Newcastle University

Faculty of Medical Sciences

TEF Gold

Athena SWAN Silver Award

IHS ION ICaMB ICM IGM NICR

2nd FMS - POst-doc Symposium

FPOS

Keynote speaker:

Prof. Ana Maria Cuervo

Professor in Developmental and Molecular Biology at the Albert Einstein College of Medicine

Title:

"Selective Autophagy: adding years of healthy life through smart recycling"

June 21st 2019 9am - 6pm

David Shaw Lecture Theatre - Medical School


Break
patients
cell
disease
Medicine
cells
DNA
human
expression
proteins
clinical
tissue
function
functional
mutations
data
role
study
protein
treatment
response
signalling
different
variants
analysis
method
risk
genes
genetic
identified
RNA
FOXO1
Cell
Results
mitochondrial
potential
bacterial
present
cases
Molecular
GalNT7
Neuroscience
Methods
development
significantly
sequencing
Genetic
Biosciences
Keywords
Research
cancer
heart
identify
immune
model
Cellular
work
gene
studies
ICM
progression
activity
essential
therapy
system
mechanism
including
specific
failure
common
significant
Conclusion
Background
POLRMT
ADAR1
NICR
T3SS
CTCs
ICaMB
IGM
RPE
lymphoma
Northern

FMS Post Doc Symposium 2019

After the resounding success of the inaugural FMS Post-doc symposium in 2018 we are back for a second year to once again to showcase the outstanding contributions and research of post-doctoral staff across all institutes. The symposium is organised by the Post-Doctoral Committee formed in 2017. We are delighted to have a keynote talk from **Prof. Ana Maria Cuervo**, co-director of the Einstein Institute for Aging Research, at Einstein College of Medicine in New York who will also present a **prize for best paper (including a £150 Amazon voucher).** Prizes will also be awarded for **best talk** and for **best poster**.

We have secured generous sponsorship from the Biochemical Society, Thermofisher Scientific, New England Biolabs, Proteintech, Integra Biosciences, Starlabs, Dixon science, and Takara who will be providing material for the conference bags as well as sponsorship for the best poster, talk and paper prizes.

We hope you will enjoy the day!

You can vote for the best poster! Go to: www.menti.com, type in the code 366207, or scan the QR code below and input the poster number of your choice!

FMS Post-Doctoral Committee

Established in 2017, we are here to organise events, provide support and information, and represent post-doctoral researchers' views to the faculty. Here you will find a list of your local institute representatives on the committee, as well as upcoming events, and important links to faculty resources.

We are continuously eager to hear from PDRAs from all institutes of FMS and open to shape the role of this committee in response to your needs, so please share your opinion via your local institute representatives, by emailing: fmspostdoccomm@newcastle.ac.uk, or using the contact form on our website. If you have some ideas or would like to join us, please also get in touch. We are always welcoming to new faces and new ideas!

FMS Post-Doctoral Committee

http://goo.gl/zeQmgN @fms_postdoccomm #nclfpos

Website: https://www.societies.ncl.ac.uk/fmspostdoccomm/

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NICR Martina Finetti Martyna Pastok Natalie Tatum **NICR – Chemistry** Suzannah Harnor Cinzia Bordoni	**IHS** Vacant spot - could this be you??

FMS Post-Doctoral Committee (June 2019)

Acknowledgements

We would like to thank everyone who helped us to make this event happen.

- Prof Derek Mann – for continued support of the committee throughout the year, and FMS for financial support.
- Kay Howes, Stephanie Maughan Jill McKenna and Judith Williams - for advice and help in organisation
- Dr Paula Salgado and Dr Kevin Waldron – for assistance, advice and help
- All our sponsors without whom the prizes would not be so generous
- All the academics who helped with the best paper selection: Dr Kevin Waldron (ICaMB), Dr Paula Salgado (ICaMB), Prof Sophie Hambleton (ICM), Prof Simi Ali (ICM), Prof Helen Arthur (IGM), Prof Melissa Bateson (IoN), Prof Craig Robson (NICR), and Dr Rhoda Stefanatos (ICaMB).
- Jeremy Domis (ICM) for being our photographer

FMS POST DOC SYMPOSIUM
Friday 21st June 2019

08:30-09:30 **Coffee and Registration**

09:30-09:40 **Welcome and Opening Remarks from the Dean**

09:40-10:40 **Morning Session**

Anastasia Hepburn (NICR): The induction of core pluripotency master regulators in cancers defines poor clinical outcomes and treatment resistance

James O'Keeffe (IoN): A robot model of praying mantis 3D vision

Martina Finetti (NICR): Integrated quantitative proteomics by SWATH-MS of Malignant Rhabdoid Tumours uncovers new therapeutically opportunities

Jack Leslie (ICM): Combined CXCR2 inhibition and Anti-PD1 therapy alters immune cell infiltration and limits HCC progression

10:40-11:00 **Coffee Break in the Sponsors' Foyer**

11:00-12:00 **Midday Session**

Kirsty McAleese (IoN): Blood-brain barrier dysfunction in Alzheimer's disease and normal ageing: Implications for use as a biomarker

Ricardo Gouveia (IGM): Skin by the metre - how to biofabricate body-size, scaffold-free human dermis for transplantation

Svetlana Cherlin (IHS): Developing and testing high-efficacy patient subgroups within a clinical trial using polygenic risk scores

Hannah Gaimster (ICaMB): Lethal depletion of a bacterial cell wall synthesis protein is rescued by slowing DNA replication

12:00-12:30 **Flash Poster Presentations: 2-Minutes Each**

12:30-13:00 **Guest Speaker: Dr. Bette Phimister, Deputy Editor of the New England Journal of Medicine**

13:00-14:00 **Lunch (Boardroom) and Poster Session (Sponsors' Foyer)**

14:00-15:00 **Keynote Speaker: Prof. Ana Maria Cuervo, Albert Einstein College of Medicine, USA**

15:00-15:05 **Paper Prize Announcement by Prof. Ana Cuervo**

15:05-15:30 **Best Post-Doc Paper Prize Talk**

Martina Miotto (IGM): 4D Corneal Tissue Engineering: Achieving Time-Dependent Tissue Self-Curvature through Localized Control of Cell Actuators

15:30-16:00 **Coffee Break**

16:00-17:00 **Afternoon Session**

Emma Scott (IGM): GalNT7 regulates prostate cancer growth through O-linked glycosylation

Marco Trevisan-Herraz (ICM): Are men and women epigenetically different?

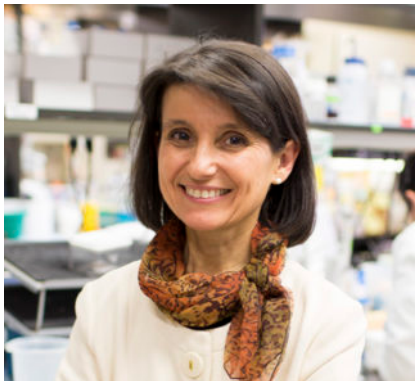
Azzeldin Madkour (ICaMB): A fishy tale packed with novelty!

Ahmad Al-Mrabeh (ICM): Restoration of insulin secretion brings about recovery of the pancreas volume in type 2 diabetes

17:00-17:30 **Poster and Talk Prizes & Closing Remarks**

17:30-19:00 **Drinks Reception**

Keynote Speaker – Prof. Ana María Cuervo

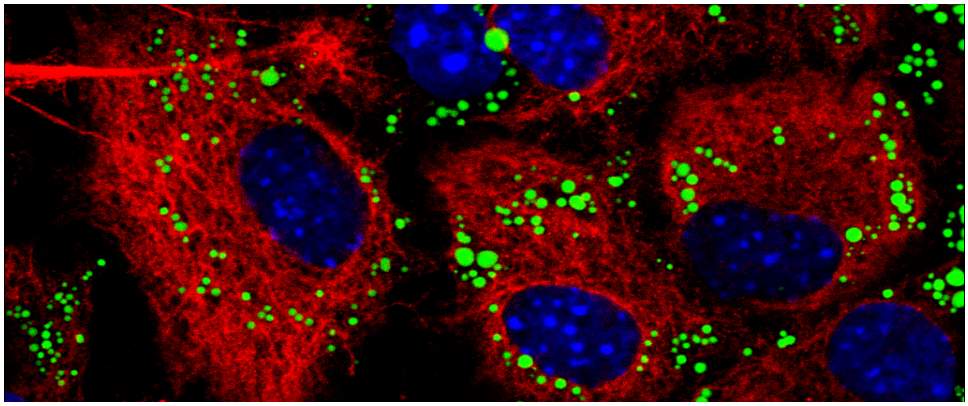


Dr. Cuervo is co-director of the Einstein Institute for Aging Research, and a member of the Einstein Liver Research Center and Cancer Center. In 2001 she started her laboratory at Einstein, where she studies the role of protein-degradation in aging and age-related disorders, with emphasis in neurodegeneration and metabolic disorders.

Dr. Cuervo's group is interested in understanding how altered proteins can be eliminated from the cells and their components recycled. Her group has linked alterations in lysosomal protein degradation (autophagy) with different neurodegenerative diseases including Parkinson's, Alzheimer's and Huntington's disease. They have also proven that restoration of normal lysosomal function prevents accumulation of damaged proteins with age, demonstrating this way that removal of these toxic products is possible. Her lab has also pionered studies demonstrating a tight link between autophagy and cellular metabolism. They described how autophagy coordinates glucose and lipid metabolism and how failure of different autophagic pathways with age contribute to important metabolic disorders such as diabetes or obesity.

Dr. Cuervo is considered a leader in the field of protein degradation in relation to biology of aging and has been invited to present her work in numerous national and international institutions, including name lectures as the Robert R. Konh Memorial Lecture, the NIH Director's, the Roy Walford, the Feodor Lynen, the Margaret Pittman, the IUBMB Award, the David H. Murdoxk, the Gerry Aurbach, the SEBBM L'Oreal-UNESCO for Women in Science, the C. Ronald Kahn Distinguished Lecture and the Harvey Society Lecture. She has organized and chaired international conferences on protein degradation and on aging, belongs to the editorial board of scientific journals in this topic, and is currently co-editor-in-chief of Aging Cell.

Dr. Cuervo has served in NIH advisory panels, special emphasis panels, and study sections, the NIA Scientific Council and the NIH Council of Councils and has been recently elected member of the NIA Board of Scientific Counselors and member of the of the Advisory Committee to the NIH Deputy Director.. She has received numerous awards for the pioneerign work of her team such as the 2005 P. Benson Award in Cell Biology, the 2005/8 Keith Porter Fellow in Cell Biology, the 2006 Nathan Shock Memorial Lecture Award, the 2008 Vincent Cristofalo Rising Start in Aging Award, the 2010 Bennett J. Cohen Award in Aging Biology, the 2012 Marshall S. Horwitz, MD Faculty Prize for Research Excellence and the 2015 Saul Korey Prize in Translational Medicine Science. She has also received twice the LaDonne Schulman Teaching Award. In 2015 she was elected International Academic of the Royal Academy of Medicine of the Valencia Community and in 2017, she was elected member of the Real Academia de Ciencias Exactas, Fisicas y Naturales. She was elected member of the American Academy of Arts and Sciences in 2018 and member of the National Academy of Science in 2019.



Best Paper winner – Martina Miotto (IGM):

4D Corneal Tissue Engineering: Achieving Time-Dependent Tissue Self-Curvature through Localized Control of Cell Actuators

(Advanced Functional Materials 2019 [epub ahead of print])

DOI link: **https://doi.org/10.1002/adfm.201807334**

Martina Miotto obtained her Bachelor and Master degrees at the University of Ferrara, Italy. Since 2018 she holds a PhD in Tissue Engineering from the Institute of Genetic Medicine at Newcastle University. During her PhD she investigated the generation of improved corneal stromal substitutes achieved via a 4D tissue engineering approach. She then started exploring the commercialisation of a technology co-developed with her supervisor, Professor Che Connon, and since late 2018 she is the co-founder and Director of CellulaREvolution Ltd that span-out from Newcastle University. She is currently an Enterprise Fellow of the Royal Society of Edinburgh funded by BBSRC. She also volunteered, both in Italy and in the UK, in engaging the public with research events.

Abstract

While tissue engineering is widely used to construct complex tridimensional biocompatible structures, researchers are now attempting to extend the technique into the fourth dimension. Such fourth dimension consists in the transformation of 3D materials over time, namely, by changing their shape, composition, and/or function when subjected to specific external stimuli. Herein, producing a 4D biomaterial with an internal mechanism of stimulus, using contractile cells as bio-actuators to change tissue shape and structure, is explored. Specifically, producing cornea-shaped, curved stromal tissue equivalents via the controlled, cell-driven curving of collagen-based hydrogels. This is achieved by modulating the activity of the bio-actuators in delimited regions of the gels using a contraction-inhibiting peptide amphiphile. The self-curved constructs are then characterized in terms of cell and collagen fibril reorganization, gel stiffness, cell phenotype, and the ability to sustain the growth of a corneal epithelium in vitro. Overall, the results show that the structural and mechanical properties of self-curved gels acquired through a 4D engineering method are more similar to those of the native tissue, and represent a significant improvement over planar 3D scaffolds. In this perspective, the study demonstrates the great potential of cell bio-actuators for 4D tissue engineering applications.

Talks

O1

Anastasia Hepburn

Title: The induction of core pluripotency master regulators in cancers defines poor clinical outcomes and treatment resistance

Northern Institute for Cancer research (NICR)

Stem cell characteristics have been associated with treatment resistance and poor prognosis across many cancer types. The ability to induce and regulate the pathways that sustain these characteristic hallmarks of lethal cancers in a novel in vitro model would greatly enhance our understanding of cancer progression and treatment resistance. In this work, we present such a model, based simply on applying standard pluripotency/embryonic stem cell media alone. Core pluripotency stem cell master regulators (OCT4, SOX2 and NANOG) along with epithelial-mesenchymal transition (EMT) markers (Snail, Slug, vimentin and N-cadherin) were induced in human prostate, breast, lung, bladder, colorectal, and renal cancer cells. RNA sequencing revealed pathways activated by pluripotency inducing culture that were shared across all cancers examined. These pathways highlight a potential core mechanism of treatment resistance. With a focus on prostate cancer, the culture-based induction of core pluripotent stem cell regulators was shown to promote survival in castrate conditions-mimicking first line treatment resistance with hormonal therapies. This acquired phenotype was shown to be mediated through the upregulation of iodothyronine deiodinase DIO2, a critical modulator of the thyroid hormone signalling pathway. Subsequent inhibition of DIO2 was shown to supress expression of prostate specific antigen, the cardinal clinical biomarker of prostate cancer progression and highlighted a novel target for clinical translation in this otherwise fatal disease. This study identifies a new and widely accessible simple preclinical model to recreate and explore underpinning pathways of lethal disease and treatment resistance.

Keywords:

O2

James O'Keeffe

Title: A robot model of praying mantis 3D vision

Institute of Neuroscience (IoN)

The estimation of depth is a fundamental task for many natural organisms and has broad applicability for autonomous systems. Stereopsis, the computation of distance via triangulation from two eyes, stands out as a robust solution to this task – having evolved in mammals, birds, and insects.

Most machine stereopsis algorithms tend to draw inspiration from humans, leading to algorithms which are computationally expensive. However, the praying mantis also uses stereopsis for depth estimation, and does so in a fundamentally different way to humans. As the mantis brain is simpler than a human's, its stereopsis algorithm is presumably also simpler – but has nonetheless made it a successful predator for millions of years.

Our work aims to reproduce mantis stereopsis and modify it for use in autonomous systems. We propose that this will be beneficial to the field of robotics and AI – particularly swarm robotics, where individual robots are necessarily simple.

Our previous behavioural experiments have shown that, whereas human stereopsis relies on comparing detailed patterns of contrast in two eyes, mantis stereopsis looks only for regions of the image where contrast is changing, and is not sensitive to the detailed pattern of contrast. Additionally, motion of the prey across the visual field is required to elicit strikes from the mantis. We have constructed a computational model of mantis motion detection that captures this behaviour.

Behavioural experiments also show that mantids have clear size preferences for their prey. Our recent neurophysiological work revealed that stereoscopic neurons in the mantis brain have receptive fields consisting of an excitatory centre and inhibitory surround. We have incorporated this centre/surround structure into our mantis motion detectors, and show that this produces a preferred target size that corresponds very closely to our empirical data. We propose these motion detectors as inputs for mantis stereopsis.

Keywords:

Vision Robotics Mantis AI Bio-Inspired

O3

Martina Finetti

Title: Integrated quantitative proteomics by SWATH-MS of Malignant Rhabdoid Tumours uncovers new therapeutically opportunities

Northern Institute for Cancer Research (NICR)

The functional consequences of SMARCB1 deletion in Malignant Rhabdoid Tumours (MRTs) have been so far characterised at the DNA and RNA level, however proteomic changes induced by SMARCB1 loss need to be revealed. In this study we developed an integrative approach to ask if SMARCB1 inactivation effects on of gene expression and methylation status also alter the composition of the MRT both in primary tumours and cell lines. Moreover to overcome the limited experimental information regarding paediatric cancer proteome, we generate a comprehensive spectral library for the analysis of MRT total, membrane and nuclear proteome by SWATH-MS. Our study shows that analysis of methylation and gene expression changes do not reliably predict proteomic changes. Notably by integration with whole genome CRIPSR screening, we described previously undistinguished SMARCB1 dependent pathway /membrane biomarkers that might be therapeutically exploited and mainly present at protein but not at mRNA/methylation level. For the first time our analysis links methylation, transcriptomic and proteome aberrations in MRT and significantly reveals the broad mechanism of SMARCB1 loss effect.

Keywords:

SWATH, paediatric cancer, Integrated quantitative proteomics

O4

Jack Leslie

Title: Combined CXCR2 inhibition and Anti-PD1 therapy alters immune cell infiltration and limits HCC progression

Institute of Cellular Medicine (ICM)

Background and Aims: Hepatocellular carcinoma (HCC) is the most common primary liver malignancy and is a leading cause of cancer-related death worldwide. Immune cells play a complex role in cancer, both promoting and limiting tumour progression. In the tumour microenvironment immunosuppressive neutrophils limit T cell activation, promoting tumorigenesis. Using a model of NAFLD associated HCC, we explored the therapeutic impact of impairing neutrophil chemotaxis into the liver, while promoting T cell activation.

Method: The DEN/ALIOS model of NAFLD-HCC was used. Male C57Bl6/j mice receiving a single intraperitoneal injection of N-Nitrosodiethylamine (DEN) at 2 weeks old were placed on to American Lifestyle-Induced Obesity Syndrome (ALIOS) diet at 8 weeks until culling at 40 weeks. Mice received 'late stage' vehicle control, a CXCR2 inhibitor and/or an anti-PD1 antibody for the final 12 weeks after tumours have already been established.

Results: Late stage treatment with anti-PD1 therapy in the DEN/ALIOS model had no effect on tumour stage or grade. Treatment with the CXCR2 inhibitor limited both tumour stage and grade, with the combination therapy reducing them further.

Treatment also had a profound effect on hepatic immune cell infiltration. CXCR2 inhibition reduced neutrophil infiltration but increased T cell numbers. Interestingly, combined therapy resulted in a large influx of neutrophils into the tumour but also a CD8+ rich T cell infiltrate. We hypothesise that combination therapy promotes the influx of anti-tumour rather than pro-tumour/ immunosuppressive neutrophils.

The effects of CXCR2 inhibition and anti-PD1 therapy alone compared to their combination highlights critical tumour promoting bidirectional crosstalk between neutrophils and T cells in the tumour microenvironment.

Conclusion: Our data provides the first evidence, in a murine model of late stage NAFLD- HCC, that dual targeting of neutrophils and T cells with the combination of a CXCR2 inhibitor and anti-PD1 therapy, combats HCC progression and has therapeutic potential.

Keywords:

inflammation liver cancer

O5

Kirsty McAleese

Title: Blood-brain barrier dysfunction in Alzheimer's disease and normal ageing: Implications for use as a biomarker

Institute of Neuroscience (IoN)

Objectives: Blood-brain barrier (BBB) dysfunction has been associated with cerebral small vessel disease (SVD), white matter lesions (WML) and the pathogenesis of Alzheimer's disease (AD). The presence of plasma-protein fibrinogen has been suggested to be a potential biomarker of AD. We aimed to determine if fibrinogen was associated with SVD, WML and AD hallmark pathologies (hyperphosphorylated tau (HPτ) and amyloid-β (Aβ)). Methods: Parietal tissue from 20 AD and 22 non-demented controls was quantitatively assessed for HPτ, Aβ, WML severity and the burden of fibrinogen in both WML and normal- appearing white matter (NAWM). SVD severity was determined by calculating sclerotic indices.

Results: WML- and NAWM fibrinogen burden was not significantly different between AD and controls nor was it associated with HPτ or Aβ pathology burden or WML severity. SVD was associated with and a predictor (both $p<0.05$) of both higher WML- and NAWM fibrinogen burden (both $P<0.05$) in controls only. In cases with minimal SVD NAWM fibrinogen burden was significantly higher in the AD cases ($p<0.05$).

Conclusions: Fibrinogen was present in both controls and AD cases and was not associated with the burden of AD pathologies or WML. Fibrinogen was strongly associated with SVD as expected, but only in controls. In AD cases with no SVD, fibrinogen was higher indicating possible additional mechanisms of BBB dysfunction associated with AD pathophysiology.

Keywords:

Alzheimer's disease, dementia, blood-brain barrier, fibrinogen

O6

Ricardo Gouveia

Title: Skin by the metre - how to biofabricate body-size, scaffold-free human dermis for transplantation

Institute of Genetic Medicine (IGM)

The human skin is a complex organ with a highly hierarchical and stratified structure. Reconstructing such an organ is therefore a challenging endeavour and requires precise and sophisticated engineering strategies to recapitulate the tissues' natural complexity. To this purpose, we developed an improved tissue templating technology that allows directing human dermal fibroblasts to bio-fabricate large-area (metre-long) tissues that closely emulate the natural dermis. This technology also allowed the new tissues to promote their own release from the template surface, thus facilitating their recovery as scaffold-free dermal equivalents solely comprising human cells and their own extracellular matrix. The structure and composition of these dermal self-lifting autogenous tissue equivalents, or SLATEs, were evaluated in detail and were shown to closely correlate to normal tissue function. Specifically, dermal SLATEs were shown to be composed of a dense collagen-based matrix interwoven with skin-characteristic elastic fibers. In addition, the mechanical properties of these tissues (i.e., robustness, elastic modulus, and resistance to contraction and enzymatic degradation) were comparable to those of the natural human dermis. Furthermore, dermal SLATEs were capable of constituting tissues with a higher-order complexity by serving as a substrate to support the growth of keratinocytes into stratified epithelia with distinct layers of differentiation. This work thus illustrates the great potential of tissue templating technologies and how these can pave the way for generating easily retrievable, body-sized, scaffold-free human skin tissues with a structure, composition, and function suitable for both fundamental and applied research, clinical testing and diagnosis, and transplant applications.

Keywords:

Tissue Engineering, Skin, Biofabrication, Templating, Serum-free

O7

Svetlana Cherlin

Title: Developing and testing high-efficacy patient subgroups within a clinical trial using polygenic risk scores

Institute of Health and Society (IHS)

Introduction It is increasingly common in clinical trials to collect a lot of data about patients such as genomic, imaging, data from wearable technologies. There is the potential for this high- dimensional information to be informative for the efficacy of a new treatment in the situations where only a subset of patients benefits from the treatment. The adaptive signature design method allows a trial to develop and test efficacy of treatment in a high- efficacy patient group (the sensitive group) using genetic data. Patients are classified to be sensitive or not based on their genetic information. The method requires specification of a set of tuning parameters for identifying the sensitive group. Selection of the tuning parameters is implemented by a time-consuming nested cross-validation procedure.

Methods We propose a variation to the adaptive signature design method that does not require selection of the tuning parameters. The method is based on polygenic risk scores that utilise weighed contribution of the gene expression levels. The sensitive group is found by applying a nonparametric clustering procedure to the polygenic risk scores. We have implemented the new method in an R package.

Results The performance of the new method is assessed for various sample sizes and response rates. The new method has substantial reduction in computational time required. In many scenarios there is a substantial improvement in the ability to correctly identify the sensitive group and the overall power of the design.

Discussion The new method for selecting a sensitive group of patients based on the polygenic risk scores shows a superior performance and drastically improves the computational time, in comparison to the existing one. Further research will focus on extending the method to incorporate different types of outcomes and a variety of types of biomarkers.

Keywords:

clinical trials, biomarkers, polygenic risk scores

O8

Hannah Gaimster

Title: Lethal depletion of a bacterial cell wall synthesis protein is rescued by slowing DNA replication.

Institute of Cell and Molecular Biosciences (ICaMB)

Cell wall synthesis is a key cell cycle process required for bacterial growth and proliferation. In bacteria the cell envelope consists of a phospholipid membrane encased within a peptidoglycan wall. Here, we report a previously unanticipated link between bacterial cell wall synthesis and DNA replication in Bacillus subtilis.

MurAA is an essential enzyme which catalyses the first step of peptidoglycan synthesis. Expression of the endogenous murAA gene was placed under the control of a xylose inducible promotor, and we show that in the absence of xylose the Pxyl-murAA strain is not viable. Depletion of MurAA causes oxidation of lipids and deoxyguanosine, indicating generation of reactive oxygen species (ROS). Moreover, depletion of MurAA activates the yneA promotor, a component of the RecA-dependent DNA damage response (DDR) regulon in B. subtilis, indicating that depletion of MurAA generates dsDNA breaks.

Unexpectedly, we have found that the lethal phenotype associated with depletion of MurAA can be suppressed by decreasing the rate of DNA replication initiation. First, deletion of the endogenous origin oriC and replacement with a significantly less active plasmid derived origin oriN provided rescue. Second, overexpression of a mutant allele of the regulator Soj (SojG12V) which specifically inhibits the activity of master DNA replication initiator DnaA also provides rescue. Excitingly, we have also found slowing the rate of DNA replication initiation in MurAA depleted cells significantly reduces the level of DNA damage.

Taken together, our preliminary data suggest that bacterial cell death associated with limiting cell wall precursor biosynthesis is caused by a combination of ROS-dependent oxidative DNA damage and high levels of DNA replication. Ongoing work will help to elucidate this molecular basis for the connection between cell wall biogenesis and DNA replication. Implications for antibiotic mechanism of action and potentiation will be discussed.

Keywords:

None given

O9

Emma Scott

Title: GalNT7 regulates prostate cancer growth through O-linked glycosylation

Institute of Genetic Medicine (IGM)

Diagnostic tests for prostate cancer (PCa) are either highly invasive or inaccurate. At current, the best treatment option available is androgen deprivation therapy (ADT), however many men will develop hormone resistance PCa. Glycosylation changes during cancer development offer the opportunity to identify novel biomarkers and therapeutic options for patients. GalNT7, a GalNAc-transferase, is responsible for the initiating steps of O-linked glycosylation and has been shown to change in a cohort of ADT-treated patients. The aims of this study were to assess the utility of GalNT7 as a PCa biomarker and to understand how its expression levels affect disease progression.

GalNT7 expression was measured in 5 PCa cohorts via tissue microarray or RT-PCR. Stable cell lines were generated for GalNT7 knockdown and GalNT7 over-expression using lenti- viral transduction, and RNA isolated from these cell lines was sent for RNA sequencing analysis. RNA sequencing data was validated by RT-PCR or western blot and in vitro behaviour assays were performed to assess cancer cell characteristics.

GalNT7 expression decreased in patients following ADT in two independent cohorts. GalNT7 expression was elevated in tumour samples compared with benign tissue, and was a significant marker of malignancy ($p=0.013$, OR =1.6). GalNT7 expression was also a significant predictive marker of PCa-metastasis ($p=0.04$). PCa cell lines over-expressing GalNT7 were more proliferative, invasive and migratory in vitro, with the reciprocal being observed with GalNT7 gene knockdown. RNA sequencing highlighted a significant decrease in FOXO1 expression in cells overexpressing GalNT7. Western blotting confirmed that GalNT7 overexpression decreased levels of phospho-AKT, a known inhibitor of FOXO1- mediated cell cycle regulation.

This data suggests that GalNT7 mediates prostate cancer progression, promoting an enhanced capacity for malignancy, through a potential interaction with the PI3K/AKT signalling cascade.

Keywords:

GalNT7 Glycosylation Glycans Prostate Cancer Biomarkers

O10

Marco Trevisan-Herraz

Title: Are men and women epigenetically different?

Institute of Cellular Medicine (ICM)

Men and women are, of course, different. For example, at the systems level we find non- obvious secondary sex differences such as in the immune system: women generally have a stronger reaction to infections, albeit they are more prone to develop autoimmune diseases [1]. The underlying mechanism behind this remains largely unknown. To address this question, we are performing an epigenome-wide analysis comparing immune cells from men and women to provide insight about the regulatory networks involved. To develop our methodology, we are using large public datasets from BLUEPRINT that include histone modification information, which is important to identify active promoters and active enhancers, such as H3K27 acetylation and H3K4 monomethylation [2]. This approach gives insight about the dynamic changes happening in chromatin. We are using machine learning techniques, such as random forest [5], to help us individuate the differential patterns in chromatin between men and women.

The machine learning algorithm was unable to predict if histone modifications in an autosome are linked to a male or female. However, our model accurately predicts the donor's sex when analysing the X chromosome alone. In some cases, we have observed this is due to the presence of H3K27 acetylations for genes of lncRNA such as Xist and Firre (involved in X chromosome inactivation) [4][5][6][7]. Nevertheless, even removing these regions, the algorithm is still able to find sex-specific patterns in histone monomethylations, which, to the best of our knowledge, had not been previously identified to have a sex- related role.

We will further work on this analysis in order to find new techniques that may allow to either find or discard more subtle differences including autosomes, allowing, in turn, the creation of a list of the key regions that will help in sex-specific diagnostics and treatments related to immune system diseases.

[1] Klein, S. L.; Flanagan, K. L. Nat. Rev. Immunol. 2016 [2] Chen, L. et al. Cells. Cell 2016 [3] Pedregosa, F. et al; Journal of Machine Learning Research 2011 [4] Pinheiro, I. et al; F1000Res. 2017 [5] Lu, Z. et al; Philos. Trans. R. Soc. Lond. B Biol. Sci. 2017 [6] Hacisuleyman, E. et al; Nature Structural & Molecular Biology 2014 [7] Yang, F. et al; Genome Biology 2015

Keywords:

bioinformatics, epigenetics, immunology, sex, histone modifications

O11

Azzeldin Madkour

Title: A fishy tale packed with novelty!

Institute of Cell and Molecular Biosciences (ICaMB)

Human enteric disease by attaching and effacing (A/E) pathogens, which include enteropathogenic and enterohemorrhagic E. coli (EPEC; EHEC), depend on a type III secretion system (T3SS) that transfers >20 'effector' proteins into infected gastrointestinal cells. Many notorious pathogens, including strains of Yersinia, Salmonella, Shigella, Pseudomonas, also require T3SS's; latter are composed of 15-20 proteins of which ~9 are highly homologous with others sharing low or no homology due to evolution-driven adaptation to different hosts/environments. The A/E pathogen T3SS is encoded on a horizontally-acquired 'LEE' region with >95% identity between T3SS proteins. Here, we describe our bioinformatics and experimental analyses of LEE from Edwardsiella tarda FCP503 (an invasive fish pathogen) reported to lack many (~20%) genes - linked to LEE genetic reorganisation - and, perhaps, non-functional T3SS. Our work reveals the loss of only non-essential genes but four T3SS proteins are encoded over 2 open-reading frames; a known regulatory mechanism. Unexpectedly, comparing the predicted T3SS proteins to their EPEC LEE counterparts revealed unprecedented divergence; 11, 8 and 9 proteins shared only 21-40%, 40-60% and 60-73% identity, respectively. Expressing each E. tarda LEE T3SS protein in EPEC revealed that most (~70%; despite many with high divergence) functionally replaced their EPEC homologue. Amazingly, divergence level and interchangeability were not correlated. Importantly, studies illustrated non-complementing proteins were expressed with defects rescued by co-expressing known partner proteins – revealing co-divergence – or swapping domains between homologues. The two LEE- encoded effectors (6 in A/E pathogens) take the record for being the most divergent. Collectively, our work reveals unexpected, adaptation-driven, reconfiguration of a LEE T3SS (with important implications) and provides a platform for studies on virulence-critical proteins shared by A/E, and other, pathogens.

Keywords:

EPEC , E. tarda, LEE, T3SS

O12

Ahmad Al-Mrabeh

Title: Restoration of insulin secretion brings about recovery of the pancreas volume in type 2 diabetes

Institute of Cellular Medicine (ICM)

Background: The pancreas is one third smaller in type 2 diabetes compared with matched control groups, possibly due to in utero developmental effects, or secondary to diabetes itself. We have investigated the changes in pancreas volume in relation to restoration of functional β-cell mass over 2 years in a subgroup of the Diabetes Remission Clinical Trial (DiRECT).

Methods: 37 people who achieved remission of diabetes (15/22 F/M, 53.8±7.0 years, BMI 34.5±4.2 kg/m2) were studied in comparison with 16 people who failed to achieve remission. Pancreas volume was measured using MRI and volume rendering techniques. Insulin secretion was measured using Stepped Insulin Secretion Test with Arginine (SISTA).

Results: Pancreas volume was low at baseline in both responders and non-responders (63.3±2.8 vs. 58.9±3.5cm3, p=0.34). This remained unchanged after 5 months in both groups (64.0±2.9cm3, p=0.18 and 60.0±3.7cm3, p=0.32, respectively). At 12 months, there was major increase in pancreas volume in responders (59.6±3.3 to 65.9±3.6cm3, p<0.0001) while the change was modest in non-responders (60.1±3.9 to 63.3±4.7cm3, p=0.06). By 24 months, pancreas volume had increased by 11.8cm3 in responders compared with 4.5cm3 in non-responders (p=0.001).

Responders were characterized by a gradual increase of functional β-cell mass at 12 months (0.60[0.13-1.95] to 0.83[0.25-2.69] nmol/min/m2, p<0.05). This was remained durable at 24 months (0.90 [0.18-4.13] nmol/min/m2, p<0.05 vs. baseline).

Pancreas volume had not changed in those who relapsed between 12-24 months (n=13). In this group, first phase insulin response was lost (0.10[0.054-0.122] to 0.04[0.027-0.052] nmol/min/m2, p=0.012), but functional β-cell mass was retained (0.94 [0.31-2.7] to 1.0[0.45-1.85] nmol/min/m2, p=0.41).

Conclusion: The low pancreas volume of type 2 diabetes is likely to be related to insulin deficiency over many years of disease progression. Long term restoration of β-cell function appears to have a trophic effect on pancreatic tissues.

Keywords:

Type2diabetes, pancreasvolume, insulinsecretion, functional β-cell mass

Posters

Selected Flash Presentations:

P2 - Bernhard Kepplinger: Utilizing chemical genetics to discover novel molecules for elucidation of biological pathways

P6 - Birthe Dorgau: Decellularised extracellular matrix enhance development of hPSC-derived retinal organoids and their light responsiveness

P15 - Charles Winterhalter: DNA replication initiation in B. subtilis: how is it done?

P19 - Fiona Malcomson: Dietary Inflammatory Index score and colorectal cancer risk markers associated with inflammation and WNT signalling

P20 - Kevin Whitley: Combining nanofabrication, microfluidics, and fancy imaging to investigate bacterial division machinery

P22 - Bas Olthof: Puncta of neuronal nitric oxide synthase (nNOS) mediate NMDA-receptor signalling in the auditory midbrain

P25 - Alistair Poll; Detection of Circulating Tumour Cells in Patients with Early Stage Oesophageal Adenocarcinoma

P29 - Peizun Zhou: Burkitt lymphoma subtypes have frequent FOXO1 mutations but distinct hotspots in the AKT recognition

P32 - Aneta Mikulasova: Epigenomic translocation of H3K4me3 broad domain: a mechanism of super-enhancer hijacking

P46 - Faye McLeod: Secreted proteins as modulators of synaptic connectivity and function: a link to autism?

P1

Oliver Shannon

Title: Mediterranean diet adherence and cognitive function in older, UK adults: The EPIC-Norfolk study

Cellular Medicine (ICM)

Background
In Mediterranean countries, adherence to a traditional Mediterranean diet (MedDiet) is associated with better cognitive function and reduced dementia risk. It is unclear if similar benefits exist in non-Mediterranean regions. We explored associations between MedDiet adherence and cognitive function in an older, UK population. Additionally, given that poor cardiovascular health accelerates cognitive decline, we explored whether associations differed between individuals with high versus low cardiovascular disease (CVD) risk.

Methods
We conducted an analysis in 8009 older individuals with dietary data at Health Check 1 (1993-1997) and cognitive function data at Health Check 3 (2006-2011) of the European Prospective Investigation of Cancer, Norfolk (EPIC-Norfolk). Associations were explored between MedDiet adherence and global and domain specific cognitive test scores and risk of poor cognitive performance in the entire cohort, and when stratified by CVD risk status.

Results
Higher MedDiet adherence defined by the Pyramid MedDiet score was associated with better global cognition (β±SE=-0.012±0.002; P<0.001), verbal episodic memory (β±SE=-0.009±0.002; P<0.001), and processing speed (β±SE=-0.002±0.001; P=0.013). Lower risk of poor verbal episodic memory (OR(95%CI)=0.784 (0.641-0.959); P=0.018), processing speed (OR(95%CI)=0.739 (0.601-0.907); P=0.004), and prospective memory (OR(95%CI)=0.841 (0.724-0.977); P=0.023) was observed for the highest versus lowest MedDiet tertiles. The effect of a three-point increase in Pyramid score on global cognitive function was equivalent to five fewer years of cognitive ageing. In stratified analyses, associations were evident in individuals at higher CVD risk only (P<0.05).

Conclusions
Higher adherence to the MedDiet is associated with better cognitive function and lower risk of poor cognition in older, UK adults. This evidence underpins the development of interventions to enhance MedDiet adherence, particularly in individuals at higher CVD risk, to ameliorate age-related cognitive decline in non-Mediterranean populations.

P2

Bernhard Kepplinger

Title: Utilizing chemical genetics to discover novel molecules for elucidation of biological pathways

Cell and Molecular Biosciences (ICaMB)

Lipoteichoic acids play an important role in bacterial cell physiology; from morphogenesis to virulence. They are essential in the human pathogen S. aureus and thus identified as a potential antibiotic target. Here we set out to identify inhibitors of LtaS and also probe the function of the MreB cytoskeleton utilizing a novel chemical genetics screen. We screened extracts from 2000 actinomycete isolates and found 3 preliminary hits. We purified the active compound of one of these and discovered a compound of novel structure. Current studies into the detailed mode of action are in progress. Analysis of the whole genome sequencing of the three strains suggests that at least one of them makes a different active compound.

P3

Karin Engelhardt

Title: Use of whole exome sequencing for the genetic discovery of inborn errors of immunity

Institute for Cellular Medicine (ICM)

Background – We see in our clinic a range of patients with inborn errors of immunity, including severe combined immunodeficiency (SCID), diverse forms of combined immunodeficiencies (CID) with varied defects in T and B lymphocytes, hemophagocytic lymphohistiocytosis (HLH), early-onset inflammatory bowel disease (EO-IBD), autoimmunity and autoinflammation. We aimed to provide these patients with a molecular diagnosis which might inform treatment options and allow for genetic counselling for the family.
Methods – We performed whole exome sequencing (WES) of DNA from 189 patients, either alone (singletons) or alongside healthy family members. Variants were called applying GATK Best Practices recommendations and analysed using the analysis platform SeqR. Candidates were selected using the following criteria: genetic hypothesis (autosomal recessive, autosomal dominant, X-linked), allele frequency (novel to rare), variant impact prediction (deleterious, damaging, possibly damaging) and known pathogenicity (ClinVar, known PID gene). Potential variants were confirmed by Sanger sequencing and if required by functional analyses.
Results – We found a genetic aetiology in at least 47% of our patients. We had an especially high success rate for patients with SCID and Omenn's syndrome (RAG1, RAG2, IL2RG, JAK3, IL7RA, LIG4, Artemis, CD3E, CD3D), CID (ZAP70, DOCK8, WAS, CARMIL2, ICOS, ATM, CECR1, CDCA7, DNMT3B) and immune dysregulation (STAT3-GOF, CTLA4, TNFAIP3, NFKB2, FOXP3, SKIV2L, TTC37), whereas we could not establish a molecular diagnosis for patients with HLH, lymphopenia or neutropenia. Mutations in 10 genes previously unknown to cause immunodeficiency were found, three of them in singletons, the pathogenic mechanisms of which were explored.
Conclusion – We successfully used WES to obtain a molecular diagnosis for nearly half of our patients. Most variants were found in known disease genes (71 patients, 34 different genes), with a higher success rate in some disease types than in others. Novel disease gene identification was more effective in trios/families than singletons.

P4

Patrick Welsh

Title: Teachers' perceptions of Restricted and Repetitive Behaviours in the classroom

Institute of Neuroscience (IoN)

Background: Restricted and Repetitive Behaviours (RRBs) are some of the most difficult behaviours to manage in children with Autism Spectrum Disorders (ASD). Although RRBs frequently occur in educational settings, we know little about the way in which teachers understand these behaviours.

Aims: The study aimed to explore the attributions, emotional response and feelings of confidence held by teachers working in different educational settings when faced with RRBs.

Methods and Procedures: A single group survey design using behavioural vignettes was adopted in order to elicit teacher beliefs and ratings.

Outcomes and Results: Analysis indicated that there were differences in the attributions and confidence ratings held for different types of RRBs. Significant differences were also observed between teachers working in mainstream and specialist educational settings. Emotional response and confidence scores were often predictive of one another alongside factors related to teaching experience.

Conclusion and implications: The findings indicate that teachers from mainstream schools potentially hold less helpful beliefs in response to RRBs and therefore are a professional group who may benefit the most from additional support and training. Further research could consider conducting a qualitative exploration of why teachers hold certain beliefs about RRBs and/or sampling those who are less experienced in working with children with ASD.

P5

Natalie Young

Title: The effect of the Histamine H3 receptor antagonist; ZPL-8680871 in the treatment of Neuropathic pain

Institute of Neuroscience (IoN)

Neuropathic pain can occur as a consequence of many common pathological diseases, examples include; diabetes, stroke and multiple sclerosis, and is the “pain arising as a direct consequence of a lesion or disease affecting the somatosensory system”. It is caused by an alteration in transmission of the sensory signal to the spinal cord and brain, which results in sensory deficits, that manifest as burning, aching and stabbing sensations, and cause symptoms such as; dysaethesia, paraesthesia, allodynia and hyperalgesia. It is reported that 8% of the UK population are affected by neuropathic pain, and as a consequence of extended life expectancy, it is predicted that the worldwide prevalence of neuropathic pain is likely to increase further, because this type of chronic pain occurs with many common age-related diseases. Despite advances in the understanding of the causes and mechanisms leading to the development and maintenance of neuropathic pain, only 33% of patients achieve pain control with existing medications and to date, no medication has shown long-term efficacy and tolerability for neuropathic pain. Consequently, there is a pressing need for the identification of new therapeutic strategies to improve management of neuropathic pain. A possible target is the histamine H3Rs, because there is increasing evidence to support the expression of these receptors in nociceptive pathways; H3Rs have been found in specific thalamic areas, and on fibres in the dorsal route ganglia and spinal cord. Our earlier studies demonstrate H3Rs on a population of A-delta fibres that regulate pain sensitivity, and a role for H3Rs in the modulation of mechanical pathological pain. We postulate that targeting peripheral H3Rs with our selective H3R antagonist; ZPL-8680871, will block sensory symptoms of neuropathic pain, with the aim of this project to define the extent to which changes in the H3R-mediated signalling, underlies the analgesic effects of ZPL-8680871.

P6

Birthe Hilgen

Title: Decellularised extracellular matrix enhance development of hPSC-derived retinal organoids and their light responsiveness

Institute of Genetic Medicine (IGM)

Tissue specific extracellular matrices (ECM) provide structural support and enable access to molecular signals and metabolites which are essential for directing stem cell renewal and differentiation. To mimic this phenomenon in vitro, tissue decellularisation approaches have been developed, resulting in the generation of natural ECM scaffolds that have comparable physical and biochemical properties of the natural tissues. In this study, RPE conditioned medium (CM RPE), decellularised ECM from neural retina (decel NR) and retinal pigment epithelium (decel RPE) were successfully generated and their impacts on differentiation of human pluripotent stem cells (hPSCs) to retinal organoids was investigated. The effects of CM RPE, decel NR and decel RPE on the on differentiation process was studied using a number of laboratory techniques including: immunohistochemistry, qRT-PCR, electrophysiological recordings and transmission electron microscopy. Culture media supplementation with decel RPE enhanced RPE generation and significantly increased the generation of rod photoreceptors. The latter one was also observed by adding CM RPE to the culture media, whilst addition of decel NR and decel RPE significantly enhanced ribbon synapse marker expression and the light responsiveness of hPSC-derived retinal organoids.
This study indicates that decellularised ECM generated from neural retina and RPE plays an important role in photoreceptor maturation, synaptogenesis and light responsiveness of pluripotent stem cell derived retinal organoids. The decellularised ECMs are easy to generate, amenable to scale up and 3D culture conditions, opening up new possibilities for generation of retinal organoids which mimic adult human retina both in cell type composition and function. These data are published data (doi: 10.1016/j.biomaterials.2019.01.028. Epub 2019 Jan 22).

P7

Lauren Walker

Title: Can early tau deposits in mixed Alzheimer's disease and Lewy body disease give insights into disease progression?

Institute of Neuroscience (IoN)

Background

Cases that neuropathologically fulfil the criteria for both Alzheimer's disease (AD) and Lewy body disease (LBD) are classified as mixed dementia (mixed AD/LBD). Interestingly, some of these cases present clinically with AD, and others with LBD. Previous work indicates cases with an AD clinical phenotype exhibit a higher hyperphosphorylated tau burden [1]. This may suggest that tau pathology has been developing for a longer period of time and is more established in these cases. Conversely, in cases with a LBD clinical phenotype tau may have been deposited at a later time point in LBD progression and still in the early stages of development. The evolutionary development of tau can be detected by different tau antibodies, therefore the 'age' of each tau tangle in human post-mortem brain tissue can be identified.

The aim of this project was to determine if mixed AD/LBD cases that presented clinically with LBD have a higher burden of tau in the early stage of development.

Methods

We quantitatively assessed post-mortem tissue sections from the hippocampus from cases that have fulfilled neuropathological criteria for mixed AD/DLB with tau marker MC1 to identify early tau conformations.

Results

Mixed AD/LBD cases with a LBD clinical phenotype had a greater MC1 burden in the hippocampus ($p<0.05$) compared to those with an AD clinical phenotype.

Conclusion

These results suggest in neuropathologically mixed AD/LBD cases, those with a clinical phenotype of LBD may have developed dementia initially caused by LBD, with concomitant AD related pathology developing later in the disease course. This highlights the importance of biomarkers for co-morbid pathologies and if identified, secondary pathologies should be considered in future treatment strategies.

[1] Walker, L., et al. Acta Neuropathol, 2015. 129(5): p. 729-48.

P8

Iffath Ghouri

Title: Generating cardiomyocytes from iPSCs to investigate mitochondrial disease

Institute of Neuroscience (IoN)

Introduction: The heart is a highly metabolic organ requiring a continuous supply of ATP in order to fulfil its role of pumping oxygenated blood to every cell in the body. It is therefore frequently affected in mitochondrial disease conditions, where ATP production does not meet demand. Human heart tissue is difficult to obtain. However, differentiation of induced pluripotent stem cells (iPSCs) to cardiac muscle cells (cardiomyocytes) provides a valuable model for studying cardiac mitochondrial function in normal and diseased states.

Methods: Differentiation of iPSCs from WT and mitochondrial disease patients was carried out by temporal modulation of the canonical Wnt signalling and nodal pathways using small molecule inhibitors. Cells were cultured for at least 7 days in appropriate media and immunofluorescence (IF) imaging carried out following fixation at different stages to determine cell type. Electrophysiology recordings from cells were obtained by high speed fluorescence imaging following loading with voltage sensitive FluoVolt dye. TMRM and MitoSox dyes were used to compare mitochondrial membrane potential ($\Delta\psi_m$) and ROS levels in WT and mutant iPSCs, with fluorescence quantified by flow cytometry.

Results and conclusions: IF staining indicated the presence of cardiac troponin T (cTnT) and NKX 2.5 positive cells within 10 days of differentiation. Presence of the atrial isoform of myosin regulatory light chain (MLC2a) was observed a few days later, with cells positive for the ventricular isoform (MLC2v) - an indicator of cardiac maturation - observed around 4 weeks post-differentiation. Electrophysiology recordings in WT cells exhibited action potentials of normal shape, indicating expression of ion channels expected to be present in adult cardiomyocytes. iPS cells obtained from mitochondrial disease patients with Complex I mutations in either NDUFS2 or high levels of mutation in the ND5 gene did not successfully differentiate to cardiomyocytes with the normal differentiation protocol; the majority of cells expressed α-smooth muscle actin. Examination of iPSCs by flow cytometry revealed increased TMRM fluorescence, indicative of increased $\Delta\psi_m$, and increased ROS levels in mutant iPSC lines compared to WTs. Strategies aimed at manipulating ROS levels, $\Delta\psi_m$, or improving metabolic status may therefore be required to trigger successful cardiomyocyte differentiation in iPSCs with particular mitochondrial disease mutations.

P9

Magdalena Mroczek

Title: MYO-SEQ2: Application of exome sequencing to a cohort of 2,000 patients with unexplained limb-girdle weakness

Institute of Genetic Medicine (IGM)

Muscular dystrophies are a heterogeneous group of rare genetic disorders that are characterised by progressive skeletal muscle wasting and weakness, and can directly precipitate premature mortality. In the first phase of the MYO-SEQ project, between 2014 and 2016, whole exome sequencing (WES) was applied to 1000 patients with unexplained limb-girdle weakness. Since 2017, we have been extending the MYO-SEQ project by a further 1000 samples.

The variant call set was uploaded onto the Broad Institute of Harvard and MIT's seqr platform and 429 muscle disease-associated genes were examined. Overall, we have already analysed data for 1484 samples and further 479 have been shipped to the Broad Institute. Likely pathogenic variants were identified in 55% of patients, with mutations identified in 101 known disease-associated genes. LGMD2A (CAPN3), LGMD2B (DYSF), LGMD2L (ANO5) and Duchenne/Becker muscular dystrophy (DMD) were the most common in our cohort, together accounting for more than a third of these patients.

Despite targeted WES analysis to detect pathogenic mutations, the disease aetiologies of approximately half of the patients in the MYO-SEQ project remain unexplained. We therefore selected 21 index cases and performed trio analysis (proband and parental DNA). It allowed us to determine if the rare variants segregated with the disease, narrow down the number of variants and diagnose cases where diagnosis could not be made based on a single exome analysis. Interrogating the unsolved cases using WES for the genetic trio helped us follow recessively acting variants e.g. compound heterozygotes.

The focus on undiagnosed patients with a defined clinical phenotype enabled an increased diagnostic rate for disease-causing mutations in known genes in this cohort. The remaining unsolved cases provide especially exciting challenges. For these patients, we will perform WES for the parental DNA aiming to discover new genes and we will discuss other possible diagnostic approaches.

P10

Nduka Okwose

Title: Opportunities and challenges of cardiac output response to stress test to enhance heart failure diagnosis

Institute of Cellular Medicine (ICM)

Objective: To explore the role of the novel Cardiac Output Response to Stress (CORS), test in the current diagnostic pathway for heart failure and the opportunities and challenges to potential implementation in primary care.
Design: Qualitative study using semi-structured in-depth interviews which were audio recorded and transcribed verbatim. Data from the interviews were analysed thematically using an inductive approach.
Setting: Newcastle upon Tyne, UK.
Participants: Fourteen healthcare professionals (six males, eight females) from primary (general practitioners, nurses, healthcare assistant, practice managers) and secondary care (consultant cardiologists).
Results: Four themes relating to opportunities and challenges surrounding the implementation of the new diagnostic technology were identified. These reflected that adoption of CORS test would be an advantage to primary care but the test had barriers to implementation which include: establishment of clinical utility, suitability for immobile patients and cost implication to GP practices.
Conclusion: The development of a simple non-invasive clinical test to accelerate the diagnosis of heart failure in primary care maybe helpful to reduce unnecessary referrals to secondary care. The CORS test has the potential to serve this purpose however, factors such as cost-effectiveness, diagnostic accuracy and seamless implementation in primary care have to be fully explored.

P11

Emine Bagdatlioglu

Title: Serotonergic innervation of the cervical spinal cord in the rhesus macaque

Institute of Neuroscience (IoN)

Serotonin (5HT) can modulate the activity of interneurones within the spinal cord and studies in the rodent have demonstrated that motoneurones (MN) also receive direct 5HT inputs. The density of serotonergic inputs received by spinal MNs remains largely unknown in the adult primate, and in particular for MNs of distal muscles in the upper limb. Knowledge of the organisation of 5HT innervation in the primate spinal cord is necessary to further our understanding of how 5HT is involved in motor control during health and following motor damage.

Mid cervical to early thoracic (C5 & T1) spinal cord sections, from two adult rhesus monkeys, were immunostained for choline acetyltransferase (ChAT) to label MNs, and serotonin transporter (SERT) to identify 5HT terminals. The perimeter of MNs, within different pools of the ventral horn, was measured and the density of SERT terminals contacting the perimeter determined.

ChAT positive MN cell bodies, within the various MN pools, between C5 and T1. We detected a significantly higher number of SERT positive synaptic terminals in contact with the perimeter of MN cell bodies in C5 than T1 (0.243 vs 0.184 contacts per µm, respectively, unpaired t-test, $p=0.001$), but found no difference between the various MN pools within the same spinal segment (one way ANOVA, $p>0.05$).

The difference in SERT density on MNs between C5 and T1 suggests that there is a rostro caudal gradient of 5HT innervation in the primate cervical cord. This implies that 5HT has a greater direct influence on more proximal muscles compared to more distal ones. This gradient is consistent with the well-established role of 5HT for locomotion but the evidence of some connectivity with distal MNs also raises questions regarding its importance for more manipulative hand movements.

P12

Katherine Johnson

Title: Investigating circulating miRNAs as biomarkers for non-alcoholic fatty liver disease

Institute of Cellular Medicine (ICM)

Background

Non-alcoholic fatty liver disease (NAFLD) affects one third of the general population and is the most common cause of liver disease in the Western world. NAFLD is characterised by fatty liver, or steatosis, in the absence of excess alcohol intake. The disease can advance from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis (F1-F4), cirrhosis and ultimately hepatocellular carcinoma. An invasive liver biopsy is the current gold standard for differentiating steatosis from NASH. MicroRNAs (miRNAs) are small (~22 nt) non-coding RNA molecules that can post-transcriptionally regulate gene expression and have in some cases been shown to be associated with NAFLD. Accordingly, we sequenced over 2,000 serum miRNAs in healthy donors and 280 patients across the NAFLD spectrum to establish a profile of circulating miRNAs from which disease biomarkers could be identified.

Methods

MiRNA libraries were prepared from 15 µl serum using HTG EdgeSeq and sequenced by Illumina NextSeq. Analyses on the resulting data were performed using the R software environment. Data were normalised to correct for batch effects and the variances in submitting centre. MiRNAs with a log2 fold-change (logFC) of >2 and a p-value of <0.05 were classified as differentially expressed.

Results

A total of 87 miRNAs were differentially expressed in each disease sub-group compared to healthy controls. No differentially expressed miRNAs were detected when mild and severe disease were compared. One hundred and two miRNAs showed differential expression between NASH F2 and healthy controls. Comparing the two extremes of phenotype – NASH F4 with healthy controls – resulted in 143 miRNA differences, including the previously reported miR-122.

Conclusions

We have identified potentially clinically significant differentially expressed miRNAs, which may enable prediction of disease progression non-invasively and enable early intervention as anti-fibrotic drugs become available. Differentially expressed miRNAs will next be replicated in independent patient cohorts.

P13

Daniel Peters

Title: Slippery and strong: critical properties of a bacterial polymer enabling Yersinia pestis to resist phagocytosis

Institute of Cell and Molecular Biosciences (ICaMB)

Y. pestis has haunted humanity for 5000 years, is responsible for three major pandemics, including the Black Death, and to this day remains endemic in several areas of the world. The high levels of virulence displayed by this organism can be attributed in part to the array of strategies it uses to evade the host's immune system. One of these strategies involves the secretion of a gel-like coat that surrounds the bacterium and prevents its uptake by macrophages through an unknown mechanism. The coat is composed of a chaperone-usher protein called Caf1, a ~ 15-kDa protein that the bacteria assemble into long, secreted, non-covalent polymers. Here, we use electron microscopy, mutagenesis, macrophage engulfment assays and single molecule AFM pulling measurements to determine the factors that underlie Caf1's anti-phagocytic activity. We identify two key properties, anti-adhesiveness and exceptional mechanostability, which are critical for this behaviour, with the reversal of either abrogating Caf1's ability to prevent phagocytosis. These findings help elucidate the mechanism of an important survival strategy for Y. pestis, and may aid in the fight against this highly dangerous pathogen.

P14

Monika Olahova

Title: POLRMT mutations impair mitochondrial transcription and are associated with a spectrum of mitochondrial disease manifestations

Institute of Cell and Molecular Biosciences (IoN)

Background: The vast majority of mitochondrial disorders result from mutations in components of the nuclear-encoded mitochondrial DNA (mtDNA) maintenance machinery and oxidative phosphorylation (OXPHOS) subunits. The role of the mtDNA transcription machinery in mitochondrial disease, however, remains relatively unknown. The mitochondrial RNA polymerase (POLRMT) is the sole RNA polymerase in mitochondria and is responsible for the transcription of the mitochondrial genome.
Aim: To characterise the clinical and molecular nature of novel POLRMT variants that underlie the mitochondrial disease-associated phenotype present in five unrelated individuals.
Patients and Methods: Using whole-exome sequencing, we identified novel recessive and dominant POLRMT variants in five individuals presenting with a variety of clinical problems, ranging from global developmental delay, hypotonia and growth defects in childhood to late onset progressive external ophthalmoplegia (PEO). Where investigated, these defects were accompanied by either a mosaic cytochrome c oxidase deficiency in skeletal muscle and/or multiple respiratory chain enzyme deficiencies. Mitochondrial mRNA and OXPHOS protein levels were assessed in mutant POLRMT fibroblasts (3 of 5 patients). In addition, recombinant mutant POLRMT proteins were generated in order to determine the effect of POLRMT variants on mitochondrial transcriptional activity in vitro.
Results: Functional characterisation of patient fibroblasts revealed a defect in mitochondrial mRNA synthesis, although no mtDNA deletions or copy number abnormalities were identified. Mild decreases in the levels of both OXPHOS subunits and fully-assembled complexes were observed in vivo, whilst functional in vitro characterisation of the investigated recombinant POLRMT variants revealed that patient mutations exhibited variable, but deleterious effects on mitochondrial transcription.
Conclusion: Our results demonstrate for the first time, that pathogenic variants in the POLRMT gene can cause a spectrum of clinical phenotypes ranging from childhood-onset developmental delay to late-onset PEO and emphasise the importance of defective mitochondrial transcription as a disease mechanism.

P15

Charles Winterhalter

Title: DNA replication initiation in B. subtilis: how is it done?

Institute of Cell and Molecular Biosciences (ICaMB)

DNA replication initiation is one of the first essential steps required for cellular proliferation. In B. subtilis, four proteins (DnaA/D/B/I) are required for DNA unwinding and loading of the helicase DnaC. However, temporal dynamics and individual functions of these proteins remain unclear. In order to get a better understanding of the replisome initiation complex, we developed a large-scale method to study DnaA, DnaD, DnaB, DnaI and DnaC protein mutants in vivo. This approach relies on complementation cassettes that allow the rescue of potentially lethal phenotypes, and is combined with a blue/white screening assay to help identify correct mutants after transformation. Here, we present preliminary data obtained for the alanine-scan of DnaD, the adaptor protein to the main initiator DnaA. Altogether, we aim to reconstruct the interaction map of the B. subtilis replisome initiation complex, which would be key to appreciate individual mechanisms that drive DNA replication.

P16

Simon Tual-Chalot

Title: Endothelial adenosine-to-inosine RNA editing is indispensable for vascular integrity

Northern of Genetic Medicine (IGM)

RNA editing is the binding to double-stranded RNA (dsRNA) of the adenosine deaminase acting on RNA (ADAR) family to catalyse the deamination of adenosine-to-inosine. We have recently reported that ADAR1 is the main RNA editor in endothelial cells (ECs) and is dysregulated in human atherosclerotic heart disease. However, the role of ADAR1 in the vascular system has not been reported so far. The goal of the present study is to evaluate the role of EC-restricted ADAR1 in vascular homeostasis.

Constitutional or inducible EC restricted ADAR1 ablation was achieved by crossing mice carrying a conditional ADAR1 allele with either a Tie2-Cre or with a tamoxifen-inducible Cdh5-CreERT2 mouse line.

Genetic loss of ADAR1 in ECs caused prenatal lethality at embryonic stage E14.5, demonstrating an essential role for ADAR1 in development. Furthermore, endothelial ADAR1 ablation in adult mice resulted in sudden death within 6-8 days due to vascular leakage, indicating a disturbance of EC barrier function in lung microvasculature. Mechanistically, ADAR1-mediated RNA editing is essential for the metabolism of long-to-short dsRNAs, while silencing of endothelial ADAR1 resulted in accumulation of cytoplasmic long dsRNAs. Long dsRNAs are recognised as a danger-associated molecular pattern by the cytosolic innate immune sensing and signalling sensors. Consequently, the cytoplasmic accumulation of endogenous long dsRNAs resulted in activation of innate immune system in ECs, as assessed by the induction of interferon-β. Concomitantly, activation of the cytoplasmic dsRNA sensors resulted in dissociation of β-catenin from VE-cadherin in EC junctions and endocytosis of VE-cadherin.

ADAR1-mediated RNA editing of long dsRNAs is essential for the long-to-short dsRNA metabolism and thus suppression of endogenous innate immune sensing and signalling. EC-restricted ADAR1 ablation results in embryonic lethality and sudden death in adult mice due to pleural effusions, which all indicate that ADAR1-mediated RNA editing is indispensable for maintenance of vascular barrier integrity.

P17

Catherine Mowbray

Title: High Molecular Weight Hyaluronic acid: A two pronged protectant against infection of the urogenital tract?

Institute of Cell and Molecular Biosciences (ICaMB)

Objectives
Recurrent urinary tract infections (rUTI) are associated with uropathogenic Escherichia coli (UPEC) ascending and infecting the urinary tract. Such infections can be treated with antibiotics, but while providing symptomatic relief, such therapy does not prevent recurrence. There is clinical evidence suggesting that intravesical glycosaminoglycan therapy, such as hyaluronic acid (HA), helps reduce UTI recurrence. This has been further investigated here using in vitro systems modelling the urogenital tract tissues.
Methods
RT4 bladder cells were preconditioned with high molecular weight (HMW) hyaluronic acid (HA) (>1500kDa) at 2 mg mL-1 and challenged with UPEC to analyse barrier protection and bacterial adherence. Untreated and HA-preconditioned VK2 E6/E7 vaginal cells were challenged with E. coli flagellin (50 ng mL-1) to mimic bacterial challenge, with media analysed for production of lipocalin-2 (LCN2), human beta defensin 2 (hBD2) and interleukin-8 (IL-8) by ELISA. Experiments were repeated after siRNA knockdown of toll-like receptors (TLRs) 2, 4 and 5, and CD44 to attempt to elucidate their roles in HA/flagellin response
Results
Microscopic analyses showed reduced bacterial adherence and urothelial disruption with HA, suggesting that HA functions as a barrier protecting the epithelium from bacterial infection. Media from cells treated with HA and flagellin simultaneously revealed increased concentrations of the host antimicrobial agent LCN2 and pro-inflammatory IL-8 protein ($P < 0.05$) compared to the media of the no HA/flagellin challenges. Increased gene expression of hBD2 ($P < 0.05$), but not the BD2 peptide, was also observed in the HA/flagellin challenged cells.
Conclusion
These data suggest that exogenous HA has potential to protect the urogenital epithelia from UPEC infection via a two-pronged approach that involves the physical enhancement of the epithelial barrier and augmentation of its innate immune response.

P18

Darin Zerti

Title: Defining the optimal stage for transplantation of pluripotent stem cell derived photoreceptor precursors

Institute of Genetic Medicine (IGM)

Retinal degeneration is one of the major causes of untreatable blindness in the developed world. Outer retinal degeneration (ORD) culminates in the loss of photoreceptors. A potential treatment for is the use of cell transplantation strategies to repair the damaged retina. Progress has been rapid, and transplanted cells from a range of types and sources have been shown to restore visual function in animal models of human disease. Recently Collin and colleagues (2015) created a hESC lines harbouring the green fluorescent protein (GFP) reporter at the endogenous loci of the Cone-Rod Homeobox (CRX) gene. This is an applicable tool for the purification of hESC-derived retinal photoreceptor precursors for transplantation. The aim of the present study is to define the most optimal differentiation stage to achieve a robust and functional integration of human photoreceptor precursors in mammalian diseased retina.
METHODS: The differentiation of the hESC CRX-GFP reporter line towards retinal organoids was performed as described in Mellough et al. 2015. The retinal organoids were dissociated, analysed and purified by fluorescence-activated cell sorting. Sorted GFP positive cells were then trans-vitreally transplanted into the sub-retinal space of rd1 mice.
RESULTS: Our results so far show that photoreceptors obtained from day 60 and day 90 of differentiation are often observed in the host inner nuclear layer and begin to form a distinct layer in direct contact with the second order neurons of rd1 mice.
CONCLUSIONS: Identification of the most favourable age of human pluripotent stem cell derived photoreceptors to achieve engraftment and functional connectivity following transplantation is a vital step in optimising this potential therapy. Further transplants as well as behavioural, electrophysiological and morphological analyses are required to determine the presence of any light response from these grafts and the feasibility of this approach.

P19

Fiona Malcomson

Title: Dietary Inflammatory Index score and colorectal cancer risk markers associated with inflammation and WNT signalling

Institute of Cellular Medicine (ICM)

Inflammation is associated with diseases, including colorectal cancer (CRC) and both inflammation and CRC risk may be modulated by diet. Abnormal WNT signalling may be causal for the development of pathologies including CRC and other inflammatory and immune diseases. The aim of this study was to investigate relationships between the inflammatory effects of diet, assessed using a Dietary Inflammatory Index(1), and markers of CRC risk that are associated with inflammation and the WNT signalling pathway.

We used samples and dietary data from 75 healthy participants recruited to the DISC Study(2). DII score was calculated using food frequency questionnaire data and included 29 food parameters. Systemic inflammation was assessed by quantifying high-sensitivity C-reactive protein (hsCRP) and local inflammation from faecal calprotectin. Expression of WNT pathway genes and regulatory microRNAs by qPCR, SFRP1 methylation by pyrosequencing and colonic crypt proliferative state were assessed in colorectal mucosal biopsies.

Mean DII score was 0.143 (-4.186 – 4.964) and mean hsCRP and faecal calprotectin concentrations were 3.6mg/L and 46.5mg/kg, respectively. We observed a significant relationship between DII score and hsCRP (ρ=0.295, p=0.011). DII score also correlated positively with expression of AXIN2 (ρ=0.358, p=0.003), CTNNB1 (ρ=0.390, p=0.001) and GSK3β (ρ=0.317, p=0.011) WNT pathway-related genes which have been previously reported to be upregulated in CRC.

The findings from this study provide evidence for detrimental effects of an inflammatory diet (greater DII score) on markers on inflammation and WNT pathway-related markers of CRC risk, suggesting that WNT signalling may be a mechanism through which diet modulates CRC risk.

References:

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P20

Kevin Whitley

Title: Combining nanofabrication, microfluidics, and fancy imaging to investigate bacterial division machinery

Institute of Cell and Molecular Biosciences (ICaMB)

During bacterial cell division, >10 different proteins assemble into a ring at mid-cell and dynamically build the cell wall inward. It was recently discovered that filaments of the tubulin homolog FtsZ treadmill circumferentially around the division ring, and that in B. subtilis these dynamics are tightly coupled with both the motion of cell wall synthesis enzymes and the overall constriction of the ring. However, it remains unclear how these dynamics are coordinated with one another. Imaging cell division protein dynamics in rod-shaped bacteria is difficult using traditional methods whereby cells are oriented horizontally—as the division ring is viewed side-on—and so in previous work cells were confined vertically in 'microholes' to orient the ring in one microscope imaging plane. Here, we present a new version of this method whereby cells are held in open-topped microholes within a fluidic chamber so that cytoskeleton and synthase dynamics can be imaged at the same time as the system is rapidly subjected to drug treatment. We use this method to investigate the dynamics of division proteins in live B. subtilis cells during instantaneous perturbations with drugs that affect FtsZ polymerization kinetics or cell wall synthesis.

P21

Ruth Cranston

Title: Candidate Oncogenes on Chromosome 21 in B-cell Precursor Acute Lymphoblastic Leukaemia

Northern Institute fro Cancer Research (NICR)

Introduction

Acute lymphoblastic leukaemia (ALL) is the most common paediatric cancer, with precursor B-cell ALL (B-ALL) accounting for around 80% of ALL diagnoses. Whole or partial copy number gain of chromosome 21 (CNG21) is the most common somatic chromosomal aberration across the differing cytogenetic subgroups. The prevalence of these copy number gains imply that these aberrations are non-random, suggesting genes present on chromosome 21 potentially have an important, oncogenic role in leukaemogenesis and disease maintenance in patients who exhibit CNG21. Intrachromosomal amplification of chromosome 21 (iAMP21)-ALL is a distinct subgroup associated with a poor prognosis which feature common regions of amplification and overexpression on 21q, offering a refined location for oncogene identification.

Methods

Clustered regularly-interspaced short palindromic repeats (CRISPR)-mediated gene editing technology was utilised as a whole genome CRISPR knock-out (GeCKO) screen to identify novel oncogenes in a B-ALL cell line panel. Model-based analysis of genome-wide CRISPR/Cas9 knockout (MAGeCK) was used to analyse screening data, GeCKO data was integrated with cell line and patient RNA-sequencing and copy number data (SNP6.0) to identify authentic candidates for functional investigation. Isogenic cell lines were developed using the lentiMPHv2 and lentiSAMv2 transcriptional activation system for the overexpression of target genes. Protein levels of isogenic cell lines were measured by Western blot.

Results

GeCKO screening in CNG21 cell line NALM16 identified DYRK1A as a novel candidate oncogene within the common regions of interest on chromosome 21. DYRK1A is implicated in a number of pathways which are commonly dysregulated in cancer including: the RB1 pathway and phosphorylation of FOXOs, and has an essential role in pre-B cell development. DYRK1A is overexpressed in B-ALL in direct association with chromosome 21 copy number, as detected by RNA-sequencing. Isogenic cell lines were successfully developed showing increased expression of DYRK1A, validated at the protein level.

P22

Bas Olthof

Title: Puncta of neuronal nitric oxide synthase (nNOS) mediate NMDA-receptor signalling in the auditory midbrain

Institute of Neuroscience (IoN)

Nitric oxide (NO) is a gaseous molecule synthesised in the brain by neuronal nitric oxide synthase (nNOS). Although the expression of nNOS in the inferior colliculus (IC) is well documented in neurons in the dorsal and lateral cortices of the IC (Coote & Rees, 2008), the functional relevance of NO in the IC in vivo remains elusive.

Using fluorescent immunohistochemistry against NMDA-R1, nNOS, sGC(α2) and PSD95, and confocal imaging on 40 µm coronal sections obtained from adult pigmented guinea pigs, we show a not previously described subcellular distributions of nNOS in the IC.

Our multi-labelling studies reveal that nNOS puncta form multi-protein complexes with NMDA receptors, soluble guanylyl cyclase (sGC), and PSD95. These complexes are found directly apposed to glutamatergic terminals indicative of synaptic function.

To investigate the functional role of these multi protein complexes in the ICC, we combined microdialysis with in vivo electrophysiological recordings of multiunit activity in the guinea pig ICC. Pigmented guinea pigs were anesthetized, pure-tone sound stimuli were delivered and drugs targeting NO signalling were applied by reverse dialysis via a microdialysis probe inserted in the IC. Neurons across the frequency laminae of the central nucleus were recorded using a 32 channel single shank electrode inserted rostral to the dialysis probe.

We found that local application of NMDA enhances sound-driven activity in a concentration-dependent and reversible fashion. This response is abolished by blockade of nNOS with L methyl arginine or sGC with ODQ indicating that the NMDA effect is mediated solely via the NO and cGMP signalling pathway.

This discovery of a ubiquitous, but highly localised expression, of nNOS throughout the ICC and demonstration of the major influence of the NMDA activated NO pathway on sound-driven neuronal activity, imply a key role for NO signalling in auditory processing.

P23

Helen Griffin

Title: Working in the Genomics England Research Environment: Analysis of Whole Genomes from Families with Immunodeficiency

Institute of Cellular Medicine (ICM)

As part of the 100,000 Genomes Project, Genomics England (Gel) have sequenced the whole genomes of 70 thousand individuals from UK families with rare diseases. The project has been delivered in partnership with the NHS where patients have been recruited through Genomic Medicine Centres (GMCs) that are also responsible for reporting the clinical genome sequencing results. In order to help improve the interpretation of genomic variants, the research community have formed Clinical Interpretation Partnerships (GeCIPs) that consist of groups interested in specific diseases or cross-cutting research themes.

The Immune disorders GeCIP are reviewing clinical information and genetic variants from a heterogeneous set of 868 families (Version 6 release 31st February 2019) with either a primary immunodeficiency or a complex condition with an immune component, the aim being to identify underlying genetic mutations. Working within the Gel research environment, it was initially difficult to access the genome variant calls and clinical information specific to the immune disorder families. We therefore developed an analysis pipeline to bring together per family phenotype data and genetic variants in a set of html pages that made the data easier to navigate for non-bioinformaticians. This has now enabled us to start a detailed interrogation of the genomic data and identify putative disease causing mutations specific to individual families. We are also using statistical methods (e.g. BeviMed, SKAT) in an attempt to identify novel genetic causes of immune disorders where damaging variants cluster in specific genes or functional groups of genes.

This knowledge will ultimately help to improve clinical interpretation pipelines. Newly discovered putative mutations will be assessed by a multi-disciplinary team (MDT) and if found to be clinically relevant, the result will be reported to patients through the GMCs.

P24

Shreya Ayyub

Title: Understanding the roles of uncharacterised release factors in human mitochondrial translation and ribosome rescue

Institute of Cell and Molecular Biosciences (ICaMB)

Mitochondria are ubiquitous organelles in higher eukaryotes responsible for ATP production and for survival of obligate aerobes including humans. Despite this critical role, there is a surprisingly rudimentary understanding of quality control mechanisms in the human mitochondrial translation system. By contrast, the clinical consequences of mitochondrial dysfunction have been extensively characterised. In order to understand the molecular pathogenesis of many of these disorders, we need to identify essential factors that monitor and maintain accurate protein synthesis. In mitochondria, there are 4 members of the translation termination release factor (RF) family: mtRF1, mtRF1a, ICT1 and C12orf65. RFs recognise stop codons and facilitate the release of the nascent peptide. Of the four human RFs, only mtRF1a is required to terminate translation of the 13 genes encoded by the mitochondrial genome (mtDNA). Interestingly, depletion of any of the remaining 3 causes loss of cell viability, and mutations in C12orf65 are responsible for mitochondrial disease. In the event of mRNA truncation or amino acid starvation, a ribosome may stall on the transcript, diminishing the cellular pool of translating ribosomes. Since bacterial RFs rescue stalled ribosomes, we decided to examine the potential role of mtRF1 and C12orf65 in rescuing mitoribosomes stalled by these or other events. We have generated and characterised relevant CRISPR/Cas9 gene knockouts and used a cell line that has a defect causing stalled mitoribosomes, to understand the regulation of translation termination and ribosome rescue in human mitochondria.

P25

Alistair Poll

Title: Detection of Circulating Tumour Cells in Patients with Early Stage Oesophageal Adenocarcinoma

Northern Institute for Cancer Research (NICR)

Many oesophageal adenocarcinoma patients present with advanced disease, for which the prognosis is poor. Consequently, it is the sixth most common cause of cancer-related death in the UK. Circulating tumour cells (CTCs) could provide an accessible source of malignant cells from which cancer could be detected, treatment decisions made, and response to treatment monitored.

Patients diagnosed with OAC being considered for curative treatment at the RVI were recruited. Peripheral blood was collected at various stages of the treatment cycle. Cells were fixed, red blood cells and platelets removed, before white blood cells were positively depleted by immuno-magnetic separation based on CD45 expression. The enriched sample was labelled with fluorescently-conjugated antibodies against epithelial markers, HER2 and CD45, and with DAPI. Cells were examined by imaging flow cytometry using an ImageStreamX. CTCs were distinguished as having a nucleus, no CD45 expression and expression of at least one epithelial biomarker. Cell morphology assessed with brightfield images assisted with identification.

CTCs were detected in the majority of curative patients recruited to the study. CTCs were present in patients with early stage T1N0M0 OAC undergoing endoscopic mucosal resection. All CTCs express either EpCAM or cytokeratins, but only ~40% express both - there is considerable heterogeneity of epithelial biomarker expression between patients of the same stage and within individual patients. One or more HER2 positive CTC was detected in 45% of patients, but again expression was heterogeneous within individual patients.

Unlike the FDA approved CellSearch® system, our method does not rely on positive selection of CTCs that express a single biomarker. Consequently, more CTCs are detectable and there is potential to analyse CTCs not expressing a specific biomarker. Similarly, absence of preselection on the basis of size does not exclude smaller CTCs, allowing a fuller characterisation of OAC CTCs and better detection, prognostic and monitoring potential.

P26

Jarmila Spegarova

Title:

Institute of Cellular Medicine (ICM)

P27

Stacey Richardson

Title: Genetic characterisation of medulloblastoma relapse

Northern Institute for Cancer Research (NICR)

Relapse following primary therapy occurs in 30-40% of patients diagnosed with medulloblastoma (MB) and is almost universally fatal. Over the last decade there has been a great increase in our understanding of MB biology at diagnosis, however, the same progress has not been made at relapse, with only a handful of biological studies published to date. Recent evidence has demonstrated that whilst molecular subgroup is unchanged at recurrence, other molecular features are altered. Therefore, advancing our understanding of MB biology at relapse is now essential to improve outcomes.

This study provides a comprehensive genetic and epigenetic characterisation of an unprecedented cohort of MB tumours sampled at relapse (n=68). Using Agilent SureSelect-Target-Enrichment panel/whole exome sequencing and Illumina Infinium methylation arrays, we have interrogated the mutation, copy number and DNA methylation landscape of this cohort.

Strikingly, mutations of the histone methyltransferase genes, KMT2C and KMT2D, are significantly enriched at MB relapse and together represent 37%. Furthermore, mutations of DNA damage response (DDR) pathway genes are also significantly enriched at relapse (e.g. TP53; 19%, ATM; 23%, ATR; 16%), and together, these DDR pathway mutations represent 58% of MB relapses. In addition, we identify the novel enrichment of PTEN deletions (4.2%), compared to MB at diagnosis. Finally, integration of mutation, copy number, and DNA methylation data from our cohort identified several converging biological signalling pathways as candidate mechanisms for MB relapse. Importantly, p53 pathway defects account for 61% of MB relapses, moreover, whilst these defects are seen across all MB subgroups, they are significantly enriched in MBSHH (85%).

In conclusion, we have identified key biological mechanisms which characterise the majority of MB relapsed disease. Future work is now essential to validate these findings, and to explore their utility as prognostic biomarkers, or as a basis for novel therapeutic interventions at relapse.

P28

Richard Howey

Title: Comparison of Mendelian Randomisation and Bayesian Network Approaches for Causal Inference

Institute of Genetic Medicine (IGM)

Mendelian randomisation (MR) is a popular method for inferring the casual relationships between variables in genetic studies. Results from genome-wide association studies often generate genetic variants that are associated with an intermediate trait. These variants can then be used as instrumental variables to investigate if the intermediate trait has a causal influence on another (outcome) trait. However, a valid instrumental variable must satisfy certain conditions, in particular, that there are no direct relationships between the instrumental variable and the outcome variable or any other potential confounders. An alternative approach for inferring the causal direction between two variables is with the use of Bayesian networks (BN), which have no such explicit causal assumptions and benefit from using more than three variables in an analysis.

We compare MR and BN using simulated data in a four variable model where X is (usually) causal on Y and G and Z are instrumental variables for X and Y respectively. We consider MR analyses using G or Z as an instrumental variable and BN analyses using only G or Z as well as both G and Z. We consider three different models: (i) a model with no confounding variables; (ii) a non-genetic confounding model, where a variable influences both X and Y, and (iii) a genetic confounding model, where G influences Y via an additional intermediate variable.

We have shown that while MR is easy to perform and widely understood, BN offers an alternative approach with some advantages over MR such as better ROC curves, ability to utilise more variables and less adverse effects when confounding is present.

P29

Peixun Zhou

Title: Burkitt lymphoma subtypes have frequent FOXO1 mutations but distinct hotspots in the AKT recognition motif

Northern Institute of Cancer Research (NICR)

FOXO1 has an oncogenic role in adult germinal center derived lymphomas, in which mutations, predominately within the AKT recognition motif, cause nuclear retention of FOXO1 resulting in increased cell proliferation. To determine the prevalence and distribution of FOXO1 mutations in pediatric Burkitt lymphoma (BL), we sequenced a large number of sporadic and endemic BL patient samples. We report a high frequency of FOXO1 mutations in both sporadic and endemic BL at diagnosis, occurring in 23/78 (29%) and 48/89 (54%) samples respectively, as well as 8/16 (50%) cases at relapse. Mutations of T24 were the most common in sporadic BL but were rare in endemic cases, in which mutations of residue S22, also within the AKT recognition motif, were the most frequent. FOXO1 mutations were almost always present in the major tumor cell clone but were not associated with outcome. Analysis of other recurrent mutations reported in BL revealed that FOXO1 mutations were associated with mutations of DDX3X and ARID1A, but not MYC, TCF3/ID3 or members of the phosphatidylinositol 3' OH kinase (PI3K) signaling pathway. We further show common nuclear retention of the FOXO1 protein, irrespective of mutation status, suggesting alternative unknown mechanisms for maintaining FOXO1 transcriptional activity in BL. CRISPR/Cas9 knockout of FOXO1 in an endemic cell line produced a significant decrease in cell proliferation, supporting an oncogenic role for FOXO1 in endemic BL. Thus, FOXO1 is frequently mutated in both sporadic and endemic BL and may offer a potential therapeutic target for pediatric BL patients worldwide.

P30

Tamara Metzler

Title: Arterial Stiffness in patients with rheumatic disease and chronic heart failure

Institute of Cellular Medicine (ICM)

Background: Rheumatic Disease (RD) is an independent risk factor for development of heart failure. Systemic inflammation reduces cardiac and vascular function and increases prevalence of cardiovascular morbidity and mortality. The aim of the present study was to compare arterial stiffness and functional capacity between patients with RD and chronic heart failure (CHF).
Methods: 36 patients (RD, n= 18; and CHF due to reduced left ventricular ejection fraction, n = 18) underwent assessment of arterial stiffness (i.e. augmentation index) using pulse wave analysis. Maximal graded cardiopulmonary exercise stress testing using cycle ergometry with non-invasive gas-exchange measurements was used to determine maximal functional capacity (i.e. peak O2 consumption).
Results: Compared to patients with CHF, patients with RD were significantly younger (age: 56 ± 8 vs. 66 ± 5 years, $p < 0.01$) and demonstrated lower Body Mass Index (24.4 ± 2.5 vs. 28.5 ± 4.6 kg/m2, $p < 0.001$). Patients with RD demonstrated significantly higher peak exercise oxygen consumption (37.1 ± 9.6 vs. 17.3 ± 3.7 ml/kg/min, $p < 0.001$; and 212 ± 76 vs 85 ± 19 watts, $p < 0.01$). No significant difference in augmentation index was found between the RD and CHF patients (25 ± 12 % vs. 29 ± 7 %, $p = 0.19$).
Conclusion: The findings of our study suggest that patients with RD demonstrate better functional capacity than those with CHF. However, augmentation index was not significantly different between the RD and CHF patients. Based on these findings it is reasonable to suggest that RD may lead to increased arterial stiffness which may increase the risk of cardiovascular mortality and morbidity. Interventions known to reduce arterial stiffness should be considered to delay vascular function decline in patients with RD.

P31

Serlie Fatrin

Title: Relationship between functional capacity, haemodynamic response to exercise and quality of life in heart failure

Institute of Cellular Medicine (ICM)

Background/Aim: Diminished functional capacity, haemodynamic response to exercise and quality of life are strong independent determinants of prognosis and mortality in chronic heart failure (HF). The aim of study was to assess the relationship between functional capacity, haemodynamic response to exercise and quality of life(QOL) in patients with chronic HF.
Methods: A single-centre prospective study recruited 42 patients (31 male, 11 female, mean age 60±10 years, body mass index 29±4 kg/m2) with stable chronic HF due to left ventricular systolic dysfunction (LVEF=25±7%) and NYHA Functional Classification II and III. All patients completed a maximal graded cardiopulmonary exercise stress testing using cycle ergometer with non-invasive gas exchange and haemodynamic monitoring. QOL was assessed using Minnesota Living with Heart Failure Questionnaire (MLHF).
Results: The average value (±SD) for functional capacity i.e. peak O2 consumption was 14.3±4.3 ml/kg/min, peak exercise haemodynamics i.e. heart rate 105±21 beats/min, mean arterial blood pressure 95±18 mmHg, cardiac output 13.9±3.6 L/min, cardiac power output, 2.87±0.83, and MLHF QOL score 27±18. There was a significant negative relationship between the MLHF QOL score and peak O2 consumption output (r= -0.50, p=0.01) and heart rate (r= -0.30, p=0.05). There was however no significant relationship between the MLHF QOL score and other exercise haemodynamic measures including peak cardiac power output (r= 0.15, p=0.38), cardiac output (r= 0.22, p=0.15), and mean arterial blood pressure (r= -0.09, p=0.57).
Conclusion: The major finding from the present study suggests that functional capacity, represented by peak exercise oxygen consumption, is a strong determinant of quality of life in patients with chronic heart failure. Results further suggest that quality of life may not depend on exercise haemodynamic measures which represent overall function and pumping capability of the heart.

P32

Aneta Mikulasova

Title: Epigenomic translocation of H3K4me3 broad domain: a mechanism of super-enhancer hijacking following oncogenic translocations

Northern Institute for Cancer Research (NICR)

Introduction: Chromosomal translocations are common events in haematological malignancies with oncogenic power generated via aberrant fusion proteins or juxtaposition of proto-oncogenes and strong regulatory regions promoting their over-expression. One of the most frequent translocations involves the immunoglobulin heavy locus (IGH) at 14q32.33, due to errors in the recombination processes; V(D)J and class-switch recombination, and somatic hypermutation. H3K4me3 is a histone mark characteristic of active promoters, but broad domains (H3K4me3-BDs) can cover entire genes, providing consistent expression. We present a new model of "epigenomic translocation", where a wild-type H3K4me3-BD disappears in malignant cells and "re-locates" into the target oncogene of the genomic translocation.
Methods: We used chromatin states of 108 BLUEPRINT samples (PMID:28934481) including healthy B-cells/plasma cells (n=15), T-cells (n=15), myeloid-lineage cells (n=55) and samples from B-cell-derived haematological malignancies (n=23). Myeloma cell line U266 was sequenced using a custom targeted-capture covering 95% of the IGH-DJC locus (https://doi.org/10.1101/515106).
Results: Using chromatin states, we fine mapped three B-cell-lineage specific enhancers within the IGH constant region: chr14:106,025,200-106,056,800 (Eα2), chr14:106,144,200-106,179,400 (Eα1) and chr14:106,281,800-106,323,000 (Eμ). We also discovered B-cell specific H3K4me3-BD present in healthy donors at chr14:106,346,800-106,387,800, telomeric from the IGH promoter. Interestingly, this H3K4me3-BD is absent in samples with known translocation, t(11;14)(p13.3;q32.33)/IGH-CCND1, including mantle-cell lymphoma and myeloma samples. These cases showed abnormal appearance of H3K4me3-BD over the CCND1. We mapped the translocation in U266 as a cut-and-paste mechanism of the Eα2 from IGH next to CCND1. We hypothesize that the translocation of the enhancer is followed by interaction between this enhancer and the CCND1 locus, generating the aberrant H3K4me3-BDs.
Conclusions: We describe how a genomic translocation of the IGH enhancer close to the CCND1 gene results in the "epigenomic translocation" of an H3K4me3-BD, resulting in CCND1 activation. This could be a wide-ranging mechanism of oncogenic activation in haematological malignancies.

P33

Bashar Adi Wahyu Pandhita

Title: Validity of bioreactance method in evaluation of cardiac output in patients with advanced heart failure

Institute of Cellular Medicine (ICM)

An accurate, easy-to-use, non-invasive method for evaluation of cardiac function may improve risk stratification, management and outcomes in chronic heart failure patients. The aim of the study was to assess accuracy/validity of bioreactance method in estimating cardiac output at rest and in response to stress. Eighteen patients with advanced heart failure with reduced left ventricular ejection fraction (LVEF, 19±7%, mean age 52±9 years, 5 females) underwent right heart catheterisation using the Swan-Ganz catheter. Thermodilution (TD) method was used to evaluate cardiac output in a supine position at rest and in response to stress. Simultaneously cardiac output was also estimated with an electrical signal processing technology i.e. bioreactance (BR). Following measurements at rest, all patients were asked to perform active straight leg raise test to volitional exertion. At rest, there was a non-significant differences in cardiac output values obtained by the TD and BR methods (4.72±1.42 vs 4.94±1.21 L/min,$p=0.17$), with strong positive relationship between the two methods ($r=0.88$,$p<0.01$). Mean duration of straight leg raise test was 142±54 seconds, and rate of perceived exertion ranging from 5-8 (“hard to very hard”, Borg scale score 6.1±1.5). In response to active leg raise test, TD cardiac output increased by 22% and BR by 21%. There was no significant difference between TD and BR cardiac outputs at peak straight leg raise test (6.04±1.70 vs 6.22±1.36 L/min,$p=0.29$). There was also strong relationship between the TD and BR cardiac outputs at peak straight leg raise test ($r=0.92$,$p<0.01$). The major findings of the study suggest that cardiac output estimates by bioreactance method are similar to those obtained by thermodilution method. Therefore, bioreactance method may be used in clinical practice to complement existing haemodynamic assessments in patients with chronic heart failure.

P34

Sanja Bojic

Title: A critical role for Dna2 at unwound telomeres

Institute of Genetic Medicine (IGM)

P35

Julia Humes

Title: Structural Analysis the CDK4-CyclinD Complex; A key regulator of the cell cycle

Northern Institute for Cancer Research (NICR)

Cyclins in complex with cyclin dependent kinases (CDKs) control progression though the cell cycle. CDK4 and CDK6 when bound to a D-type cyclin (D1, D2, D3) are involved in the G1 to S progression and therefor committing the cells to the cell cycle. Loss of this regulation leading to uncontrolled cell cycle is observed in many types of cancer. To this end inhibitors that interact with CDK4/6 have been developed which have been shown to inhibit tumour growth and are now used in the treatment of ER+ HER2- breast cancer, and is in trials for a number of other cancer types.
However, studies have suggested that CDK4 and CDK6 have tissue specific functions as well as their redundant roles in the cell cycle, such as that of CDK6 in white blood cells. Due to the duel inhibition of CDK4 and 6 by current drugs they cause side effects such as neutropenia so can only be used in a dose limited manor.
Although crystal structures of both proteins in complex with cyclin D have been solved, and show subtle differences, there is no robust crystal system to obtain high resolution CDK4-cyclin D structures. At present I am optimising the crystal system for CDK4 in the presence and absence of inhibitors. Understand the differences between the structures of CDK4 and CDK6 should inform future studies in which drugs that target CDK4 and not CDK6 can be generated therefor reducing the side effects of the CDK inhibitors used in treatment.

P36

Faye Mcleod

Title:

Institute of Neuroscience (IoN)

P37

Manuel Pazos

Title: Z-ring membrane anchors associate with cell wall synthases to initiate bacterial cell division

Institute of Cell and Molecular Biosciences (ICaMB)

During the transition from elongation to septation the rod-shaped cells of Escherichia coli establish a ring-like zone for peptidoglycan growth at the future division site. This preseptal peptidoglycan synthesis does not require the cell division specific peptidoglycan transpeptidase PBP3 or most of the other essential cell division proteins and has been termed PIPS for PBP3- (or Penicillin-) independent peptidoglycan synthesis. Only FtsZ, its membrane-anchor ZipA and at least one of the bi-functional transglycosylase-transpeptidases, PBP1A or PBP1B, were known to be required for preseptal peptidoglycan synthesis. Here we show that PBP1A and PBP1B interact with ZipA and localize to preseptal sites in cells with inhibited PBP3. ZipA stimulates the glycosyltransferase activity of PBP1A and PBP1B. The membrane-anchored cell division protein FtsN does not interact with ZipA but localizes at preseptal sites and stimulates both activities of PBP1B. ZipA and ftsN can be individually deleted in cells with an ftsA* mutation but the depletion of both genes is lethal and cells do not establish preseptal sites. Model: ZipA and FtsN-FtsA have semi-redundant roles in connecting the cytosolic FtsZ ring (Z-ring) with the membrane-anchored peptidoglycan synthases during the preseptal phase of envelope growth.

P38

Katharina Peters

Title: Copper inhibits peptidoglycan LD-transpeptidases suppressing β-lactam resistance due to by-pass of Penicillin-binding proteins

Institute of Cell and Molecular Biosciences (ICaMB)

Penicillin-binding proteins (PBPs) synthesize the essential DD-cross-links in peptido-glycan (PG) and are inhibited by β-lactam antibiotics. Some clinical isolates and laboratory strains of Enterococcus faecium and Escherichia coli achieve high level β-lactam resistance by utilizing β-lactam insensitive LD-transpeptidases (LDTs) to produce exclusively 3-3 (LD) cross-links in PG, by-passing the PBPs. In E. coli, other LDTs covalently attach Braun's lipoprotein (Lpp) to PG to stabilize the envelope and maintain the permeability barrier function of the outer membrane. Here we show that sub-MIC concentration of copper chloride sensitizes E. coli cells to sodium dodecyl sulphate and impair survival upon LPS transport stress, indicating reduced cell envelope robustness. Cells grown in the presence of copper chloride lacked LD-cross-links in the PG and reduced the covalent attachment of Lpp and incorporation of a fluorescent D-amino acid, suggesting inhibition of LDTs. Copper dramatically decreased the minimal inhibitory concentration of ampicillin in E. coli and E. faecium strains with a resistance mechanism relying on LDTs and inhibited purified LDTs at sub-mM concentration. Hence, our work reveals how copper affects bacterial cell envelope stability and counteracts LDT-mediated β-lactam resistance.

P39

Declan Gray

Title: Understanding nutrient transport across the outer membrane by members of the human gut microbiota

Institute of Cell and Molecular Biosciences (ICaMB)

P40

Pradeep Dheerendra

Title: Dynamics underlying detection of auditory object boundary

Institute of Neuroscience (IoN)

Auditory object analysis requires the fundamental perceptual process of detecting boundaries between auditory objects. However, the dynamics underlying the identification of discontinuities at object boundary is not well known. We investigated the cortical dynamics underlying this process by employing a synthetic stimulus composed of frequency modulated ramps knowns as "acoustic textures", where boundaries were created by changing the underlying spectrotemporal coherence. We collected magnetoencephalographic (MEG) data from 14 subjects in 275-channel CTF scanner.
We observed a very slow (<1 Hz) drift in the neural-magnetic signal that started 430 ms post boundary between textures that lasted for 1330 ms before it decayed to baseline no-boundary condition. The response evoked by this drift signal was source localized to Heschl's Gyrus bilaterally which was shown in the previous BOLD study to be involved in the detection of object boundaries. This low frequency drift signal is consistent with a precision based predictive coding account of perceptual inference.

P41

Lizah van der Aart

Title: Thermoactinomycetaceae are tip-growing, multicellular Firmicutes

Institute of Cell and Molecular Biosciences (ICaMB)

Firmicutes and Actinobacteria are two major groups of bacteria. Both are extremely diverse, including organisms of clinical and environmental importance. The groups generally differ in several fundamental properties including G+C content of DNA, spore production, tip growth versus lateral wall growth, and branching vs straight chain growth and mid-cell division. The aims of this project are to investigate the molecular cell biology of an important group of Firmicutes – Thermoactinomycetes – that show features of both Firmicutes and Actinobacteria. Although these organisms display the tip growth and branching mycelial habit typical of Actinobacteria, they are Firmicutes as judged by both DNA sequencing and endospore formation. Studies of these unusual organisms will shed light on an important phylogenetic step in bacterial evolution, as well as providing important insights into the mechanisms and evolution of tip growth and branching.

P42

James Wordsworth

Title: TGF-beta in the Ageing and Senescence of Human Skin

Institute of Cell and Molecular biosciences (ICaMB)

As we age, the epidermis thins and the dermis is depleted of multiple extracellular matrix (ECM) components including collagen and elastin, while the wound healing capacity is reduced. The TGF-β pathway plays a major role in wound healing and skin constitution, and is thought to contribute to the ageing process, potentially via its role in the induction of senescence and the associated secretory phenotype, which has been shown to have detrimental effect on the surrounding tissue.

We conducted differential expression analysis of neonatal, adult (56+ years), and senescent cells stimulated with TGF-β from 0-96 hours, discovering that the profile of senescent cells was more similar to that of neonatal cells than adult cells. The mRNA for multiple ECM components was lowest in the adult cells, while matrix degrading factors such as matrix metalloproteinases (MMPs) were significantly higher. Factors associated with myofibroblast differentiation, necessary for the fibrotic state during wound healing, were also lowest in adult cells. Analysis of protein levels and the phenotypic consequences of the different cell types is ongoing, but the current findings suggest an interesting relationship between senescence and ageing in skin, where under some conditions senescence might even protect against the degradative phenotypes of replicative adult cells.

P43

Rachel Queen

Title: Spaniel: an R package for analysis and interactive sharing of Spatial Transcriptomics data

Institute of Genetic Medicine (IGM)

Spatial Transcriptomics allows the sequencing of the complete transcriptomes from barcoded regions of intact tissue. By linking gene expression of specific cell types and spatial location the method has the potential to answer a wide range of biological questions concerning cellular function. The analysis of the data presents a number of challenges unique to the technology which are not met by existing bioinformatics analysis tools. Therefore, I designed Spaniel which provides a framework for analysing and sharing Spatial Transcriptomics data. Spaniel is an R package which provides functions to plot gene expression onto a histological image. It can be used for quality control, and interpretation of data and as a close collaboration between a computational biologist analysing the data and the biologist with specialised knowledge of the tissue type is required to interpret the experiments a web app, written in Shiny, is included with the package for interactive sharing of data.

P44

Adrian Santos-Ledo

Title:

Institute of Genetic Medicine (IGM)

P45

Marina Garcia Macia

Title: Autophagic approaches to prevent fatty liver disease

Institute of Cellular Medicine (ICM)

Aberrant accumulation of lipid droplets (LDs) can lead to diseases such as non-alcoholic fatty liver disease, which is the most frequent liver pathology in western countries. The Perilipin (Plin) family is the group of proteins that coat LDs, controlling their biogenesis, stabilization, and preventing their degradation. Among perilipins, Plin3 is important to initiate LD maturation and is a marker of early stage of fatty liver disease. Recent studies have revealed that autophagy is involved in LD degradation and, therefore, may be crucial to avoid lipid accumulation. High levels of fatty acids activate autophagy, but the mechanism is poorly understood. Here, we describe a key role of Plin3 in the selective degradation of LDs after oleic acid treatment in fibroblasts, primary hepatocytes and human liver slices. Our results show that LC3-II and Lamp1 accumulate in the LDs after lysosomal inhibitors treatment, therefore autophagy machinery is selectively recruited in the surface of the LD. Interestingly, Plin3 phosphorylation increases when autophagy is inhibited, by lysosomal inhibitors or when Atg7 is silenced, suggesting that it is a key component of lipophagy. When Plin3 is silenced, autophagic machinery recruitment to the LD surface is reduced. Plin3 pulldowns revealed interaction between this perilipin and the autophagy initiators proteins (FIP200, Atg16L). Therefore, Plin3 could work as a docking protein for lipophagy. Our study indicates that Plin3, specifically its phosphorylation, is crucial in the degradation of LDs mediated by autophagy and this may lead to the discovery of new clinical targets to prevent or treat fatty liver disease

P46

Faye Mcleod

Title: Secreted proteins as modulators of synaptic connectivity and function: a link to autism?

Institute of Neuroscience (IoN)

Efficient synaptic communication between neurons is fundamental for every brain function. Breakdown in synaptic connections is linked to neurological diseases during development including autism spectrum disorders (ASDs). Therefore, it is crucial to understand the mechanisms that modulate the formation, function and maintenance of synapses in the healthy and diseased brain.

Wnt secreted proteins are prominent synaptic modulators in the brain, and de novo mutations in canonical Wnt signalling have been implicated in ASD. To date, the exact contribution of Wnt signalling in ASD has not been characterised. Wnts regulate synapse formation and maintenance, glutamate release and receptor function. Using a combination of confocal microscopy, live imaging and electrophysiological techniques, I have now shown that Wnt7-Fz7 signalling is required for activity-mediated dendritic spine plasticity, AMPA receptor localisation and synaptic strength, all key cellular events involved in learning and memory. Thus, Wnt signalling is essential for the structural and functional plasticity of synapses.

Given the integral role that Wnts have at the synapse and their genetic and functional link to ASD, targeted research into the role of Wnts in ASD could provide novel insight into the disease origin. Future experiments will explore this in greater detail utilising human foetal and mature cortical tissue containing Subplate neurons (SPNs). SPNs present early in the developing cortex, show precocious synaptic connectivity, are enriched in Wnts and have been linked to ASD. SPN-containing regions will be preserved in culture under optimised conditions for days to weeks to extend the viability and productiveness of the tissue. Synaptic structure and function will then be assessed through gain and/or loss of Wnt proteins. Ultimately, this research could provide valuable insight into the early cellular events leading to neurodevelopmental disorders.

P47

Tamara Modebadze

Title: Modulation of human and rodent neuronal activity via Kv7 potassium channels ex vivo

Institute of Neuroscience (IoN)

The M currents are generated by the activity of the Kv7 potassium channels which are abundant in the central nervous system. These currents play a crucial role in controlling neuronal excitability. Conditions such as benign familial neonatal seizures, early onset epileptic encephalopathy, and peripheral nerve hyperexcitability have been associated with a disrupted function of the Kv7 channels. Cortical hyperexcitability has also been linked to other conditions such as autism spectrum disorders and Fragile X syndrome. Enhancement of M currents has been gaining interest as a potential treatment strategy in the hyperexcitability-associated disorders. We, therefore, explored the effects of ezogabine, a Kv7 channel opener, on cortical neuronal network oscillations and the activity of individual cortical neurons in human and rodent brain slices.
Human neocortical brain slices were obtained from patients during the tumour debulking procedure. For the rodent study, male Lister Hooded rats (6 – 8 weeks old) were used to obtain prefrontal brain slices. Brain samples were kept in ice-cold sucrose-based aCSF solution and cut at 350 – 450 um thickness for both human and rodent experiments. Local field potential was used to record neuronal network oscillations induced by application of 600 – 800 nM kainic acid. The effect of ezogabine on the gamma frequency and amplitude was evaluated. Whole-cell patch-clamp experiments were used to assess the effect of ezogabine on the firing properties of neurons. Data are presented as mean (standard deviation).
Our preliminary findings demonstrate that ezogabine produces an inhibitory effect on the firing of human cortical neurons reducing the firing by 66.2 (30.4) % in response to a depolarizing stimulus ($n = 8$ cells, 6 patients, $p<0.001$, paired t-test). Moreover, ezogabine reduced the amplitude of gamma frequency oscillations in a concentration-dependent manner in the rat prefrontal cortex. Ezogabine (100 nM) insignificantly reduced the gamma by 3.9 (29.7) % ($n = 5$, $p>0.05$, One-way ANOVA), while 1 uM and 10 uM ezogabine decreased the oscillations by 37.2 (13) % ($n = 5$, $p<0.01$, One-way ANOVA) and 87 (10.4) % ($n = 5$, $p<0.0001$, One-way ANOVA), respectively. Future experiments will determine whether the Kv7 channel opener alters neuronal activity in human and rodent brain slices in a comparable manner.

P48

Sanja Bojic

Title: Cryopreservation of murine neuroretina at different sub-zero temperatures

Institute of Genetic Medicine (IGM)

The prospect of whole organ preservation remains a great scientific challenge in cryopreservation research. To investigate how complex tissues can be cryopreserved, we here chose to study the retina. In particular, we examine the effects of slow freezing to different sub-zero temperatures on adult murine neuroretina. Neuroretina from C57BL/6 mice, with addition of 10% dimethyl sulphoxide (ME2SO) as a cryoprotective agent, was frozen to various sub-zero temperatures at 1°C/min cooling rate using a VIA Freeze TM Research controlled rate freezer (Asymptote, UK). The tissue was quickly thawed to 37°C immediately after reaching the desired sub-zero temperature and kept in organ culture at 37°C. Retinal explants were evaluated after 1 to 4 days in organ culture by immunofluorescence (IF) and hematoxylin & eosin (H&E) staining. Immunohistochemical markers for photoreceptors, ganglion, horizontal and amacrine cells were examined. No or minor differences in anatomy, retinal layer organisation and presence of different cell types were observed between tissue frozen to limited sub-zero temperatures and controls by either IF or H&E staining up to 2 days spent in the organ culture system. In contrast, slow freezing to low sub-zero temperatures induced injury detectable at the cellular and macro-scale level.
In conclusion, our findings indicate that murine neuroretina can be effectively cooled down to certain sub-zero temperatures and kept in organ culture up to 2 days without significant impact on the tissue organization and cell morphologies. Further experiments will need to confirm functionality of these tissues.

P49

Masood Zaka

Title: Investigation potential therapeutic role of TP53 in paediatric aggressive B-cell non-Hodgkin lymphoma

Northern Institute for Cancer Research (NICR)

Burkitt lymphoma (BL) is an aggressive mature B cell Non-Hodgkin lymphoma with poor clinical outcome. High resolution genomic analysis revealed that MYC-TP53 are the primary deregulated pathways in BL and therapeutic targeting of TP53 gene is remains unexplained burkitt lymphoma. Therefore, we performed mutational analysis on a large cohort of patients and cell lines of B-NHL, primarily focusing on paediatric BL sequenced using whole genome and whole exome technology.

Mutational analysis shows presence of TP53 (range 3-100%) mutation in 54/100 (54%) of the cases at diagnosis and 2/3 (66%) cases with disease progression in burkitt lymphoma. Most of the mutations occurred in DNA binding domain of TP53, and include hotspot mutations R248 (n=6), R273 (n=6) and G245 (n=3). Almost all of the identified genomic variants were missense mutations showing medium or high functional impact when compared against disease associated and polymorphic variants database. Additionally, we have also identified a major shift in subclonal frequencies of TP53 gene, indicating a late event in the tumour evolution in cases with disease progression.

Mutations in TP53 gene is associated with increased risk of relapse (hazard ratio 3.6, 95% CI 1.2-10, p=0.018), and existence of subclonal mutations makes an ideal candidate for therapeutics intervention for patient with disease progression. However, further investigation is essential to elucidate the role of this phenomenon and TP53 in aggressive lymphoma.

P50

Suzannah Harnor

Title: Development of a Potent Class of Small Molecule Inhibitors of the MDM2-p53 Protein-Protein Interaction

Northern Institute for Cancer Research (NICR)

In response to cellular stress, the p53 tumor suppressor is activated to modulate cell cycle progression, DNA repair, and cell death. The activity of p53 is tightly regulated by MDM2, an E3 ubiquitin ligase that targets p53 for proteasomal degradation. Inhibition of the MDM2-p53 interaction in tumors carrying wild-type p53 can therefore reactivate p53 and elicit an anti-cancer effect. Small molecule inhibitors of the MDM2-p53 interaction remains a promising strategy for cancer therapy and a number of these compounds are in clinical development.

An isoindolinone series, identified by Newcastle University, has been used as a starting point for the development of potent MDM2-p53 inhibitors. Structure based drug design was applied during lead optimisation to gain potency whilst also focusing on stabilizing the main metabolically labile position and reducing lipophilicity. Substitute groups were identified to block metabolism, however, lower potency against the target or an increased risk of drug-drug interactions via CYP3A4 inhibition tended to be observed. Alternatively, deuteration of the metabolically labile position appeared to reduce metabolism, without impacting other properties. This approach led to potent compounds with EC50 <1 nM against MDM2 in cell-free ELISA assays and EC50 <30 nM for p53 induction in SJSA-1 osteosarcoma cells. Further analyses of the compounds demonstrated an increase in the levels of p53 and p53 transcriptional targets as a result of inhibiting the MDM2-p53 interaction. Using three pairs of isogenic cell lines, the compounds were shown to be specific for cell lines with wild-type p53. Key compounds were also characterized in pharmacokinetic and pharmacodynamic studies in mice bearing the SJSA-1 tumor xenograft where they displayed strong induction of p53, 3 hours post oral administration, together with an increase in the expression of p53 target genes p21 and MDM2. These potent MDM2-p53 inhibitors have also shown significant in vivo efficacy in the SJSA-1 xenograft model at well tolerated oral doses. Thus, promising lead compounds were identified, meriting further optimization of the series.

P51

Diana Papini

Title: Preventing chromosome segregation errors in anaphase

Institute of Cell and Molecular Biosciences (ICaMB)

The effective segregation of chromosomes is essential for genome integrity. Errors can lead to birth defects and may contribute to carcinogenesis.
To prevent mis-segregation, sister kinetochores must be attached to microtubules emanating from opposite spindle poles and anaphase onset must be delayed until all chromosomes are correctly attached in this bi-oriented state.
Even in normal cells, numerous attachment errors occur during chromosome alignment. These errors are generally detected during prometaphase by surveillance mechanisms that allow their release and correction, and which require the centromeric kinase Aurora B (AurB). However, some errors, such as merotelic attachments, can persist and lead to the formation of lagging chromosomes. These are observed at the midzone during anaphase and are a potential source of aneuploidy. It remains unclear how the cell deals with lagging chromosomes once they are formed, and whether they are subjected to any type of error correction after anaphase onset.

At anaphase onset, AurB transfers from chromosomes to the central spindle, which results in an activity gradient across the dividing cell, centred on the midzone. Because AurB is removed from centromeres in anaphase, it is commonly assumed that its role at kinetochores and in error correction ends at this point. Our data show that AurB substrates involved in kinetochore-microtubule attachments prior to anaphase (eg Dsn1) are phosphorylated on lagging chromosomes after onset. Strikingly, the phosphorylation pattern at kinetochores shows a clear gradient: centromeres near the midzone are most highly phosphorylated.

We hypothesize that the AurB gradient causes phosphorylation of kinetochore targets on lagging chromosomes persisting near the central spindle and that this might function as a previously unidentified mechanism by which the cell can detect and potentially resolve lagging chromosomes in space and time.
To determine whether kinetochore phosphorylation is dependent on midzone AurB and involved in controlling lagging chromosome fate, we perturbed CPC localization and activity during anaphase using Mklp2 or PRC1 siRNA-mediated knockdown or acute AurB inhibition. We found that the pattern of anaphase phosphorylation gradient on the kinetochores of lagging chromosomes depends on the location and activity of Aurora B in the central spindle region. We are now investigating whether AurB modulates chromosome attachments by phosphorylating kinetochores on lagging chromosomes, and if this can influence their fate and limit chromosome segregation errors.

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