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**RNA Bristol (online) 2021**

**Thursday 3rd June 2021**

*Keynote Speaker:*

Prof. Claudio Sette, Università Cattolica del Sacro Cuore, Rome Italy

**‘A dynamic splicing program ensures proper synaptic connections in the developing cerebellum’**

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**The Meeting starts at 9.30am and finishes at 3pm, UK time.**

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|  | **TIME**  | **SPEAKER** | **INSTITUTION** | **TALK TITLE** |
| **Welcome and Introduction:**Michael Ladomery & Sebastian Oltean | 9.30-9.40 |  |  |  |
| **Session 1**Chair: James Uney | 9.40-10.00 | Ryan Frankum | University of Exeter | Genotype specific changes in isoform usage may explain the association between FOXO3 and extreme longevity |
|  | 10.00-10.20 | Monica Lamici-Ayine | University of Exeter | Novel compounds found to regulate VEGF-A alternative splicing in diabetic podocytes |
|  | 10.20-10.40 | Jinxia Zheng | University of Exeter | Investigation of properties and signalling pathways of compounds that switch FGFR2 splicing and regulate EMT in prostate cancer cells |
|  | 10.40-11.00 | Zhuofan Mou | University of Exeter | A differential gene expression signature for prostate cancer |
| **Tea/Coffee** | 11.00-11.30 |  |  |  |
| **Session 2**Chair: Sebastian Oltean | 11.30-11.50 | Merlin Davies-Abraham | University of Exeter | Persistence of clinically-relevant levels of SARS-CoV2 subgenomic RNA (sgRNA) in non-immunocompromised individuals |
|  | 11.50-12.10 | Manni Emad | University of Exeter | Depletion of Senescence-associated miRNA may drive senescence by disruption of constitutive splicing and stress fibre formation |
|  | 12.10-12.30 | Dr Daniel Smith | University of Cardiff | Electrochemical detection of urinary microRNAs in diabetic kidney disease. |
|  | 12.30-12.50 | Dr Helen Scott  | University of Bristol | Interaction between SAFB1 and mRNAs encoding polyglutamine repeat expansions, a role in polyglutamine disease pathology? |
|  | 12.50-13.10 | Victoria Kemp | Qiagen | QIAGEN RNA Sequencing solutions - including new Geneglobe analysis portal |
| **Lunch** | 13.10-14.00 |  |  |  |
| **Session 3****KEYNOTE LECTURE**Chair: Michael Ladomery | 14.00-15.00 | Prof. Claudio Sette | Università Cattolica del Sacro Cuore, Rome | **A dynamic splicing program ensures proper synaptic connections in the developing cerebellum** |
| **Closing remarks:**Michael Ladomery & Sebastian Oltean | 15.00-15.10 |  |  |  |

**Genotype specific changes in isoform usage may explain the association between FOXO3 and extreme longevity**

Ryan Frankum1, Tom S.O. Jameson2, Bridget A Knight3, Francis B. Stephens2, Benjamin T Wall2, Timothy A. Donlon4,5, Trevor Torigoe7, Bradley J. Willcox6,8, Richard C. Allsopp7 and Lorna W. Harries1.

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The rs2802292, rs2764264 and rs13217795 variants of FOXO3 have been associated with extreme longevity in multiple human populations, but the mechanisms underpinning this remain unclear. We aimed to characterise potential effects of longevity-associated variation on the expression and mRNA processing of the FOXO3 gene.

We performed a comprehensive assessment of FOXO3 isoform usage across a wide variety of human tissues and carried out a bioinformatic analysis of the potential for longevity-associated variants to disrupt regulatory regions involved in isoform choice. We then related the expression of full length and 5’ truncated FOXO3 isoforms to rs13217795 genotype in peripheral blood from two human populations and in skeletal muscle.

Different FOXO3 isoforms displayed considerable tissue specificity. We determined that rs13231195 and its tightly aligned proxy rs9400239 may lie in regulatory regions involved in isoform choice. The longevity allele at rs13217795 was associated with increased levels of full length FOXO3 in peripheral blood, but a decrease in truncated FOXO3 isoforms in skeletal muscle RNA.

We suggest that the longevity effect of FOXO3 SNPs may in part derive from a shift in isoform usage in skeletal muscle away from the production of 5’ truncated FOXO3 isoforms lacking a complete forkhead DNA binding domain which may have compromised functionality.

**Novel compounds found to regulate VEGF-A alternative splicing in diabetic podocytes**

Monica Lamici Ayine, Yihuan Liu, Jake Hunter, Megan Stevens, Sebastian Oltean

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Vascular endothelial growth factor A (VEGF-A) has an alternatively spliced variant, VEGF-A165b, formed when a distal 3’ splice site in exon 8 is selected. The anti-angiogenic and anti-permeability VEGF-A165b has reno-protective properties and has been shown to rescue kidney function in diabetic nephropathy (DN) mouse models. The aim of this study is to investigate in renal podocytes, three novel compounds that regulate VEGF-A splicing.

Trovafloxacin and 10058-F4 **(5-[(4-Ethylphenyl) methylene]-2-thioxo-4-thiazolidinone),** were identified to switch VEGF-A splicing in a previous screen using the Library of Pharmacologically Active Compounds LOPAC (Sigma). The third compound, delphinidin, was found to be the key compound regulating VEGF-A splicing in a natural blueberry and sea buckthorn extract drug (DIAVIT).

Trovafloxacin, 10058-F4, and delphinidin (all at 10 μg/ml) significantly increased VEGF-A165b relative to VEGF-A165a in podocytes exposed to a normal glucose environment. Furthermore, 10058-F4 and delphinidin were also found to increase the VEGF-A165b/VEGF-A165a ratio in podocytes exposed to a diabetic environment. Preliminary data suggests that 10058-F4 and delphinidin may influence the expression of Clk-1, a kinase known to regulate VEGF-A165b splice site selection, in diabetic podocytes. Delphinidin was found to significantly increase phosphorylation of SRSF6, a splice factor known to promote VEGF-A165b splice site selection, in diabetic podocytes. 10058-F4 was found to downregulate SRSF1, which may be through c-myc inhibition.

In conclusion, we have identified three novel compounds that regulate VEGF-A splicing to promote the expression of VEGF-A165b in podocytes. This study will further investigate their mechanism of action. The final goal of this project is to identify whether these novel splicing-modulating compounds can be used to develop new therapeutic strategies in DN.

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

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**Introduction:**

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

**Materials and methods:**

Various properties of different PCa cell lines treated with chemicals were assessed by AlamarBlue assay, Boyden chamber assay, and growth curve. Signalling pathways were investigated by inhibiting specific steps of possible pathways.

**Results and Discussion:**

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

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

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

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

**Conclusions:**

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

**A differential gene expression signature for prostate cancer**

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Prostate cancer (PCa) is one of the leading causes of male death worldwide, and its pathology remains to be fully explored. We aimed to use a whole transcriptome approach to identify potential prognostic or predictive biomarkers for PCa prediction or prognosis. We carried out whole transcriptome profiling on the Clariom D Pico™ platform on paired and histologically confirmed benign and malignant prostate tissue from 9 PCa cases obtained from routine radical prostatectomy from the Royal Devon and Exeter Hospital, Devon, UK. We determined that 22165 transcripts were expressed in our sample set, of which 47 were differentially expressed in PCa tissue (differentially expressed genes; DEGs). BIO-ENV correlation analysis was then applied to identify the most representative subsets of the complete DEGs based on Spearman correlation, which confirmed a module of 6 potential genes (*DANCR*, *ERBB3*, *MYC*, *PILRB*, *TRPM4* and *TRPV6*) that were significantly associated with PCa. Independently, weighted gene co-expressed analysis (WGCNA) was used to explore potential associations between co-expressed gene modules and prostate tumour status. A total of 7 modules were identified, 4 of which were considered as key modules based on Pearson correlation between module eigengenes and benign-malignant tumour status. Three hub genes (*PCBP2*, *MIR1228* and *MIR574*) were identified based on their intramodular connectivity. Moreover, GO function and KEGG pathway enrichment analyses of the DEGs indicates that the DEGs are enriched in lyase and carbon-oxygen lyase activities; fructose and mannose metabolism, base excision repair, AMPK and insulin signalling pathways. GO-based gene set enrichment analysis (GSEA) suggested that the pre-processed genes were enriched in mitotic sister chromatid segregation, cell cycle checkpoint, G2/M transition of mitotic cell cycle and ubiquitin ligase complex activities. Our studies suggested that the 6 genes identified in our sample set may have future utility as biomarkers of the presence of PCa, but their predictive or prognostic capabilities remain to be further assessed.

**Persistence of clinically-relevant levels of SARS-CoV2 subgenomic RNA (sgRNA) in non-immunocompromised individuals**

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Prevention of ongoing transmission of SARS-CoV-2 is an essential component of managing the current pandemic. A key prerequisite for this is an accurate understanding the kinetics of viral clearance, likely infectious duration and the circumstances under which clinically relevant levels of active and potentially infectious virus may persist.

We carried out an assessment of E gene and sgRNA viral load as a function of disease severity in a retrospective collection of swab samples from 176 PCR-confirmed individuals. We then determined the viral clearance dynamics of E gene and sgRNA viral sequences in serial samples from a subset of 17 individuals, and assessed duration of positive test result in a further set of 65 E gene positive individuals and 32 sgRNA positive individuals.

We found that both E gene and sgRNA viral load correlated with the presence of COVID-19 symptoms, and that whilst sgRNA viral load declined before E gene viral load, some individuals retained both sgRNA and E gene positivity for extended periods of time up to 68 days. 13% of sgRNA positive cases still exhibited clinically-relevant levels of virus after 10 days, and that these individuals were clinically unremarkable.

Our results suggest that care needs to be taken in the assumption that people without immunocompromised or those who are not elderly will be clear of infectious virus after 10 days. In some cases, active virus may persist after this period, which may lead onward transmission in these cases. We suggest when onward transmission would pose a serious public health threat, for example return to residential care after hospitalisation.

**Depletion of senescence-associated miRNA may drive senescence by disruption of constitutive splicing and stress fibre formation**

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The accumulation of senescent cells has emerged as a key regulator of ageing and the deterioration of tissue function. MicroRNAs are a key component of the molecular machinery governing the emergence of cellular senescence, with several known to regulate important genes associated with this process. Here, we have carried out a whole miRNome characterisation of the patterns of miRNA expression in early and late passage human primary endothelial cells, as well as late passage cells that have undergone cellular rejuvenation following treatment with the polyphenol resveratrol. We have identified some miRNAs that demonstrate association with senescence and reversal following correction for multiple testing, one of these miRNAs, demonstrates antagonistic pattern of expression in senescence and rescue. It is predicted to target 65 genes, 32 of which demonstrated dysregulated expression in senescent cells. Enrichment analysis or GO pathways demonstrated that targets of this miRNA were enriched in pathways regulating tri-snRNP formation and action, ubiquitination and microfilament-mediated vesicle trafficking. These data indicate that this miRNA may contribute to cellular senescence in human primary endothelial cells by dysregulation of the major spliceosome and subsequent disruption to the proteasome.

**Electrochemical detection of urinary microRNAs in diabetic kidney disease**

Daniel A. Smith,\*1,2 Kate Simpson,1 Matteo Lo-Cicero,2 Lucy J. Newbury,1 3 Phillip Nicholas,3 Nigel Caiger,3 Donald J. Fraser1, James E. Redman2,Timothy Bowen1

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We have developed an RT-qPCR-based method for urinary microRNA (miRNA) quantification [1], and recently identified expression profiles to identify diabetic kidney disease [2] and predict graft function following renal transplantation [3]. In parallel, we are investigating the use of electrochemical detection to quantify urinary miRNAs. Firstly, we established proof of principle using glassy carbon electrode-based biosensors, which detected urinary microRNAs with increased sensitivity compared to RT-qPCR [4]. We are now developing disposable screen printed carbon electrode (SPCE)-based miRNA sensors with comparable performance that are potentially adaptable to the clinical laboratory/point of care environment. A linear response was observed for our SPCE sensors across physiologically relevant concentrations of exogenous miR-21, achieving an analogous limit of detection to our previously published data [4,5]. We then used these sensors to analyse patient and control urine samples, and detected a diabetic kidney disease-associated miR-192 decrease we identified previously using RT-qPCR [2,5]. Sensor surface analyses using histochemistry and atomic force microscopy showed the predicted deposition of sensor components at the electrode surface, while uric acid measurements provided evidence that the responses obtained were not due to urinary interferents[5]. Our protocol is readily adaptable to routine analysis of human urine samples, and this method has clear potential for development into a high throughput and parallel diagnostic platform for urine and other body fluids in a variety of clinical disorders.

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**Interaction between SAFB1 and mRNAs encoding polyglutamine repeat expansions, a role in polyglutamine disease pathology?**

Helen L Scott, Nicola Buckner, Caroline Rivers, Kevin Kemp, James Uney

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Scaffold attachment factor B (SAFB) 1 and 2 are RNA and DNA binding proteins with roles in chromatin organisation, transcriptional control, alternative splicing, the cellular response to stress and miRNA biogenesis. iCLIP experiments showed a strong interaction between SAFB1 and ATXN1 mRNA with binding occurring in close proximity to the polyglutamine encoding CAG repeat. We hypothesised that the SAFB1 interaction with SCA1 mRNA may be altered if as in spinocerebellar ataxia 2 (SCA2) there is an expansion in the CAG repeat region.  Co-immunoprecipitation experiments revealed an increased affinity for the expanded ATXN1-85 Q mRNA transcript compared to the wild type.  Intrigued as to whether this might lead to altered SAFB1 function in SCA we examined the expression of SAFB1 in the cerebellum of SCA cases.  In tissue sections from SCA patients we observed an increase in SAFB1 immunostaining in the nucleus of cerebellar Purkinje cells compared to control tissue. We also observed SAFB1 in the dendrites of Purkinje cells from SCA but not control tissue.  Furthermore, we observed a similar altered expression of SAFB1 in the striatal neurons of Huntington’s disease cases, another neurodegenerative disease caused by the expansion of a CAG repeat.  Together these data point to a role for SAFB1 in the processing and/or trafficking of CAG repeat containing mRNA.  Furthermore, the interaction with disease associated expanded repeats leads to a miss-localisation of SAFB1 which may play a role in the aetiology of the disease.