regulating the splicing of cardiac troponin T and insulin receptor. MBNL3 is typically expressed during fetal development, however the expression of MBNL3 is associated with tumorigenesis in hepatocellular carcinoma, ovarian cancer, pancreatic cancer and non-small cell lung cancer. Human cancer cells (MG-63 and PANC-1) were transfected with a morpholino designed to knock down MBNL3 (MBNL3.204). Morpholino knockdown of MBNL3 effects on cell biology were measured based on cell survival, cell proliferation, migration (wound healing assay, Boyden chamber) and invasion (in both a cell model and spheroid model). MBNL3-regulated alternative splicing events were assessed with high-throughput next-generation RNA sequencing, analysed on rMATs and confirmed with PCR. MBNL3.204 morpholino resulted in a 4- and 10-fold downregulation change in MBNL3 expression in MG-63 and PANC-1 cells respectively. MBNL3 knockdown resulted in a significant decrease in cancer cell migration and invasion. MBNL3 knockdown resulted in a ~80% decrease in MG-63 spheroid invasion. Changes were also seen in the expression of proteins associated with epithelial-mesenchymal transition (EMT) N-cadherin and E-cadherin consistent with a reduction in EMT when MBNL3 was knocked down. Moreover, MBNL3 knockdown in PANC-1 cells resulted in the expression of alternative splice events occurring in 713 genes, including the alternative splicing of lncRNA *PXN-AS1* (a known MBNL3 target). The knockdown of MBNL3 with a translation-blocking morpholino offers a potential option for the development of an anti-cancer therapeutics as there was a reduction in critical aspects of cancer progression, cell survival and migration.

# ***Investigating functions for syntenic long non-coding RNAs during zebrafish melanocyte development and human melanoma.***

*Stephanie M. E. Jones[1], Elizabeth Coe[1], Igor Ulitsky[2], Robert N. Kelsh[1] and Keith W. Vance[1].*

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Long non-coding RNAs (lncRNAs) are involved in embryonic development and cancer tumorigenesis. However, assessment of evolutionarily conserved functions of lncRNAs is challenging due to the lack of sequence conservation between vertebrates. Here, we identified melanoma and melanocyte expressed lncRNAs, predicted to be regulated by the key MITF melanocyte and melanoma transcription factor, based on conserved human-zebrafish synteny. These lncRNAs hold promise for the development of new melanoma treatments, but first we to understand their functions in development and disease.

We identified 506 human melanoma and melanocyte expressed lncRNAs whose loci are bound by MITF and that have a positionally equivalent zebrafish lncRNA transcript. We prioritised one of these lncRNAs, *Differentiation Antagonizing Non-Protein Coding RNA* (*DANCR),* transcribed from syntenic regions in human and zebrafish genomes. We have shown that melanoma patients with high *DANCR* expression display decreased survival and that *DANCR* expression is regulated by oncogenes MITF and c-MYC. Consistently, depletion of the *DANCR* transcript reduces cell proliferation and migration of human melanoma cells. *DANCR* is a cytoplasmically-enriched regulator of genes involved in cell proliferation, migration, cell cycle and stress response. Zebrafish *dancr* is also cytoplasmically-enriched and expressed as a gradient in early development with highest expression in the tail. *CRISPR*-mediated deletion of the zebrafish *dancr* promoter leads to a dorsalisation-like phenotype where tail structures are lost. We have identified conserved regulatory functions of *Dancr* for several genes between humans and zebrafish. Together our work indicates that *Dancr* is a conserved lncRNA oncogene that is crucial during early vertebrate development.

## MICRORNA EXPRESSION PROFILING IN PARKINSON'S DISEASE BRAINS

Klokkaris, Anna Migdalska-Richards (and 2nd supervisor is Prof Jon Mill).

Parkinson's disease (PD) is a heterogeneous disorder and the aetiology is still incompletely understood. The most common genetic risk factor for PD, *GBA1* (encoding glucocerebrosidase), shows incomplete penetrance with only a minority of carriers developing the disease. Additional factors beyond genetics are likely to play a role and previous work has identified microRNA (miRNA) expression alterations in the disease. We start to investigate whether miRNA expression in the brain varies according to both PD and *GBA1* mutation status.

RNA was extracted from the prefrontal cortex of 79 individuals consisting of PD with dementia, PD without dementia and controls. Each group was subdivided into individuals with and without *GBA1* mutations. The TaqMan OpenArray MicroRNA Panel was used to quantify the expression of 754 miRNAs. A linear regression model was used to identify differentially expressed miRNAs between the different subgroups.

Several miRNAs were differentially expressed ($P < 0.05$) in PD brains as well as between *GBA1* and non-*GBA1* samples, with a number of these previously associated with PD and other brain disorders. In particular, miR-181a-5p and miR-374a-3p were significantly differentially expressed (passing FDR correction) between PD-*GBA1* with dementia and control-*GBA1*. miR-374a-3p was previously found to be downregulated in PD peripheral cells.

This is one of the first miRNA studies to subdivide PD individuals by *GBA1* status. We have also generated genome-wide DNA methylation data in different brain cell types on these samples. We plan to integrate these datasets and functionally validate the top changes to identify novel genes involved in PD and improve mechanistic understanding.

**Urinary MicroRNA Changes in Living Kidney Donors**

Hughes, S., Smith, D. A., Dighe, S., Fraser, D. J., Khalid, U., Bowen, T.

## Introduction

MicroRNAs (miRNAs), global regulators of gene expression at the post-transcriptional level, hold promise as biomarkers of function in health and disease, tools to gain insight into functional mechanisms, and monitors of responses to treatment.

Following kidney donation, the donor's remaining kidney exhibits hyperfiltration, a benign, compensatory process resulting in increased kidney function that subsequently returns to pre-donation baseline. However, little is known about the processes that regulate post- donation hyperfiltration, and no studies on miRNAs have so far been published.

Here, we profiled miRNAs in living kidney donor (LKD) urine samples collected at time points before and after donation.

## Methods

MiRNAs were profiled in LKD urine samples (n = 16) collected immediately before donation and then at 1 day, 2 weeks and 3 months post donation. Direct quantification was carried out with nucleic-acid hybridisation-based nCounter multiplex analysis (NanoString) to quantify 827 miRNAs. We compared these outputs with data for 754 miRNAs generated using the RT-qPCR-based Taqman Low Density Array system (TLDA; Thermo Fisher Scientific).

## Results

Our NanoString data revealed that detection of 14 miRNAs decreased significantly in LKD urine samples post-donation. In addition, sex-specific differences were observed. TLDA analysis is currently underway, and our comparison of differences between hybridisation- based and RT-qPCR-based urinary miRNA detection will be presented at RNA Exeter 2024.

## Conclusion

Analyses of urinary miRNAs have the potential to reveal the molecular mechanisms underlying hyperfiltration. Hyperfiltration mimics recovery from acute kidney injury, which in 2023 was estimated to cost approximately 1.6% of the total NHS budget (>£3 bn).

**Title:** Characterisation of iPSC-derived Renal Proximal Tubule Epithelial Cells Using Single Cell RNA Sequencing

**Authors:** Elliot Ruf, Shrinivas Dighe, Catia Das Neves Neto, Sumukh Deshpande, Mark Gumbleton, Tim Bowen, Donald Fraser

## Introduction:

Renal proximal tubular epithelial cells (PTCs) constitute >50% of the human kidney's cortical mass and are central to renal function and response to injury. Recent studies using single cell RNA sequencing (scRNA-seq) have identified novel PTC subtypes. Developing effective *in vitro* model systems is crucial to understanding PTC biology in health and disease. In the current study we characterised iPSC-derived PTC cells and compared them to the established PTC line HK-2.

## Methods:

PTCs were generated from one iPSC line derived from commercial KOLF-2 cells and from two iPSC lines derived in-house from PBMCs. iPSCs were pre-treated with 10 µM RI for 1 hr at 37°C and dissociated into single cells (40,000 cells/well, 6 µm CHIR99021) before seeding into laminin-521 (20-40 µg/mL) 12-well plates with mTeSR-1 medium and 10 µM Ri (2 wells) for 5 d. Monocultures were incubated in mTeSR-E6 media with 200 ng/mL FGF9 and 1 µg/mL heparin for 3 d, then mTeSR-E6 with CDBLY2 for a further 5 d. For spheroid generation, 250,000 cells were transferred to 0.4 µm Transwell polyester membranes in 6-well ALI plates, generating up to 9 spheroids/Transwell with TeSR-E6/2% FBS. Spheroids were incubated with TeSR-E6 with 5 µm CHIR for 1 hr at 37°C, then TeSR-E6 media with 200 ng/mL FGF9 and 1 µg/mL heparin. Spheroid medium was changed to TeSR-E6 without growth factors on day 5, then refreshed every second day until harvest. Spheroids were harvested and used for RT-qPCR, immunofluorescence, and scRNA-sequencing. iPSC-derived PTCs and HK-2 cells were characterised by RT-qPCR, immunocytochemistry, and immunoblotting. Comparative scRNA-sequencing was performed using Parse Biosciences WT Mini v2 kit, sequencing used the NovoSeq-S200 platform and data processed employing Cardiff University supercomputing facilities.

## Results:

HK-2 cells and iPSC-PTCs exhibited PTC and epithelial characteristics under light microscopy, and PTC markers expression was detected by RT-qPCR. Injury markers including HAVCR1 were expressed by HK-2 cells but was undetected in iPSC-PTCs. iPSC-PTCs exhibited differential expression of markers in the spheroid centre compared to the periphery. The scRNA-seq dataset comprised 69,284 cells. Major clusters exhibiting PTC gene profiles were common to HK-2 and iPSC-derived PTCs. Additional HK-2 clusters expressed dedifferentiation, proliferation, and injury markers. iPSC-PTCs clusters expressing similar gene profiles were detected, as were other clusters with predominantly mesenchymal gene expression profiles.

## Conclusion:

Our data support the utility of iPSCs to generate PTCs and characterise previously unrecognized phenotypic heterogeneity in HK-2 cells.

**An atlas of expressed transcripts in the prenatal and postnatal human cortex**

Rosie Bamford and Jon Mill

University of Exeter Medical School

Alternative splicing is a post-transcriptional mechanism that increases the diversity of expressed transcripts and plays an important role in regulating gene expression in the developing central nervous system. We used long-read transcriptome sequencing to characterise the structure and abundance of full-length transcripts in the human cortex from donors aged 6 weeks post-conception to 83 years old. We identified thousands of novel transcripts, with dramatic differences in the diversity of expressed transcripts between prenatal and postnatal cortex. A large proportion of these previously uncharacterised transcripts have high coding potential, with corresponding peptides detected in proteomic data. Novel putative coding sequences are highly conserved and overlap de novo mutations in genes linked with neurodevelopmental disorders in individuals with relevant clinical phenotypes. Our findings underscore the potential of novel coding sequences to harbor clinically relevant variants, offering new insights into the genetic architecture of human disease. Our cortical transcript annotations are available as a resource to the research community via an online database.

**Insights into downstream translation initiation from 33,000 start-loss variants**

Stuart J Cannon, Patsy Ralls, Prof. Caroline F. Wright

University of Exeter Medical School

**Background**

Population cohorts enable unique insights into genetic constraint across different classes of variation. Start-loss variants could result in complete loss-of-function or be rescued by downstream initiation. It is unknown in which genes reinitiation can act as a possible rescue mechanism, hampering accurate variant classification.

**Methods**

We identified several likely reinitiation features and assessed their contributions to the incidence of start-loss variants (n~33,000) from gnomAD v4 exome sequencing data (n~750,000 participants). Findings were replicated in whole genome sequencing data from UK Biobank (n~500,000 participants) and All of Us (n~250,000 participants).

**Results**

Loss-of-function intolerant genes are more likely to have start-loss variants in population cohorts if the distance from the canonical start to the next in-frame ATG is short (OR=1.44, $p=6.83x10^{-8}$). This signal increases when the next in-frame ATG is before an out-of-frame ATG (OR=1.91, $p=3.32x10^{-17}$), before the first predicted protein domain (OR=2.22, $p=1.41x10^{-14}$), or has a strong Kozak sequence strength (OR=2.57, $p=3.29x10^{-13}$). Finally, transcripts in which the canonical start codon has a weak Kozak sequence strength are more likely to have start-loss variants (OR=1.89; p=0.0001), suggesting more stochastic start codon usage.

**Conclusion**

Our data support the hypothesis that start-loss variants can sometimes be rescued by downstream reinitiation, depending upon transcript-specific features. These findings will enable the development of a pathogenicity prediction model based on the reinitiation potential of transcripts, that could aid classification of start-loss variants in disease-associated genes.

**Can environmental RNA characterise the physiological status of the European eel?**

Robyn Murdoch, Michael Ladomery, Laura Weldon, Mark Steer

The European eel, *Anguilla anguilla*, is a critically endangered species which is hard to survey using standard methods.

This PhD project aims to build upon the established environmental DNA (eDNA) work on European eels by identifying environmental RNA (eRNA) recovered from water samples and monitor the expression of genes related to ecologically relevant traits.

RNA is extracted from water samples collected from eel tanks. The RNA is then amplified and analysed using RT-PCR to assess if specific genes related to life stage and physiological stress are being expressed. So far in the project it has been possible to extract RNA from water samples and look for housekeeping genes specific to *A. anguilla*.

If successful in a captive context, the method will then be tested on water samples from rivers with known eel populations to assess whether species-specific eRNA detection is possible and what the RNA can tell us about the physiological status of eels without the need for invasive handling.

Title: scRNAseq of *Drosophila pseudoobscura* testis shows development of multiple sperm morphs

Authors: Dr. Fiona Messer, Prof. Helen White-Cooper (Cardiff University)

Abstract: Males of the fruit fly *Drosophila pseudoobscura*, unlike most *Drosophila* species, make three different types (morphs) of sperm: long, medium, and short. The three sperm morphs produced simultaneously within the same testis, and throughout the lifetime of the fly. Each sperm type has a specific function. Long sperm are fertilising, whereas medium and short sperm are non-fertile. The non-fertile medium and short sperm have roles in sperm competition and protecting the fertilising sperm from damaging spermicides present in the female reproductive tract.

Very little is known about the processes underlying the development of the three sperm morphs, particularly the processes regulating sperm length – a key determinant of fertility. In previous work, we have used RNA-seq of pre-meiotic cells (spermatocytes) to identify genes which are differentially expressed between the developing sperm morphs, finding several key transcriptional regulators which may have roles in morph-specific development. However, this offers a snapshot of transcription at a single stage in the germline, and does not allow for exploration of earlier and later stages of development, or potential interactions between the soma and the germline.

Single cell RNA sequencing of the whole *D. pseudoobscura* testis shows two clear trajectories in the germline, likely representing fertile/non-fertile morph development, and which diverge earlier in development than previous data has shown. Furthermore, somatic lineages (support cells, epithelium and muscle) are also represented in the data, allowing us to build a richer understanding of the testis transcriptome in this species.

Future work will further validate the cell-type markers identified in this dataset, and RNAi of identified candidate genes involved in morph-specific transcriptional regulation and sperm elongation processes.