Brunel Bioscience PhD Conference
7th July 2026

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BIO-RAD

STEMCELL™ TECHNOLOGIES

Brunel University of London

PROGRAMME

9:30 - 10	Registration and Poster
10 – 10:10	Welcome and Opening Remarks – Prof Paola Vagnarelli DDR Bioscience
10:10 -10:25	Opening address by Prof Patrick Leman – Executive Dean CHMLS
10:30 -10:50	Rita Torres Pereira - The Impact of Disease-Specific Lamin A Mutations in Peripheral Chromatin Organisation and Nuclear Envelope Function.
10:50 -11:11:10	Rebecca Collinson - Cilia regulate the crosstalk between cancer cells and associated fibroblasts in malignant pleural mesothelioma.
11:10 - 11:30	**Coffee break**
11:30 -11:50	Emine Efendi - Oncogenic roles of CHRDL2 in Colorectal Cancer
11:50 -12:10	Zoi Grigoriou - VPS33B regulates cilia function in kidney epithelial cells.
12:10 -12:30	Paulina Farrant - Understanding the molecular mechanisms of centromere activity in human Robertsonian translocations
12:30-14	**Lunch**
14:00-14:10	Telvin Gyamfi - Account Manager I London & South East England- Life Science Group - Bio-Rad Laboratories
14:10 - 14:30	Amina N Ali - Therapeutic Efficacy of Ac2-26 in Driving Thromboinflammatory Resolution in Zebrafish Larvae
14:30 – 14:50	Thomas Williams
14:50 -16:00	Poster viewing and 2 min Flash talks
16:00 – 16:15	**Prize Awards**
16:15 – 16:30	Concluding Remarks

Acknowledgements

Organisers
Prof Paola Vagnarelli – DDR Bioscience
Dr Ines Ines J. de Castro – Lecturer in Genomics

We are grateful to the following sponsors

For sponsoring the Best Project on stem cells Prize

For sponsoring the Best Talk Prizes

For sponsoring the Best Poster and Flask talk Prizes

We are grateful to the following Centres at Brunel University of London for sponsoring Luch and Coffee break

- **Centre for Genome Engineering and Maintenance**
- **The Antimicrobial Innovations Centre**
- **Centre for Inflammation Research and Translational Medicine (CIRTM)**

We thank our prizes **Judges:**

Best Stem Cells Project
Dr Sybille Ermler
Dr Camilla Cerutti

Best Talks
Dr Ahmet Ucar
Dr Doreen Lau

Best Posters and Flash Talk
Dr Barbara Tanos
Dr Joseph Hetmanski

Talks

The Impact of Disease-Specific Lamin A Mutations in Peripheral Chromatin Organisation and Nuclear Envelope Function.

Rita Torres Pereira, Jose I. de las Heras, Paulo Caldas,Eric C. Schirmer, Joanna M. Bridger(1st), Ines J. de Castro (2nd)

Genome Organisation and Dynamics Cluster, Center for Genome Engineering and Maintenance, Department of Biosciences, College of Health, Medicine and Life Sciences, Brunel University of London, Uxbridge, UB8 3PH.

Beyond providing structural integrity to the nucleus, the nuclear lamina is a key regulator of 3D genome architecture by tethering large, typically repressed heterochromatin regions - lamina-associated domains (LADs) - to the nuclear periphery. While the discovery and mapping of LADs have shed light on lamina-chromatin interactions, their contribution to disease expression remains poorly understood. Laminopathies, such as Hutchinson-Gilford Progeria Syndrome (HGPS) and Dilated Cardiomyopathy (DCM), encompass a broad range of clinical phenotypes but share the prominent cellular hallmark of nuclear structure abnormalities. Here, we investigated the impact of LMNA mutations, associated with HGPS and DCM, on LAD organisation and periphery gene expression. We utilised DamID constructs carrying HGPS and DCM mutations to map the LADs in fibroblasts and hiPSC-derived cardiomyocytes. We validated that these constructs recapitulated nuclear morphology defects associated with disease states using elliptical Fourier analysis. Furthermore, we also observed changes in the levels of other nuclear envelope lamin A interactors, further mirroring the disease environment. Regarding LAD architecture, our preliminary DamID data revealed differential LADs not only between WT and mutants but also between HGPS and DCM. The potential identification of disease-specific chromatin patterns may uncover novel disease mechanisms and, consequently, highlight new therapeutic targets.

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Cilia regulate the crosstalk between cancer cells and associated fibroblasts in malignant pleural mesothelioma

Rebecca Collinson, Arturo Sala (2nd) and Barbara Tanos (1st)

Malignant pleural mesothelioma (MPM) is a rare cancer of the mesothelial cells lining the lungs with very poor prognosis. Patient symptoms are largely due to the extensive fibrosis caused by the accumulation of fibroblasts and their secreted extracellular matrix (ECM). It has been shown that a functional crosstalk between MPM cells and fibroblasts alters the behaviour of both cell types and drives therapeutic resistance. Primary cilia are sensory organelles found on the surface of most cells which coordinate several signalling events; cilia have been implicated in the progression of different cancer types, contributing to many of the hallmarks of cancer. We therefore hypothesised that cilia could mediate this crosstalk between MPM cells and fibroblasts.

By growing fibroblasts in MPM-cell-conditioned media, we found that MPM cell secretions activate fibroblasts, as seen by increased expression of activation markers, formation of smooth muscle actin (SMA) stress fibres, and a metabolic switch permissive for ECM production. In these activated fibroblasts, we have also observed increased ciliogenesis. Notably, removing cilia from fibroblasts reduces activation marker expression and SMA stress fibre formation. MPM cells secrete distinct cytokines compared to normal pleural cells. We are

currently examining the effects that these cytokines elicit in fibroblasts, investigating their effects on ciliogenesis and fibroblast activation, and whether this in turn activates specific pathways in MPM cells. Our work has uncovered a novel role for cilia in regulating the crosstalk between MPM cells and fibroblasts and places cilia and ciliary signalling pathways as potential therapeutic targets for MPM.

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Oncogenic roles of CHRDL2 in Colorectal Cancer

Emine Efendi , Evgeny Makarov (2nd) and Annabelle Lewis (1st)

Colorectal cancer (CRC) is the second leading cause of cancer-related fatalities worldwide. CHRDL2 is a bone morphogenic protein (BMP) antagonist, regulating BMP signalling, an important pathway for maintaining normal intestinal function. CHRDL2 is known to be upregulated in CRC, with high expression causing poorer clinical outcomes. The CHRDL2 gene produces multiple transcripts due to alternative splicing. We are interested in investigating the functional effects of CHRDL2 transcript variants in CRC to evaluate its potential as a therapeutic target. Two protein coding CHRDL2 transcripts differ by the presence/absence of exon 10. Our first aim was to evaluate if a colorectal cancer-associated genetic variant impacts CHRDL2 splicing and the expression levels of these two transcripts. We then explored whether the two transcripts have differential binding to BMPs and how increased expression of each CHRDL2 transcript influenced cancer-related behaviour in CRC cell lines and patient-derived CRC organoids. Our findings showed that the CRC risk variant, rs34695217, did not alter CHRDL2 splicing but computational modelling predicted that exon 10 inclusion is likely to alter protein structure and interactions with BMPs. Elevated expression of both CHRDL2 transcripts in CRC cells impacted several cancer-associated characteristics, such as cell survival, resistance to radiotherapy and chemotherapy, migration. Gene expression analysis further demonstrated activation of pathway involved in cell proliferation, survival and tumour progression. Lastly, treatment with high levels of CHRDL2 enhanced tumour organoid growth, increased WNT signalling activity and facilitated stemness. Together, these findings reveal that CHRDL2 may have oncogenic functions in CRC and may serve as a potential therapeutic target.

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VPS33B regulates cilia function in kidney epithelial cells.

Zoi Grigoriou, Victor Hernandez (2nd) and Barbara Tanos (1st)

Arthrogryposis renal dysfunction cholestasis (ARC) syndrome is a rare autosomal recessive disorder affecting multiple organ systems. Symptoms include cholestatic jaundice, renal tubular dysfunction, aminoaciduria, glycosuria, renal tubular acidosis and skeletal defects. ARC syndrome affects children, that usually do not survive the first year of life. Mutations in the Vacuolar Protein Sorting 33 homolog B (VPS33B) gene and in the VPS33B interacting protein, apical–basolateral polarity regulator (VIPAR), represent the primary genetic causes of ARC syndrome. Primary cilia are signalling organelles and key regulators of kidney function. Cilia defects result in a wide range of diseases, known as ciliopathies. It has been shown that a canonical RAB cascade, involving Rab8 and Rab11a, all small G proteins of the Ras superfamily, is associated with cilia formation. VPS33B has been shown to interact with RAB11A and other cilia-related proteins. However, a connection between VPS33B and cilia formation has never been explored. Here, we have downregulated VPS33B using RNA interference and examined the effect on cilia formation. We find that depleting VPS33B in RPE-1 and in IMCD3 and RPTEC kidney epithelial cells results in ciliogenesis defects. We

are currently examining how this is regulated and how cilia and ciliary function can affect kidney tubule morphogenesis in in vitro models of ARC syndrome.

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Understanding the molecular mechanisms of centromere activity in human Robertsonian translocations

Paulina Farrant, Sabrina Tosi (2nd) and Paola Vagnarelli (1st)

Genome Organisation and Dynamics Cluster, Center for Genome Engineering and Maintenance, Department of Biosciences, College of Health, Medicine and Life Sciences, Brunel University of London, Uxbridge, UB8 3PH.

Robertsonian translocations are common chromosomal rearrangements in humans, occurring in approximately 1 in 1000 live births. They arise from fusion of the p-arms of two acrocentric chromosomes and contain two centromeres. These chromosomes are typically stable because one centromere is inactivated, allowing proper chromosome segregation. However, Robertsonian translocations involving chromosome 21(mainly with chromosome 14 and chromosome 15) are associated with an increased risk of developing acute lymphoblastic leukaemia (ALL). The underlying mechanisms remain unclear but likely involve chromothripsis triggered by mis-segregation of the Robertsonian chromosome. This suggests that, in children carrying the constitutional rob(15;21) who develop ALL, the silent centromere is re-activated. In contrast, other chromosome 21 Robertsonian translocations segregate stably across generations without increased cancer risk. To investigate this difference and extract information of diagnostic and prognostic value, we examined centromere distances and activity in stable versus unstable Robertsonian chromosomes. Our findings suggest that these chromosomes become functionally stabilised, often behaving as monocentric despite being structurally dicentric. Notably, rob(15;21) displays greater centromeric plasticity, with shifts in centromere spacing and a bias in activity towards chromosome 21 in ALL, alongside low-levels mis-segregation. Due to the paucity of patient derived material, we are developing the first human cellular model of rob(15;21) to directly investigate mechanisms of centromere re-activation.

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Therapeutic Efficacy of Ac2-26 in Driving Thromboinflammatory Resolution in Zebrafish Larvae

Amina N. Ali[1], Conor A. Treacy[2], Michelle Sahai[3], Alessandro Esposito[2], Luigi Margiotta-Casaluci[4] (2nd), Felicity N. E. Gavins[1] (1st)

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Introduction

Thromboinflammation is a key driver of cardiovascular and inflammatory disease, yet mechanistic discovery and therapeutic development are constrained. We

investigated whether zebrafish larvae can provide a New Approach Methodology (NAM)-aligned by investigating the therapeutic potential of Annexin A1 mimetic peptide Ac2-26 to resolve thromboinflammation in zebrafish larvae via targeting Formyl Peptide Receptor (FPR) signalling.

Methods

To quantify inflammation, transgenic [Tg(mpx:gfp)] zebrafish larvae were anaesthetised (4.2% tricaine), and the distal tip of the notochord was removed using micro-scissors. Neutrophil migration was quantified within 200 μm^2 of the tail-end using intravital microscopy between 0 and 24 hours. At 6-hours post-injury, larvae were re-anaesthetised and injected with the following treatment groups: vehicle (phosphate buffered saline (PBS)), Ac2-26 (10, 100 or 1000nM); Ac2-26 (1000nM) + Boc2 (10nM); Ac2-26 (1000nM) + CSH (20nM); or Ac2-26 (1000 nM) + WRW4 (10 nM). Agonist treatments with AG-09/1 (FPR1-selective) or ACT-389949 (FPR2-selective) were administered to further confirm FPR-mediated resolution effects. Data was analysed using a one-way repeated-measures ANOVA followed by Dunn's multiple comparison

Thrombosis was induced using Rose Bengal (800 µg/mL) injected into the circulation via the duct of Cuvier for 30 min, with localised exposure to two-photon, 80 MHz pulsed, 900 nm laser light triggering ROS-mediated endothelial injury and thrombus formation. Visualisation within the caudal vein was achieved using a Leica SP5 confocal laser scanning microscope equipped with 8KHz resonant scanners. $AnxA1_{Ac2-26}$ was administered to the yolk sac 30 min prior to thrombosis, and time to first cell adherence (TTA) and full vessel occlusion (TTO) were recorded. Data were analysed using an unpaired t-test.

Complementary molecular docking studies investigated Ac2-26 binding to FPRs using High Ambiguity Driven protein-protein DOCKing (HADDOCK) 2.4 (https://rascar.science.uu.nl/haddock2.4/).

Results

Zebrafish larvae displayed robust neutrophil recruitment and thrombus formation, enabling simultaneous visualisation of thromboinflammatory and haemostatic processes. $AnxA1_{Ac2-26}$ treatment accelerated resolution-associated responses, reducing neutrophil recruitment to inflammatory sites and preventing vessel occlusion compared with untreated controls. Docking studies predicted favourable Ac2-26 binding to FPR-associated sites, thereby supporting engagement of pro-resolving pathways.

Conclusion

These findings demonstrate that Ac2-26 resolves inflammation and limits thrombosis by engaging FPR-dependent resolution pathways and establish zebrafish larvae as a tractable *in vivo* model for screening resolution-targeted therapeutics.

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TBA

Thomas Williams, Martin Scholze (2^{nd}) and Felicity Gavins (1^{st})

Population ageing is a global phenomenon occurring at unprecedented rates, especially in developed nations. With ageing comes increasingly complex heath conditions such as cardiovascular disease (CVD), diabetes, arthritis, and dementia, all of which are characterised by chronic low-grade inflammation.

Classically inflammation has been treated through suppressants, reducing its peak, but this often leads to low grade chronic inflammation and off target effects including immunosuppression. However, an alternative to having a drug suppress inflammation is to aid in inflammation resolution, i.e. rather than lowering the peak of inflammation a drug treatment allows the peak to be reached before mechanistically resolving inflammation through endogenous mediators (e.g. annexin A1, and lipoxin A4) and pathways such as the Formyl Peptide Receptor (FPRs)-pathways.

Mounting evidence suggests that, in aged organisms, neutrophils (our most abundant immune cell) adhere to distinct sites in the micro-vasculature where they promote inflammation by shaping the rheological environment. Additionally, neutrophils are emerging as decision-shapers during chronic inflammation and the age-related decline in neutrophil function is thought to contribute to the decreased immunity.

Using annexin-A1 mimetic peptide Ac2-26 to treat IL-6 and TNF-α simulated neutrophils we have seen reduction in abundance of neutrophil adhesion markers. As well as this we have observed protective effects when pre-treating neutrophils with Ac2-26 before IL-6 and TNF-α stimulation, maintaining a healthier phenotype. We have also seen that neutrophil sub-populations respond to Ac2-26 in different ways depending on neutrophil maturity and inflammatory status.

Posters

1 Resolvin D1 Attenuates Inflammation-Driven Thrombus Formation in a Human Microfluidic Thromboinflammation Model

Magdalini Malliari, Camilla Cerutti (2nd), Felicity N. E. Gavins(1st)

Department of Biosciences, Centre for Inflammation Research and Translational Medicine (CIRTM), Brunel University of London, Uxbridge, Middlesex, UB8 3PH, UK.

Cardiovascular disease (CVD) remains a leading cause of morbidity and mortality worldwide. A key mechanism underlying many cardiovascular complications is thromboinflammation, in which inflammation and thrombosis become interlinked through reciprocal activation of endothelial cells, platelets, neutrophils and coagulation pathways. Understanding how these interactions can be modulated is critical for identifying novel therapeutic strategies for CVD.
A human-relevant thromboinflammation microfluidic model was optimised and used to investigate, in real time, the effects of pro-resolving mediators on thrombi formation and composition. Whole blood from healthy adult volunteers was fluorescently labelled for fibrin, platelets and neutrophils, and perfused through commercial 2D microfluidic devices that were seeded with primary human umbilical vein endothelial cells (HUVECs), under unstimulated or TNF-α-stimulated (10ng/ml) inflammatory conditions. Thrombus formation was captured in real time using a Nikon CrestOptics spinning disk Confocal microscope.
TNF-α stimulation of the endothelium significantly promoted thrombus formation, doubling fibrin deposition, neutrophil recruitment and platelet accumulation compared to unstimulated controls, thereby validating the model as a reproducible platform for inflammation-driven thrombosis. Treatment of whole blood with the pro-resolving mediator Resolvin D1 (RvD1) significantly attenuated coagulation, reducing total fibrin surface area and fluorescence intensity within the thrombus to levels comparable to unstimulated conditions ($p<0.05$).
Collectively, these findings demonstrate the potential of pro-resolving mediator (RvD1) as a therapeutic strategy to attenuate thromboinflammation and highlights the utility of this human microfluidic model as a physiologically relevant platform for preclinical drug screening.
Funding: This work was supported by The British Heart Foundation (FS/PhD/24/29557) and The Royal Society Wolfson Foundation (RSWF\R3\183001).

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2 Potential Role of Myostatin in Placentation

Jovile Kazileviciute, Sabrina Tosi (2nd) and Emmanouil Karteris (1st) - Year 2

Myostatin (MSTN), also known as Growth Differentiation Factor 8 (GDF-8), belongs to a large family of multifunctional Transforming Growth Factor-beta (TGF-β) proteins that regulate cell growth, differentiation and immune responses. Myostatin is produced and secreted by skeletal muscle cells and can exert its effects in an autocrine, paracrine and endocrine manner. Despite considerable research on myostatin in tissues such as adipose tissues, the heart and the liver, its overall biological and physiological function in human placental development remains poorly understood due to the limited number of studies in this area. The present study investigates the effects of myostatin on the transcriptome of the BeWo and JEG 3 placental cell lines and assessed the expression of Activin type II receptors ActRII A (ACVR2A) and Activin type II receptors ActRII B (ACVR2B) in these in vitro cell models. Several methods, including reverse transcription quantitative polymerase chain reaction (RT-qPCR), RNA sequencing, differential gene expression analysis, and gene set enrichment analysis, were applied. RNA sequencing analysis revealed 3,462 differentially expressed genes in BeWo cells and 242 differentially expressed genes in JEG 3 cells following myostatin treatment, with 99 genes commonly differentially expressed in both in vitro models, including FST and

SMAD6. The predominantly dysregulated signalling pathways involved TGF-beta signalling pathway and Growth Hormone Synthesis, Secretion and Action pathway. The present study provides a novel insight into the actions of myostatin at placental level. Future studies should focus on investigating its role in pregnancy related complications, such as pre-eclampsia and gestational diabetes mellitus.

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3 Investigating Tertiary Lymphoid Structure Formation and Function in Colorectal Cancer

Margot Rame, Doreen Lau (2nd) and Gudrun Stenbeck (1st) - Year 1

Colorectal cancer (CRC), the third most common cancer globally, is a leading cause of cancer-related deaths. While immune checkpoint inhibitors have shown success in microsatellite instable (MSI) CRC, most patients develop microsatellite stable (MSS) tumours with poor immune infiltration and responsiveness to immunotherapy. Understanding the mechanisms regulating anti-tumour immunity in MSS CRC is a significant clinical challenge. Tertiary lymphoid structures (TLSs), organised clusters of immune cells that develop in non-lymphoid tissue due to chronic inflammation, play a crucial role in regulating local immune responses in tumours. Their presence is associated with better prognosis and improved immunotherapy responses in various cancers, including CRC. However, the mechanisms behind TLS formation, maturation, and function, especially in MSS CRC, are still not fully understood.
This PhD project investigates the hypothesis that TLS formation and function are regulated by the coordination of tumour antigen presentation, immune cell crosstalk, and signals derived from the tumour-associated microbiota. Disruption of these processes may contribute to immune evasion and resistance to immunotherapy in MSS CRC.
A comprehensive literature review was conducted in the first nine months to assess the current state of knowledge in colorectal cancer immunobiology, antigen presentation, TLS biology, and microbiota-driven immunity. Extensive training in tissue culture, immune cell isolation, flow cytometry, microscopy, and scaffold-based cell culture systems was undertaken. Preliminary studies successfully developed workflows for isolating and immunophenotyping T-cells and demonstrated the suitability of three-dimensional scaffold systems for maintaining fibroblast viability.
Future research will combine in vitro and in vivo bioinformatics approaches to characterise antigen presentation in TLS, investigate immune cell communication networks, and identify molecular mechanisms driving TLS formation and maturation in colorectal cancer. The goal is to enhance our understanding of local anti-tumour immunity and discover novel strategies to boost immune responsiveness in immune-resistant microsatellite instability colorectal cancer.

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4 One Pore, Many Faces: Heterogeneity at the Nuclear Basket

Lukas Serapinas, Paola Vagnarelli (2nd) and Ines J. de Castro (1st) - Year 1

Genome Organisation and Dynamics Cluster, Center for Genome Engineering and Maintenance, Department of Biosciences, College of Health, Medicine and Life Sciences, Brunel University of London, Uxbridge, UB8 3PH.

The nucleus is enclosed by a double-membrane nuclear envelope, intercalated by nuclear pore complexes (NPCs) that act as dynamic, bidirectional transport channels. NPC is composed of approximately 30 proteins, termed nucleoporins, arranged in multiple copies across three main structures: cytoplasmic filaments, central ring and the basket, which

protrudes towards the nucleoplasm and is composed of NUP153, TPR and NUP50. Beyond their canonical role in controlling the entry and exit of RNA and proteins, the basket nucleoporins are increasingly recognised for non-canonical functions including mRNA splicing, chromatin organisation, and transcriptional regulation, though the precise mechanisms underlying these roles remain unclear.

Current literature presents the NPC as an evolutionarily conserved structure; however, recent work in budding yeast has shown that the NPC exhibits far greater heterogeneity and dynamism, with populations of basket and basketless pores. Nup60, the yeast counterpart of NUP153 and one of the anchors of the basket, also undergoes acetylation. Mass spectrometry databases of acetylation modifications show 5 key lysines in NUP153 that are acetylated; however, it remains to be studied whether mammalian cell lines also have NUP153-acetylated pores or subpopulations of pores lacking NUP153 and TPR.

This poster presents the conceptual background of my PhD project, which aims to determine whether there are distinct basket/basketless nuclear pores, whether NUP153 acetylation similarly drives nuclear pore heterogeneity in mammalian cells, and to assess the functional consequences of this heterogeneity. Data collection is ongoing; this poster outlines the biological context and open questions motivating the project, rather than presenting experimental findings.

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5 Expression, purification and immunological characterisation of recombinant PPE50 proteins from Mycobacterium tuberculosis

Sajika Sritharan, Jacqueline Cliff (2nd) and Anthony Tsolaki (1st) - Year 1

Within Mycobacterium tuberculosis (Mtb), 10% of its genome consists of PE/PPE proteins. These proteins are involved in the acquisition of nutrients and other host–pathogen interactions. The function of PPE50 is unknown. Recently PPE50 was found to have 8 distinct variants, which are Mtb lineage-specific. This lineage specificity distribution suggests that PPE50 may be involved in host-pathogen co-evolution. This study aims to express and purify the 8 PPE50-variants and then investigate their immunological properties, perhaps giving insights into a role they may play in host adaptation by the pathogen.

All 8 of the PPE50 variants were expressed in LPS-free E. coli cells (Clearcoli) and following lysis it was purified using a nickel-sepharose gravity column recognising a N-terminal histidine tag. THP-1 cells were then challenged with two purified recombination proteins variants, rPPE50-132 and rPPE50-381 at different concentrations (ranging from 1-50ug/ml). qPCR when then performed on RNA extracted from the THP-1 to measure the expression of pro- and anti-inflammatory cytokines.

The results from the immunoassays by using rPPE50-132 (recently evolved truncated variant) and rPPE50-381 (evolutionary conserved variant) highlight differences in cytokine expression levels of TGF-beta, IL-6 and IL-1beta. These data seem to show that rPPE50-132 induced an anti-inflammatory response, while rPPE50-381 induces balanced inflammatory response. This differential response suggests that PPE50 variants may influence the host immune response in pathogenesis.

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6 Investigating Chromosome Organisation in Breast Cancer Cell Nuclei

Hoda Alizadeh, Sabrina Tosi (2nd) and Joanna Bridger (1st)

Breast cancer is the second-leading cause of death in women and is characterised by widespread chromosomal and genetic abnormalities. Within the nucleus, there are filamentous proteins called lamins that make up the nuclear lamina, that are part of the Linker of Nucleoskeleton and Cytoskeleton (LINC) complex, which is a structure that connects the cytoskeleton and the nuclear interior, and crucial in maintaining normal structure and function of the nucleus. Chromosomal territories (CTs) are at the top of the hierarchical organization of the genome, which is followed by topologically associated domains (TADs), and the gene body or chromatin loops. Dysregulation of nuclear lamins has been implicated in alterations of nuclear structure and chromatin organisation and reduced expression of lamins has been observed in breast cancer. This study investigates the hypothesis that localisation of chromosomes is altered due to the disrupted structure of the nuclear lamina in breast cancer cells. Using the MDA-MB-231 cell line as a model, chromosome positioning and nuclear architecture are examined through a combination of fluorescence in situ hybridisation, immunofluorescence and fluorescence microscopy supported by image analysis.

This work aims to provide insight into the relationship between nuclear structural integrity and genome organisation in breast cancer, contributing to a better understanding of how nuclear architecture influences tumour biology.

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 # 7 H2A.Z.2, a histone variant beyond CENP-A essential for centromere maintenance

Amin Hashemloo Denise Ragusa, Paulina Farrant and Paola Vagnarelli -
Molecular Genetics MSc

Genome Organisation and Dynamics Cluster, Center for Genome Engineering and Maintenance, Department of Biosciences, College of Health, Medicine and Life Sciences, Brunel University of London, Uxbridge, UB8 3PH.

Centromeres are epigenetically defined and maintained. Beside the key centromeric H3-like histone CENP-A, we have identified the histone variant H2A.Z.2 as another important epigenetic feature necessary to maintain a functional centromeric chromatin. Using a HCT116 cell line where both H2A.Z.2 alleles were endogenously tagged with RFP- and AID (auxin-inducible degron system), we showed that this histone variant, but not the very similar H2A.Z.1, is enriched at centromeric chromatin, and its depletion in G2 or M leads to chromosome segregation defects. Mechanistically, H2A.Z.2 is essential for localising and maintaining Aurora B and Shugoshin-1 at centromere in a H2AS121ph- independent manner and depletion of H2A.Z.2 in Nocodazole arrested cells, leads to silencing of the SAC and exit from mitosis with Cyclin B degradation and chromatin de-condensation. It has been shown that H2A.Z binds HP1 in vitro and our data suggest that HP1 pulls down H2A.Z.2-containing chromatin more efficiently that the H2A.Z.1 one. Interestingly, artificially tethering HP1 to centromeres via a CENP-B DNA-binding domain-HP1 fusion construct, fully rescued Sgo1 localisation in H2A.Z.2-depleted cells.
These data altogether indicate that H2A.Z.2 is an essential epigenetic feature of the mitotic centromeric chromatin.