Brunel University London

Centre for Genome Engineering and Maintenance

CenGEM

Virtual symposium on Epigenetics in Cancer

Book of Abstracts

10th-11th May 2021

Organisers Cristina Pina and Annabelle Lewis

Table of Contents

Day 1

Keynote speaker: Doug Higgs.....3

Session 1: General epigenetics and haematological malignancies

Speaker: Kristina Kirschner.....4

Short talk: Denise Ragusa.....5

Speaker: Luciano di Croce.....6

Speaker: Sahand Hormoz.....7

Short talk: Shikha Gupta 8

Speaker: Maria Figueroa.....9

Day 2

Session 2: Epigenetics of childhood cancers

Speaker: Paolo Salomoni.....10

Speaker: Giovanni Perini.....11

Short talk: Aakila Sammy.....12

Speaker: Katrin Ottersbach.....13

Short talk: Alexia Tsakaneli.....14

Session 3: Epigenetics of solid tumours

Speaker: Jessie Hsu.....15

Speaker: Daniela Furlan.....16

Short talk: Laura Libera.....17

Speaker: Adele Murrell.....18

Switching genes on and off in health and disease

Keynote speaker: *Doug Higgs*
University of Oxford, United Kingdom

Researchers now have access to over 100,000 human genomes and the genomes of an ever-increasing number of other organisms spanning the evolutionary tree of life. The next major task in biomedicine is to decode this information and provide a link between the DNA sequence and cell behaviour during development and differentiation. We currently know for certain of three classes of regulatory elements that are active in interphase: enhancers, promoters and insulator elements. Using the globin cluster as a tractable model we are studying how these fundamental elements work individually and in combination, linking gene expression, epigenetic modifications and the 3-D genome. We are also interested to understand how these elements and gene expression are perturbed in acquired and inherited human genetic diseases.

Myeloproliferative Neoplasms vs Clonal Haematopoiesis: using single cell analysis to study the ageing human haematopoietic system

Speaker: *Kristina Kirschner*
University of Glasgow, United Kingdom

Maria Terradas Terradas, Ana Rodriguez-Meira, Neil A. Robertson, Sarah E. Harris, Simon R. Cox, Ian J. Deary, Adam J. Mead, Tamir Chandra, Kristina Kirschner

Clonal haematopoiesis (CH) is apparent in the general population from age 60 with a steady increase to 18-20% over age 90 driven by somatic mutations in leukaemic driver genes, leading to reduced diversity of the blood pool. One of those mutations can be found in the tyrosine kinase Janus 2 (*JAK2V617F*). CH carries an increased risk for leukaemia.
Patients with myeloproliferative disease (MPN), a pre-neoplastic, clonally derived cancer which is associated with increased age, also carry a *JAK2V617F* mutation driving disease. Disease in a subset of patients go on to transform into full blown leukaemia.
In this study, we aim to understand molecular and functional characteristics of *JAK2V617F* in different disease and ageing context, by contrasting a pre-disease state with a pre-neoplastic state.

Disturbed nuclear organisation and gene expression in paediatric leukaemias with chromosome 7 rearrangements

Presenter: *Denise Ragusa*
Brunel University London, United Kingdom

Denise Ragusa, Sabrina Tosi
Brunel University London, United Kingdom

Chromosomal rearrangements can disturb the distribution of chromatin in the nucleus by altering the location of the affected regions. Nuclear organisation plays an important role as epigenetic regulator of gene expression, with transcriptionally inactive regions residing towards the nuclear periphery, and active regions in the nuclear interior. Previous studies demonstrated an altered nuclear repositioning of the *MNX1* gene in leukaemia patients harbouring the chromosomal translocation t(7;12)(q36;p13). This relocation is thought to be responsible for the overexpression of *MNX1* mapping at 7q36. To investigate the link between nuclear repositioning and gene expression, we analysed patients with haematological disorders harbouring chromosomal rearrangements involving the 7q region. In interstitial 7q deletions upstream of *MNX1*, we observed a nuclear repositioning of *MNX1* that is dependent on the GC-content of the affected chromosomal bands. Deletions with breakpoints in the GC-rich band 7q22 resulted in a relocation of *MNX1* to the nuclear interior, while breakpoints in the GC-poor band 7q21 brought *MNX1* to the periphery. Interestingly, the localisation of *MNX1* in these patients was also correlated with its transcriptional status. These observations suggest that gene expression patterns changes in these leukaemia patients may be dependent on the interactions between the newly juxtaposed chromosomal bands resulting from the rearrangement. This work highlights the importance of nuclear organisation as epigenetic mechanism behind the dysregulation of gene expression in leukaemia.

Epigenetic regulation of adult stem cells and cancer cells

Speaker: *Luciano di Croce*
Centre for Genomic Regulation (CRG) and Catalan Institution for Research and Advanced Studies (ICREA), Spain

Polycomb (PcG) and Trithorax (TrxG) group proteins are transcriptional regulators involved in embryonic development, cell differentiation, and maintenance of cell identity. Deregulation of PcG or TrxG has been linked to anomalous activation of differentiation pathways, carcinogenesis and cancer progression. PcG machinery has been subdivided into two main complexes: Polycomb repressive complex 1 (PRC1) and PRC2. PRC1 is responsible for the deposition of H2AK119ub1, while PRC2 catalyzes H3K27 methylation. This view has been greatly clarified and expanded in the last decade revealing that the PcG system is much more diverse than initially anticipated.
The antagonistic function is performed by Trithorax/MLL complexes that through deposition of methyl marks on lysine 4 of histone H3 positively regulate enhancer activation and transcription, thus counteracting the activity of Polycomb complexes.
I will discuss how Polycomb and MLL proteins (including novel associated factors) determine adult stem cell identity, control differentiation, and – when mis-regulated/mutated – facilitate cancer progression.

Inferring the dynamics of blood cancers from single-cell measurements

Speaker: *Sahand Hormoz*
Harvard University, United States of America

I will talk about our recent efforts to determine when cancer first occurs and the dynamics of its expansion in individual patients with a type of blood cancer called myeloproliferative neoplasm. We do this by reconstructing the lineage trees of individual cancer cells. Surprisingly, the driver mutation for this type of blood cancer occurs decades before diagnosis. In addition, we use single-cell sequencing to decipher the differentiation dynamics of the cancer cells, which occur on the much shorter time-scale of days. Finally, I will discuss tools from synthetic biology that can help us reconstruct lineage trees of single cells.

Transcriptional heterogeneity governs cell fate diversification during pre-leukaemia to leukaemia progression

Presenter: *Shikha Gupta*
University of Cambridge, United Kingdom

Shikha Gupta [1], Ana Filipa Domingues [2,3,4], Oliwia Cyran [1], George Giotopoulos [2,3], Sudhakaran Prabakaran [1,5], Brian J. Huntly [2,3], Oliver M. Dovey[4], George S. Vassiliou [2,3,4], Cristina Pina [1,6]
University of Cambridge [1]Department of Genetics, [2]Department of Haematology and [3]WT-MRC Stem Cell Institute, UK; [4]Wellcome Trust Sanger Institute, UK; [5]IISER-Pune, India; [6]Brunel University London College of Health and Life Sciences – Biosciences

Acute myeloid leukaemia (AML) is a heterogenous clonal disorder of haematopoietic progenitor cells with a dismal survival. It has a strong reliance on epigenetic and transcriptional factors for disease progression. Accordingly, we have previously identified KAT2A, a histone acetyl-transferase, as a requirement for AML maintenance; where chemical inhibition of KAT2A promotes differentiation of AML cell lines (Tzelepis et al., 2016, *Cell Reports* 17, 1193–1205). More recently, using a conditional knockout (KO) mouse model for *Kat2a* we showed that it sustains KMT2A/MLLT3 AML stem cells. *Kat2a* is a classical regulator of transcriptional variability, it's loss leads to cell-to-cell heterogeneity in transcription levels specifically from genes involved in ribosomal biogenesis and translation (Domingues et al., 2020, *eLife* 9:e51754). No recurrent mutations in the KAT2A gene have been described in AML, and it is unclear if and how it participates in preleukemia-to-AML progression.
Herein, we use our conditional *Kat2a* knockout mouse model to analyze the effects of *Kat2a* loss in biology of *RUNX1-RUNX1T1(9a)* and *Idh1*R132H-initiated AML. These models represent forms of human disease with a prolonged pre-leukaemia phase that typically require additional mutations for leukaemia progression. We observed that loss of *Kat2a* accelerates leukaemia initiation and progression in vivo. This acceleration was a consequence of fixation of transformed *Kat2a* KO cells in vivo which reflects as enhanced self-renewal capacity in vitro as measured by serial re-plating colony forming assay.
Given the central role of *Kat2a* in limiting cell-to-cell transcription heterogeneity, we interrogated a potential link between loss of *Kat2a*, its consequent increase in transcriptional heterogeneity and pre-leukaemia progression. For this, we performed single-cell RNA sequencing (scRNA-seq) of early-stage *Kat2a* WT and *Kat2a* KO *RUNX1-RUNX1T1(9a)* pre-leukaemia. Compatible with our previous observation, we observed that *Kat2a* KO cells were more heterogeneous transcriptionally. Interestingly, this was accompanied by diversification of cell fates towards B-lymphocytes and monocytes. Furthermore, pseudo-temporal ordering of single *Kat2a* KO cells revealed highly branched trajectory heavily populated with intermediate stages of transformation; including accumulation of leukaemia progenitors with RUNX1-RUNX1T1 signature. In contrast, *Kat2a* WT cells have linear normal haematopoiesis trajectory with minimal branching and an abrupt transition towards candidate leukaemia progenitor state.
Pathway analysis of *Kat2a* KO leukaemia progenitor cells indicated perturbation of ribosomal biogenesis and translation associated genes. In order to test how these changes contributed to transformation, we performed S6K1 inhibition on *Kat2a* WT cells which transiently promoted transformation *in vitro* in both *RUNX1-RUNX1T1(9a)* and *Idh1*R132H cells, thus, phenocopying the effects of *Kat2a* loss. This suggested a mechanistic contribution of observed transcriptional changes in protein synthesis machinery towards leukaemia progression.
Taken together, our work suggests that loss of *Kat2a* results in diversification of cell fates, including with increased accessibility to cell states prone to transformation. Furthermore, these cells, prone to transformation, may benefit from a low biosynthetic activity that promotes their progression to leukaemia state. We hypothesize that *Kat2a* loss may function similarly in the context of other malignancies. In the future, this knowledge may aid in development of early diagnostic tools and suggest bespoke therapeutic interventions.

Aging Human Hematopoietic Stem Cells Manifest Profound Epigenetic Reprogramming of Enhancers That May Predispose to Leukemia

Speaker: *Maria Figueroa*
University of Miami Health Systems, United States of America

Emmalee R. Adelman[1,2,3], Hsuan-Ting Huang[1,2,#], Alejandro Roisman[1,2,#], André Olsson[4], Antonio Colaprico[1,2], Tingting Qin[5], R. Coleman Lindsley[6], Rafael Bejar[7], Nathan Salomonis[8], H. Leighton Grimes[4], Maria E. Figueroa[1,2 *]

[1] Dept. of Human Genetics and [2] Sylvester Comprehensive Cancer Center, University of Miami Miller School of Medicine, [3] Dept. of Pathology and [5] Dept. of Computational Medicine and Bioinformatics, University of Michigan Medical School [4] Div. of Immunobiology and Center for Systems Immunology and [8] Div. of Biomedical Informatics, Cincinnati Children's Hospital Medical Center, [6] Dept. of Medical Oncology, Div. of Hematological Malignancies, Dana-Farber Cancer Institute, [7] Division of Hematology and Oncology, Moores Cancer Center, University of California San Diego,
[#] These authors contributed equally

Aging is associated with functional decline of hematopoietic stem cells (HSC) as well as an increased risk of myeloid malignancies. We performed an integrative characterization of epigenomic and transcriptomic changes, including single-cell RNA-seq, during normal human aging. Lineage$^-$CD34$^+$CD38$^-$ cells (HSC-enriched, HSCe) undergo age-associated epigenetic reprogramming consisting of redistribution of DNA methylation and reductions in H3K27ac, H3K4me1 and H3K4me3. This reprogramming of aged HSCe globally targets developmental and cancer pathways which are comparably altered in AML of all ages; encompassing loss of 4,656 active enhancers, 3,091 bivalent promoters, and deregulation of several epigenetic modifiers and key hematopoietic transcription factors, such as KLF6, BCL6 and RUNX3. Notably, in vitro downregulation of KLF6 results in impaired differentiation, increased colony forming potential and changes in expression that recapitulate aging and leukemia signatures. Thus, age-associated epigenetic reprogramming may form a predisposing condition for the development of age-related AML.

Investigating the Role of Onco-Histones in Cancer

Speaker: *Paolo Salomoni*
Nuclear Function Group, DZNE, Bonn, Germany

Chromatin perturbations are pervasive in cancer. However, proof of their tumour-driving roles had been missing for a long time. The most compelling evidence for epigenetic changes as tumour drivers came from the identification of mutations affecting the very basic building blocks of chromatin, histones. These alterations are found at very high frequency in brain and bone neoplasms and mostly target a histone variant called H3.3. One of them, $H3.3^{K27M}$ is a strong gain-of-function (GoF) mutant found in the majority of pediatric high-grade gliomas (pHGGs) that inhibits trimethylation of lysine 27 on H3 histones, a key repressive mark that is produced by the Polycomb Repressive Complex 2 (PRC2), itself genetically altered in other cancers. By using *in-utero* genetic modification of neural stem cells, we have demonstrated that $H3.3^{K27M}$ along with $p53^{LoF}$ is sufficient to drive formation of invasive brain neoplasms recapitulating the hallmark features of human tumours. Interestingly, this effect is seen only if these genetic alterations are introduced during development and not in the postnatal brain, suggesting that these tumors have an embryonic origin. Interestingly, a number of H3.3 mutations have been found also in developmental syndromes. Furthermore, our more recent work showed that $H3.3^{K27M}$ promotes derepression of retrotransposable elements, which on one hand may contribute to transformation while on the other may represent an Achille's heel of these tumour entities. Finally, our ongoing work is focused on understanding other onco-histones in the context of pHGG and bone neoplasms. Overall, by generating new state-of-the art preclinical models, we have shed light on the pathogenesis of these epigenetically driven cancers while also highlighting new potential vulnerabilities of onco-histone driven tumours.

MYCN promotes tumorigenesis by expanding E2F3a interactome(s) to multiple Histone Acetyl transferase complexes.

Speaker: *Giovanni Perini*
University of Bologna, Italy

The most aggressive subtype of neuroblastoma (NB) which carries the worst overall prognosis occurs where MYCN is amplified. Although, recent studies have shown that MYC as well as MYCN factors can act as wide transcriptional amplifiers and participate in super enhancer complexes, many questions remain regarding what distinguishes MYCN-amplified from non-amplified tumors. Both published studies and several preliminary findings from our lab indicate that high MYCN can establish a strong functional axis with E2F3 in neuroblastoma that is pivotal to the development of a high-risk cancer phenotype. The action of MYCN on E2F3 can apparently occur despite the RB1 status of the neuroblastoma cell and how this is achieved is not clearly understood. It should be noted that E2F3 consists of two main isoforms, E2F3a and E2F3b which result from usage of alternative promoters and that differ for just the first 109 AA of their N-terminal tails. Interestingly, survival curves of neuroblastoma patients suggest that E2F3a correlates with poor outcome, whereas E2F3b correlates with a better prognosis. To understand how MYCN controls E2F3a/b activity we used a proximity biotinylation assay to map E2F3 interactome(s) either in low or high MYCN cell backgrounds. Results show that the global E2F3a/b interactome is extensively wider than previously described and above all that E2F3a and E2F3b, despite sharing many protein interactors, display relevant differences that may in part explain patients'prognosis. Finally, the analyses also reveal that high MYCN expands E2F3a interactome by promoting and reinforcing E2F3a interactions with Histone Acetyl transferase complexes such as SAGA, ATAC and TIP60/NuA4 which are known to play a critical role in oncogenesis.

Understanding Ovarian Cancer and Chemoresistance through Chromosome Spatial Organisation and Nuclear Motors

Presenter: *Aakila Sammy*
Brunel University London, United Kingdom

Dr Aakila Sammy & Prof Joanna Bridger

Due to the silent aggressive progression of ovarian cancer (OC), 20% of patients diagnosed would not be able to receive treatment at all, and the 80% that receives treatment, as much as 90% can relapse in less than six months and, by the end of two years fail to respond to treatment as a result of multi-drug resistance. As the genetic landscape of OC remains chaotic, the epigenetic factor of non-random chromosome spatial organisation against different disease states and perturbations of its functional organisers such as lamins and nuclear myosins (NMs) can provide a stable outlook on the disease.

Using Fluorescent in situ hybridisation; three chromosome panels of four key chromosomes (chr1, chr13, chr17 and chrX) in four OC cell lines (SKOV-3, PEO-1, PEO-4 and MDAH-2774) was constructed based on diseased state (against the control cell line HOSEpi), and after two RNAi knockdowns (NM1 and NM6). A fourth panel was built to investigate the phenomenon in chemo-resistance by using MDAH-2774$_{CR}$, a cisplatin-resistant counterpart to the naïve MDAH-2774 was induced by in-vitro chemotherapy. Internal localisations were observed for chr1, chr13 and chr17, and a peripheral localisation was observed chrX. Post-NM1/6 knockdowns revealed that chromosome territories relocalised similar to the localisations of the control cell line,and following the acquisition of platinum-resistance of MDAH-2774, all four chromosomes predominated centrally.

This research broadened the comprehension of the possible mechanisms involved in the rapid development and progression of OC from an epigenetic standpoint and lays the groundwork for the role of spatial genome organisation in chemo-resistance.

The developmental origins of infant leukaemia

Speaker: *Katrin Ottersbach*
University of Edinburgh, United Kingdom

Infant leukaemias are rare and very aggressive blood malignancies that affect children under the age of 1 and that have their origin in foetal development. They are most commonly associated with chromosomal rearrangements involving the MLL gene, which codes for a histone methyltransferase that is an important regulator in haematopoietic development. The translocation results in the generation of a fusion protein, the most frequent of which is MLL-AF4, which causes wide-spread epigenetic dysregulation and a general upregulation of transcription.
While MLL-AF4+ leukaemia in older children and adults requires additional, cooperating mutations, the MLL-AF4 fusion appears to be sufficient to drive the full leukaemic phenotype in infant patients. This suggests that the foetal origin is an important contributing factor.
My group has a long-standing interest in embryonic and foetal blood development and has, together with several other groups, over the years described a number of unique features of foetal blood progenitors that may contribute to the cells being more susceptible to MLL-AF4-mediated transformation. We have also recently reported how MLL-AF4 expression subverts normal blood development, resulting in the generation of a pre-leukaemic state. We have been able to define the developmental window and the likely cell(s)-of-origin in which this occurs. We have now transcriptionally further analysed these potential cells-of-origin and have defined a conserved foetal expression signature that persists in leukaemic blast cells and that is required for disease maintenance. This has demonstrated the dependency of the leukaemia on the presence and maintenance of foetal expressed genes and foetal epigenetic features, some of which may prove to be novel targets for therapy.

MYCN regulates the oncogenic activity of exosomes in neuroblastoma: potential role of PKM2

Presenter: *Alexia Tsakaneli*
Brunel University London, United Kingdom

Alexia Tsakaneli[1]; Giuseppe Palmisano[2]; Arturo Sala[1,3]

[1]Department of Biosciences, College of Health & Life Sciences, Brunel University London, UB8 3PH Uxbridge, United Kingdom.

[2]Institute of Biomedical Sciences, Department of Parasitology, University of Sao Paulo, Brazil.

[3]Department of Psychology and Health, University of Chieti-Pescara, Chieti 66100, Italy.

MYCN amplification is a key molecular aberration in high-risk neuroblastoma and predictive of poor outcome. In this study, we investigated the role of MYCN in regulating the protein cargo of exosomes, vesicles secreted by tumour cells that can be picked up by recipient cells with important functional consequences. Using a switchable MYCN system coupled to mass spectrometry, we have verified that MYCN regulates a set of proteins in the exosomes secreted by neuroblastoma cells. Pathway analysis suggests that the MYCN-regulated proteins belong to 3 main functional groups: a) extracellular matrix-cells interactions, b) glycolysis and c) ribosome biogenesis. We delivered exosomes from MYCN positive or negative neuroblastoma cells to MYCN non-amplified cells and tested their biological activity. Interestingly, exosomes secreted by MYCN-positive cells promoted proliferation, metabolism and activation of AKT via phosphorylation of the Ser473 residue in recipient cells. We also found that the glycolytic enzyme and oncogene PKM2 is enriched in exosomes secreted by MYCN activated cells or purified from the plasma of neuroblastoma patients. We are currently investigating the role of PKM2 in mediating the oncogenic activity of neuroblastoma exosomes. In conclusion, our results support the hypothesis that non-cell autonomous effects of MYCN could be mediated by exosomes.

EED-Targeted PROTACs Degrade EED, EZH2, and SUZ12 in the PRC2 Complex

Speaker: *Jessie Hsu*

Astra Zeneca, Boston, United States of America

Deregulation of the PRC2 complex, comprised of the core subunits EZH2, SUZ12, and EED, drives aberrant hypermethylation of H3K27 and tumorigenicity of many cancers. Although inhibitors of EZH2 have shown promising clinical activity, preclinical data suggest that resistance can be acquired through secondary mutations in EZH2 that abrogate drug target engagement. To address these limitations, we have designed several hetero-bifunctional PROTACs (proteolysis-targeting chimera) to efficiently target EED for elimination. Our PROTACs bind to EED ($pK_D \sim 9.0$) and promote ternary complex formation with the E3 ubiquitin ligase. The PROTACs potently inhibit PRC2 enzyme activity ($pIC_{50} \sim 8.1$) and induce rapid degradation of not only EED but also EZH2 and SUZ12 within the PRC2 complex. Furthermore, the PROTACs selectively inhibit proliferation of PRC2-dependent cancer cells (half maximal growth inhibition [GI_{50}] = 49-58 nM). In summary, our data demonstrate a therapeutic modality to target PRC2-dependent cancer through a PROTAC-mediated degradation mechanism.

The current paradigm of colorectal cancer

Speaker: *Daniela Furlan*
University of Insubria, Italy

One of the best characterized cancer models for epigenetic alterations is colorectal cancer (CRC). In this tumour aberrant DNA methylation is very common, occurs early and involves virtually all the major signalling pathways altered during the progression of CRC. These findings, coupled with the reversibility of DNA methylation have generated interest in the development of new clinical tests for early detection of CRC and for prognostic and therapeutic purposes. At the whole genome level, CRCs have 10-40% lower levels of absolute methylation compared with normal colonic mucosae. This is primarily due to loss of methylation in megabase-scale domains of repressive chromatin characterized by low gene density, low GC density and late replication timing. Hypomethylation of Long Interspersed Nuclear Element-1 (LINE-1) has been largely studied in CRC and it is thought to contribute to CRC initiation by enhancing chromosomal instability and by deregulation of gene expression. In addition to global hypomethylation, CRCs are characterized by hypermethylation of a subset of gene promoters. Global methylation profiling enables to identify subgroups of CRCs with distinct molecular and clinical features that exhibit increased rates and specific patterns of promoter methylation, termed the CpG-island methylator phenotype (CIMP).
Our understanding of the CRC epigenome has been largely developed over the last decade and it is now believed that among thousands of epigenetic alterations, a small subgroup of these may be considered as a CRC driver event. Major advances in our capacity to detect these driver markers have the potential for great implications in clinical practice.

The *MLH1* polymorphism rs1800734 and risk of small bowel carcinomas with microsatellite instability

Presenter: *Laura Libera*
University of Insubria, Italy

Libera L.[1], Sahnane N.[1], Bombelli R.[1], Aliprandi M.[1], Arpa G.[2], Di Sabatino A.[2], Vanoli A.[2], Sessa F.[1] and Furlan D.[1]
1. Pathology Unit, Department of Medicine and Surgery, University of Insubria-ASST Sette Laghi, Varese, Italy
2. Departments of Molecular Medicine and Internal Medicine, University of Pavia, and San Matteo Hospital Foundation, Pavia, Italy

The *MLH1* promoter polymorphism -93G>A (rs1800734) is associated with microsatellite instability (MSI) in colorectal cancer (CRC) exhibiting *MLH1* promoter methylation. Recently, it has been proposed that BRAF/MAFG pathway may be driver of *MLH1* methylation in CRC1 and that rs1800734 influences methylation acquisition in *MLH1* promoter2.
Recently, *MLH1* hypermethylation and MSI have been demonstrated also in small bowel carcinomas (SBCs) at high frequencies3, but the role of rs1800734 has never been investigated in this site.
In this study, we genotyped *MLH1* rs1800734 (GG, GA and AA) in a well-characterized multicenter series of non-familial, non-ampullary SBCs (70 cases) and in a CRC cohort (300 cases) for comparison, in order to verify whether rs1800734 is associated with the risk of MSI-SBCs.
Among CRCs we observed MSI in 52/300 tumors (17,3%) that showed a significantly higher frequency of AA genotypes compared with MSS-CRCs (23% MSI-CRC vs 9% MSS-CRC, p=0.008). Interestingly, we found that AA patients developed CRCs at a significantly lower age than GA/GG patients (p=0.0017).
On the contrary, in SBCs we identified 25 (35.7%) MSI cancers (all with *MLH1* methylation); however, no significant association was found between MSI-SBCs and rs1800734 AA genotype (8% MSI-SBC vs 12% MSS-SBC). Moreover, no correlation was observed between rs1800734 and age of SBCs onset.
Our findings confirmed that rs1800734 risk allele is associated to MSI-CRC and seems to correlate with a possible accelerated tumorigenesis. By contrast, rs1800734 is not associated with a risk of MSI-SBC, suggesting that *MLH1* hypermethylation may occur by different mechanisms in SBC and CRC.

References

1. Fang et al. Mol Cell. 2014 Sep 18;55(6):904-915. doi: 10.1016/j.molcel.2014.08.010
2. Thomas et al. Sci Rep 2019 Sep 17;9(1):13463. doi: 10.1038/s41598-019-49952-x
3. Vanoli et al. Mod Pathol. 2017 Oct;30(10):1453-1466. doi: 10.1038/modpathol.2017.40

Two phases of hydroxymethylation changes during colon cancer progression. Should we target TETs?

Speaker: *Adele Murrell*
University of Bath, United Kingdom