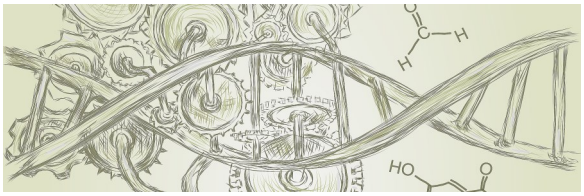


Short talk presentations

HISTONE VARIANTS LINK METABOLISM TO 3D CHROMATIN ARCHITECTURE

Dr Marcus Buschbeck, **Josep Carreras Leukaemia Research Institute (IJC)**

A novel supradisciplinary field is emerging at the intersection of nuclear organization, epigenetics and metabolism. Key developmental phases such as embryogenesis and juvenile growth are windows of phenotypic susceptibility to environmental triggers, most notably nutrition, which directly impacts on metabolism. These windows coincide with cell fate transitions on the cellular level.

Studying histone variants my lab has made two observation that position us at an exciting intersection between metabolism and chromatin architecture. Three histone variants called macroH2A are unique in having two additional domains: (i) a linker domain, which regulates 3D chromatin architecture (Kozlowski, Corujo et al., 2018, EMBO Rep), and (ii) a globular macrodomain able to sense and affect metabolism (Posavec Marjanovic, Hurtado-Bagès et al., 2017, NSMB).

We found that cells have evolved a mechanism involving the isoform macroH2A1.1 that allows to couple changes in chromatin composition to alterations in energetic needs. Specifically, we found that during differentiation muscle cells upregulate the levels of macroH2A1.1 in chromatin. The macrodomain of macroH2A1.1 but not those of the other isoforms, is able to bind NAD+ derived ADP ribose and ADP-ribosylated PARP1. In differentiated but not proliferating cells this leads to PARP1 inhibition and a net reduction of nuclear NAD+ consumption promoting NAD+ dependent respiration in mitochondria.

I will present published and unpublished data showing how macroH2A histone variants link chromatin composition and 3D architecture to metabolism.

SETD5, A NEW CONNECTION BETWEEN EPIGENETIC AND MITOCHONDRAIL FUNCTION

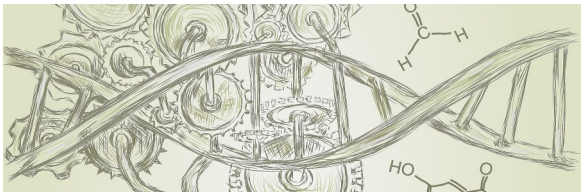
Mattia Zaghi, **Fondazione Centro San Raffaele**

Rationale. Chromatin remodelling has a crucial role in gene regulation and alterations of its molecular elements are closely associated with neurodevelopmental disorders such as intellectual disability (ID) and autism spectrum disorders (ASD). *De novo* and inherited mutations in one SETD5 allele have been associated to both ID and ASD in recent years. A clear pathological mechanism has not yet been described. In this study, we determined the exact role of SETD5 in chromatin regulation and how its dysfunction contributes to the onset of these diseases.

Methods. SETD5 protein function was investigated through biochemical assays, genome-wide approaches while its downstream effects were assessed in the neural lineage both on *in vitro* and *in vivo* conditions.

Results. We demonstrated that SETD5 has an intrinsic enzymatic activity and operates as a specific methyltransferase for H3K36. Setd5 inactivation in neural stem cells, zebrafish and mice leads to a significant H3K36 hypomethylation that is mostly detectable in the gene bodies. Consequently, this chromatin alteration perturbs RNA processing during elongation, which compromises the transcriptional output. This gene dysregulation is particularly affecting transcripts related to the mitochondrial signature. We functionally confirmed an impairment of mitochondrial compartment by revealing enhanced mitochondrial fragmentation, decreased membrane potential, ATP production and mitochondrial associated protein expression. Interestingly, these defects lead to a different distribution of mitochondria within mutant neurons, namely a reduced density along neurites.

Conclusions. Our evidences suggest possible mechanisms in ASD/ID pathogenesis due to mitochondrial hypofunctionality driven by altered chromatin landscape.



ABSTRACTS

METHIONINE METABOLISM IMPACTS MAINTENANCE OF AML WITH CHROMATIN REGULATION

Alice Victoria Taylor, **Department of Haematology, University of Cambridge**

Acute Myeloid Leukaemia (AML) is an aggressive cancer characterised by epigenetic dysregulation. Intermediary metabolism participates in epigenetic control through production of histone and DNA-modifying residues, including methyl group synthesis through methionine metabolism. MAT2A is an obligatory member of the Methionine Adenosyl-transferase enzyme, which catalyses synthesis of the universal methyl donor, S-Adenosyl-Methionine (SAM). *MAT2A* is upregulated in liver and colorectal cancer, which both depend on methionine availability. We recently identified *MAT2A* in a CRISPR drop-out screen as a novel candidate vulnerability in AML.

We show that MAT2A inhibition and SAM reduction result in markedly decreased DNA methylation and a specific erosion of H3K36 tri-methylation, which is in contrast with the preferential loss of H3K4 tri-methylation observed in colon cancer. The data suggest a dependence of AML on gene body modifications which may be integral to high expression of leukaemia-propagating signatures and/or splicing control. Functionally, MAT2A inhibition and/or expression knockdown in cultured AML cells result in decreased growth and enhanced apoptosis, with no effect on cell differentiation. Methionine depletion had similar results. *MAT2A* is required for AML cell propagation *in vivo*. Its ablation in AML patient samples results in reduced colony formation and hindered propagation in stromal co-cultures, with loss of stem/progenitor-like CD34+ leukaemia cells. Significantly, healthy blood progenitors expand and differentiate independently of MAT2A activity, indicating a requirement that is specific to AML.

Targeting of S-Adenosyl-Methionine synthesis can thus be exploited as a novel therapeutic strategy in AML, with disruption of epigenetic control.

THE HISTONE METHYLTRANSFERASES SUV420H REGULATE PPAR-γ AND ENERGY EXPENDITURE IN RESPONSE TO ENVIRONMENTAL STIMULI

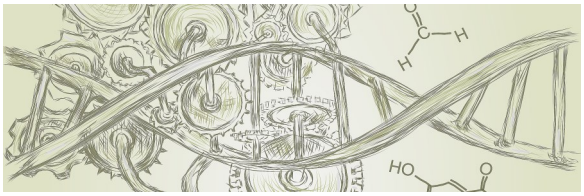
Dr Simona Pedrotti, **Ospedale San Raffaele**

The rising prevalence of obesity and its associated metabolic abnormalities have become a global emergency with considerable morbidity and mortality1-3. While there is certainly an important genetic component, extensive human epidemiologic and animal model data suggest an epigenetic contribution to obesity4. Nevertheless, the cellular and molecular underpinnings of these pathways and how they contribute to the development of obesity remain to be elucidated.

Suv420h1 and h2 are histone methyltransferases responsible for chromatin compaction and gene repression5-7. Through *in vivo*, *ex-vivo* and *in vitro* studies, we found that Suv420h1 and h2 respond to environmental stimuli and regulate metabolism by downregulating PPAR-γ, a master transcriptional regulator of lipid storage and glucose metabolism8,9. Accordingly, mice lacking Suv420h proteins activate PPAR-γ target genes in brown adipose tissue to increase mitochondria respiration, improve glucose tolerance and reduce adipose tissue to fight obesity.

We conclude that Suv420h proteins are key epigenetic regulator of PPAR-γ and the pathways controlling metabolism and weight balance in response to environmental stimuli.

1 Verma *et al*. *Diabetes Metab Syndr* **11**, 73; 2 Bhaskaran *et al. Lancet* **384**, 755; 3 Hossain *et al. The New England journal of medicine* **356**, 213; 4 S. J. van Dijk *et al. Clinical epigenetics* **7**, 66; 5 Schotta *et al. Genes & development* **18**, 1251; 6 Schotta *et al. Genes & development* **22**, 2048; 7 Hahn *et al. Genes & development* **27**, 859; 8 Soccio *et al. The Journal of clinical investigation* **127**, 1451; 9 Lapa *et al. Scientific reports* **7**, 16795.



Poster presentations

MATERNAL OBESITY AFFECTS THE EPIGENOME OF OOCYTES AND PREIMPLANTATION EMBRYOS

Poster presented by: António Galvão, **Babraham Institute**

Rationale. The oocyte epigenome has the potential to control initial reprogramming events in the early embryo, as well as metabolic outcomes in offspring. We are investigating the impact of maternal obesity on the oocyte and blastocyst methylome and transcriptome.

Methods. Using a combined method we performed single-cell RNA-sequencing and single-cell bisulfite sequencing in MII-oocytes from C57Bl/6J (B6) mice fed chow diet (CD) or high-fat diet (HFD) for 16 weeks. Subsequently, we performed in vitro fertilisation on oocytes and addressed how metabolically-driven changes in the oocyte epigenome could affect the E3.5 embryo methylome and transcriptome at a single blastocyst level.

Results and Conclusions. DESeq2 analysis identified 195 differently expressed genes in oocytes ($p<0.05$). Interestingly, transcripts affecting early development, such as *Dppa3* and *Plac1*, were significantly increased in HFD oocytes. Unbiased CpG methylation analysis of the oocytes revealed 450 differently methylated regions (DMRs), with an absolute cut-off of 20% ($p<0.05$): 61% of these DMRs overlapped genes and lost methylation ($p<0.05$), whereas the remaining 39% were intragenic and gained methylation in HFD oocytes ($p<0.05$). Whole genome methylome analysis in blastocysts revealed 205 DMRs ($p<0.05$), with 10% of blastocyst DMRs coinciding with those in the oocyte, suggesting potential longer-term consequences of methylation changes induced in oocytes. In conclusion, we give the first critical evidence that the oocyte epigenome senses maternal metabolic changes and show that methylation changes can persist at least during preimplantation development.

METFORMIN'S POTENTIAL AS A BREAST CANCER PREVENTATIVE AGENT BY ALTERING EPIGENETIC PATTERNS

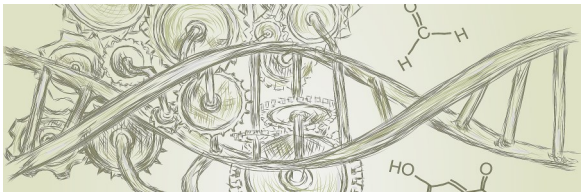
Poster presented by: Caitriona Tyndall, **Imperial College London**

Metformin, a drug used to treat type II diabetes mellitus (T2DM), has been shown to reduce breast cancer risk in long-term diabetic users by 2-fold. This study aims to investigate the novel hypothesis that metformin acts as a preventative agent by altering epigenetic patterns in non-cancerous breast epithelial cells.

Two non-cancerous breast epithelial cell lines, MCF12A and MCF10A, and primary breast epithelial cells from 4 individuals obtained from Breast Cancer Now Tissue Bank were investigated. Cell lines were treated for 3 days with 2.5mM and 5mM metformin in a range of glucose and acetate conditions. Primary cells were treated for 7 days with 1mM and 1.5mM metformin. RNA sequencing and qRT-PCR, Illumina MethylationEPIC array, crystal violet and western blot were used to assess the response of normal breast epithelial cells to metformin treatment.

We show that normal breast epithelial cells are sensitive to metformin (IC50 3-4mM) and that low glucose concentration (5mM) significantly impacts the sensitivity (IC50 1-2mM). Metformin significantly changes gene expression in MCF12A ($n=367$ genes, FDR<0.05) but less in MCF10A ($n=3$ genes, FDR<0.05), 7/8 selected genes validated. MethylationEPIC array suggests metformin induces subtle dose dependent differential DNA methylation changes. However, metformin treatment increases p-AMPK in normal breast epithelial cells and significantly decreases histone modification H3K27ac (0.5 fold, $p=0.035$), which is modulated by glucose and acetate levels but return to baseline levels after 24 hours without treatment.

In summary, this study shows a potential mechanism of long-term metformin action through altering of epigenetic patterns in non-cancerous breast epithelial cells.



ABSTRACTS

A SIMPLISTIC MODEL OF EFFECTS OF PATERNAL GLUCOCORTICOID RECEPTOR ACTIVATION ON THE GERMLINE AND OFFSPRING PHENOTYPE

Poster presented by: Katharina Gapp, **Gurdon Institute, University of Cambridge**

Single traumatic events that elicit an exaggerated stress response can lead to the development of post-traumatic stress disease and related neuropsychiatric conditions[1]. Evidence in humans shows that ancestral trauma can even confer a heightened risk for disease to their descendants[2,3].

Mouse studies including our own have accumulated data that suggest germline RNA as mediator of effects of chronic environmental exposures to the progeny[4]. The effects of an acute paternal stress exposure on the germline and their potential consequences on offspring remain unknown. Here we investigate the consequences of a single stress receptor activation on the sperm transcriptome and on the offspring molecular and metabolic phenotype. Using next-generation sequencing of mature sperm and single-embryo sequencing of 2 cell embryos we find that activation of stress pathways affects the RNA payload of postmeiotic maturing sperm collected 2 weeks later and early embryonic transcriptional trajectories. In vivo phenotyping of weight, size and glucose metabolism reveals sex specific effects on glucose clearance and insulin sensitivity in the offspring leaving body composition unaffected. We conclude that a single acute stress receptor activation can induce epigenetic germline inheritance and hence have persistant consequences on offspring metabolism. Germline effects are dependent on timing between paternal exposure and fertilisation.

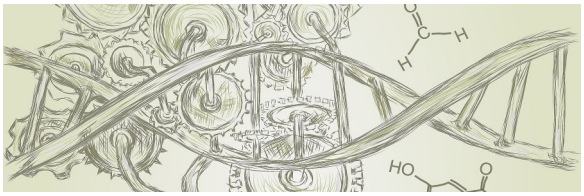
References. 1. Bowers et al., *Neuropsychopharmacology* **41,** 232–244 (2016). 2. Heijmans *et al., Proc Natl Acad Sci U S A* **105,** 17046–17049 (2008). 3. Pembrey *et al., Eur. J. Hum. Genet.* **14,** 159–166 (2006). 4. Gapp *et al.*, *Genes Brain Behavior* **17**, 1-12 (2018).

ACCURATE PROFILING OF HISTONE MODIFICATIONS UPON ENZYME-MEDIATED METHIONINE DEPLETION IN NORMAL AND COLORECTAL ADENOCARCINOMA CELLS

Poster presented by: Samanta Raboni, **Università di Parma**

Rationale. Cancer cells exhibit unique metabolic properties, including the elevated requirement for methionine. This feature has been exploited for the development of a tailored anticancer therapy based on methionine gamma-lyase (MGL), a methionine-degrading enzyme that causes cancer cells growth inhibition due to methionine starvation. Methionine is essential for the formation of S-adenosyl-L-methionine (SAM), required for histone and DNA methylation, which controls DNA readout, including gene expression, DNA repair and chromosome condensation. **Methods.** By applying mass spectrometry-based proteomics, the relative abundance of >200 histone acetylations and methylations were determined on histone proteins in fibroblasts (Hs27) vs colorectal adenocarcinoma (HT29) cells in the absence and presence of MGL. **Results.** MGL-treated cells showed a global decrease in histone H3 and H4 methylation, as expected. Specifically, all three methylation states of histone H3 on lysine 9 (H3K9me) significantly decreased in MGL treated HT29 cells, but not in non-malignant Hs27 cells. H3K9me is a fundamental silencing mark, mostly decorating highly repetitive DNA domains that are commonly kept sequestered in heterochromatin in all human cell types. Interestingly, this regulation suggests a more disordered chromatin, but a significant increase in the global acetylation levels, commonly benchmarking open chromatin, was not observed.

Conclusions. Our data suggest that selected histone methylation marks exhibit primary roles in reducing the fitness of cancer cells and hint at a potential as complementary target for cancer cells subjected to methionine starvation. Validation studies including susceptibility to epigenetics inhibitors and knock-down of selected histone methyltransferases (HMTs) are in progress.



ABSTRACTS

AGE-DEPENDENT ASYMMETRIC APPORTIONING OF MITOCHONDRIA DURING DIVISION DETERMINES CELL FATE

Poster presented by: Ana Amaral, **Karolinska Institute**

The maintenance of tissue renewal requires a balance between self-renewal and differentiation, which is achieved by asymmetric cell divisions. To ensure homeostasis throughout the lifespan, asymmetric divisions must be tightly regulated but it remains unknown which early decision mechanisms determine the fate of adult stem cells during this process. Metabolic characteristics of adult stem cells are distinct from their differentiated progeny, and cellular metabolism is emerging as a potential driver of cell fate conversions. Our work identifies inherited metabolism imposed by functionally distinct mitochondrial age-classes as a fate determinant in asymmetric division of epithelial stem-like cells. While chronologically old mitochondria support oxidative respiration, new organelles are immature and metabolically less active. Upon cell division, selectively segregated mitochondrial age-classes elicit a metabolic bias in progeny cells, with older mitochondria (imposing oxidative energy metabolism) inducing differentiation. Our results demonstrate that fate decisions are susceptible to intrinsic metabolic bias imposed by selectively inherited mitochondria. Ongoing work is addressing the epigenetic and transcriptomic signatures induced by the inherited metabolic phenotypes in order to describe the sequence of early molecular events determining cell fate upon asymmetric divisions in epithelial stem-like cells.

EPIGENETIC MODIFICATIONS INDUCED BY DIETARY DIVERSITY IN INDIA: A COMPARATIVE STUDY OF DIFFERENT STATES AND UNDERSTANDING THE IMPLICATIONS ON HEALTH

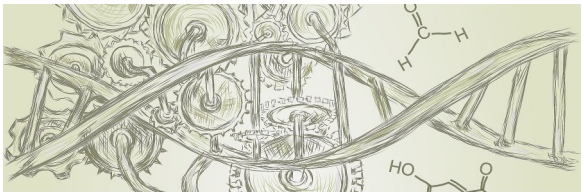
Poster presented by: Anwesha Lahiri, **IIT Bombay**

Rationale. Evidence from a growing body of research suggest that chronic diseases have been associated with epigenomic alterations that occur without changes in the DNA sequence itself. Dietary factors are likely to influence metabolic and epigenetic markers, but there are limited studies of such implications in developing countries.
Method. Systematic review of existing studies on diet induced epigenetic changes was used to model the implications of the present dietary factors (micronutrients and non-nutrient dietary components) on the disease incidence in different states in India. Dietary data as inputs from nationally representative surveys on food intake, and a state-wise comparative study was done to investigate the impact of diet on various metabolic pathways epigenetic modifications.
Results. Possible mechanisms involved in nutritional modulation of epigenetic changes, thereby leading to the prevalent diseases were established. Through this analysis, important nutritional deficiencies were identified, which are specific to the disease burden for the state, and which may help to explain the diet-epigenome-health relationship.
Conclusions. There is limited understanding about specific epigenetic signatures that are most prominent in response to dietary exposures. The interplay of diet and lifestyle factors in the aetiology of diseases, epigenetic markers and major cellular stressors implicate that further studies (both clinical cohort and animal model based) are necessary to reveal the underlying causal mechanisms in different populations. Such information will further aid in health care interventions that address ante and prenatal care, childhood, adolescent and adulthood health directives in a nation that is plagued with the dual burden of malnutrition.

ADVANCED MATERNAL AGE AND ASSISTED REPRODUCTIVE TECHNOLOGIES IMPACT MITOCHONDRIA AND GENOMIC IMPRINTING IN MOUSE PREIMPLANTATION EMBRYOS

Poster presented by: Audrey Kindsfather, **University of Pittsburgh School of Medicine**

Rationale. As the age of first-time mothers has risen over past decades, so has the use of assisted reproductive technologies (ARTs) by women over 35 years of age. ARTs including superovulation (SO) and embryo culture (EC) have been shown to alter imprinted DNA methylation in blastocysts.



ABSTRACTS

Since mitochondria provide metabolites necessary for DNA methylation, we hypothesized that ARTs and advanced maternal age, separately and together, perturb mitochondria and imprinted methylation in mouse blastocysts.
Methods. Female C57BL/6(CAST7) mice were split into four age (<2, 2-6, 6-10, and >10 months) and treatment groups (no ARTs; SO; EC; SO+EC). Spontaneously ovulating and superovulated females were mated with C57BL/6 males. Embryos were recovered at blastocyst- or 2-cell stages. The latter were cultured in Whitten's medium at 37ºC until the blastocyst stage. Blastocysts were stained with MitoTracker Green and Red to quantify total and active mitochondrial mass, respectively. Following imaging, *Snrpn, Kcnq1ot1,* and *H19* imprinted methylation were assessed with bisulfite mutagenesis and clonal sequencing.
Results. Treatment with any ART decreased imprinted methylation in blastocysts from both young and aged mothers. However, maternal age, with or without ARTs, had no effect on imprinted methylation. Both ARTs and/or advanced maternal age altered total and active mitochondrial mass. These mitochondrial variations did not correlate with loss of imprinted methylation.
Conclusions. Our results indicate that ARTs and advanced maternal age altered mitochondrial mass in blastocysts. However, only ARTs perturbed imprinted methylation. Further investigation will examine mitochondrial function to assess any linkage with perturbations in genomic imprinting.

REGULATION OF LINE-1 RETROTRANSPOSONS BY VITAMIN C IN EMBRYONIC STEM CELLS

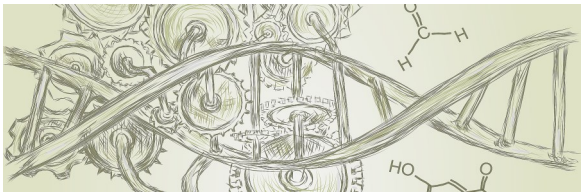
Poster presented by: Miguel R Branco, **Queen Mary University of London**

Nearly half of the mouse and human genomes are made up of retrotransposons, which have the potential to replicate and move around the genome. A relatively small subset of these elements retains the capacity for mobility, in particular LINE-1 elements. We have previously shown that TET enzymes target young LINE-1 elements in mouse embryonic stem cells (ESCs) to drive demethylation of their 5' UTR (i.e., promoter) region, a pattern that appears to be conserved in human ESCs. Given the dependence of TETs and other 2-oxoglutarate-dependent dioxygenases on metabolites for their activity, we asked whether these co-factors impact on LINE-1 expression. We found that vitamin C upregulates LINE-1 expression in naïve mouse ESCs. Unexpectedly, this effect is independent of DNA methylation and TET activity, and instead is mediated by H3K9me3 histone demethylases of the JmjC family. LINE-1 protein expression in also enhanced upon vitamin C treatment in human ESCs, but curiously this occurs through a distinct post-translational mechanism. Our work suggests that retrotransposon expression can be modulated by nutrients, and that the common use of vitamin C in iPS cell derivation may compromise genome integrity.

THE ROLE OF DIFFERENTIALLY METHYLATED CTCF BINDING SITES IN TOPOLOGY, GENE REGULATION AND METABOLISM

Poster presented by: William Watkinson, **Department of Genetics, University of Cambridge**

The Dlk1/Gtl2 domain is subject to genomic imprinting, expressing a different set of genes in a parental-origin-specific manner. Mis-regulation of the genes in this domain leads to embryonic and placental growth defects as well as postnatal perturbations in nutrient metabolism and energy homeostasis.
It has become apparent that the 3D organisation of the genome plays an important role in regulating transcription. Using the Dlk1/Gtl2 domain as a model, we performed 4Cseq to assess chromosome topology in the region. Tissue and parental-origin-specific chromatin conformation were identified that are demarcated by differentially methylated CTCF binding sites within the Gtl2 gene.
Using CRISPR/Cas9 technology we have deleted two CTCF sites within Gtl2 both separately and together in mice. Transcriptional analysis of these mice suggest subtle changes in transcriptional levels of genes in the region. Preliminary phenotypic results show the mice become obese as adults. Together these results indicate a role for chromatin topology in the subtle regulation of metabolic genes leading to possible metabolic implications in later life.



ABSTRACTS

DECIPHERING A METABOLISM-SENSITIVE READOUT OF HISTONE CROTONYLATION

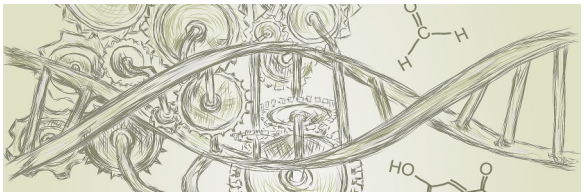
Poster presented by: Elena Stoyanova, **Babraham Institute**

Rationale. Histone posttranslational modifications (HPTMs) are major regulators of chromatin dynamics and gene expression. Knowledge about the molecular machinery involved in interpreting a HPTM is crucial for understanding its functional significance. Histone crotonylation is a conserved PTM, structurally similar to the well-studied acetylation, which characterises active chromatin and stimulates gene expression. Our lab explores the role of this modification in intestinal biology, as we found that it is abundant in the intestinal epithelium and its levels are dependent on the presence of the microbiota and microbiota-derived metabolites, therefore, making it a candidate that links diet, the gut microbiome and cellular metabolism to gene regulation (1). Pivotal for our understanding of the role of histone crotonylation in this metabolism-epigenetics interplay is the identification of 'reader' molecules, which selectively recognise it and are able to direct specific expression programs. Thus far, an extensive screen of crotonyl-binding factors is missing, and the few known readers were identified using target-based approaches.
Methods. I have adopted a combination of two unbiased techniques exploring protein-protein interactions - proteome microarrays and stable isotope labeling with amino acids in cells in culture (SILAC) pull-down experiments which compile a robust and unprecedented way to identify proteins capable of binding crotonyllysine over acetyllsyine discriminatively.
Results. Using proteome microarrays and SILAC pull-downs, I have identified candidate chromatin factors as putative selective crotonylation readers. Validation of some of these interactions are under way.
Conclusions. Further characterisation of these readers and the chromatin events they mediate will help unravel the role of histone crotonylation in intestinal biology.
References. 1. Fellows, R. et al., 2018. Microbiota derived short chain fatty acids promote histone crotonylation in the colon through histone deacetylases. Nature Communications, 9(1).

AKT MODULATES EZH2 ACTIVITY AND H3K27ME3 LEVELS IN MYOTUBES OF OBESE INSULIN-RESISTANT WOMEN

Poster presented by: Catherine Bisbal & Karen Lambert, **INSERM**

Rationale. Diet can alter epigenetic profile in obese subjects inducing a significant change in skeletal muscle genes expression favouring modifications of its physiology and metabolism and leading to comorbidities as insulin-resistance (IR), the central feature of type 2 diabetes. These genes expression modifications can be transferred across generations or through cell division. In fact, muscle stem cells retain molecular informations and metabolic characteristics of the donor even after isolation outside of their environmental niches. We have previously shown that skeletal-muscle (SM) IR is associated with increased basal Akt activation in myotubes of grade I obese women. Since Akt can inactivate EZH2 by phosphorylating it at serine 21 we assessed P_{ser21}-EZH2 and H3K27me3 levels in myotubes of grade I obese insulin-sensitive (OIS) or insulin-resistant (OIR) women.
Methods. Muscle stem cells were purified from *vastus lateralis* biopsies of 11 OIS and 9 OIR women. After differentiation, myotubes were treated or not with palmitate to mimic free fatty acid overload observed *in vivo*. Protein expressions were analysed by western blot.
Results. P_{ser21}-EZH2 was increased in OIR myotubes even in non-treated cells. However H3K27me3 levels were not modified between OIS and OIR myotubes. Surprisingly, when Akt was inhibited in myotubes by LY294002 pre-treatment, H3K27me3 was abolished as P_{ser21} of EZH2.
Conclusions. Our preliminary results indicated that in myotubes of OIR subjects, Akt regulates P_{ser21} of EZH2. However, Akt controls H3K27me3 levels *via* inhibition of demethylases.



ABSTRACTS

MLL3 MUTATIONS OVERCOME GLYCOLYTIC STRESS IN BREAST CANCER BY UTILISING THE PENTOSE PHOSPHATE PATHWAY

Poster presented by: Philip Bland, **ICR**

Rationale. Aggressive breast cancers are characterised by regions of hypoxia and limited nutrient availability, which can be modelled through the use of cancer cell line spheroids. Glycolytic cancer cells utilise the pentose phosphate pathway (PPP) to meet anabolic demand and relieve oxidative stress. Here we sought to use this spheroid model of aggressive breast cancer to identify novel tumour suppressor genes in different subtypes of breast cancer.
Methods. We performed an unbiased functional genomics siRNA spheroid screen of the 200 most frequently mutated genes in unselected breast cancers. This screen identified that loss of the methyltransferase gene *MLL3* is a putative driver of 3D growth across the MCF10A cell line progression series. Spheroid cultures of WT and MLL3-HAP1 isogenic and breast cancer cell lines were used to evaluate growth over time and assess sensitivity to the BET domain inhibitor iBET672. Mass spectrometry of total protein isolates from spheroid cultures showed an induction in PPP associated proteins in MLL3- cells.
Results. We found that MLL3 loss in HAP1 and cancer cell lines promotes a significant growth advantage specifically in 3D, which involves Transketolase-Like Protein 1 (TKTL1) induction and the PPP. MLL3 loss leads to iBET762 sensitivity in multiple models, reducing cell viability and activating apoptosis. Furthermore, inhibition of TKTL1 increased sensitivity to iBET762.
Conclusions. MLL3 loss is advantageous under spheroid conditions and induces the PPP to aid glycolytic cancer cells. The use of BET inhibitors may have therapeutic benefits in breast cancer patients with *MLL3* mutations.

SIGNALLING AND METABOLIC PATHWAYS MODULATING STABILITY OF UHRF1 IN MOUSE EMBRYONIC STEM CELLS

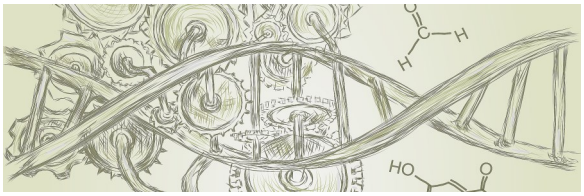
Poster presented by: Gabriella Ficz, **Barts Cancer Institute**

Genomic hypomethylation (loss of 5-methylcytosine) is a hallmark of naïve stem cells, primordial germ cells and cancer cells. 5-methylcytosine is normally propagated during cycles of cell division, and this mechanism relies on a functional DNA methyltransferase 1 (DNMT1) protein and its enzymatic partner Ubiquitin Like With PHD And Ring Finger Domains 1 (UHRF1). Substantial loss of genomic DNA methylation has been observed during reprogramming of “formative” (or Serum/LIF grown) mouse embryonic stem cells (mESCs) to naïve state trough inhibition of Erk and Gsk3b pathways. The mechanism of this global loss of 5mC is mainly achieved by the degradation of UHRF1 at the protein level, in addition to some locus specific active demethylation by TET proteins. We took advantage of this model system to dissect upstream signalling pathways leading to degradation of UHRF1. We carried out an shRNA kinase library screen in a mESC transgenic line expressing a UHRF1-GFP fusion protein, which we use a proxy for the endogenous protein regulation, and found components of the Pi3K/mTOR and PKA mediated pathways to have a role in UHRF1 stability. Furthermore, some of the pathways identified and subsequently validated are involved in metabolic regulation, particularly of glucose metabolism, and several candidates are mediating oxidative phosphorylation. This study will help connecting signalling pathways to genomic DNA methylation and identifying factors responsible for the loss of DNA methylation in cancer.

WEIGHT LOSS IN PEOPLE WITH IMPAIRED GLUCOSE REGULATION AFFECTS ADIPOSE TISSUE CAV1 EXPRESSION AND DNA METHYLATION

Poster presented by: Helene A Fachim, **Salford Royal Hospital**

Rationale. There is growing evidence of external factors modulating epigenetic modifications and their contribution to the development of obesity and type 2 diabetes (T2DM). Impaired glucose regulation (IGR) is a major risk factor



for T2DM development. We investigated whether a lifestyle intervention could influence gene expression and associated epigenetic regulation.

Methods. To improve the understanding of biological markers of progression from IGR to T2DM, participants with IGR (n=20) were recruited and underwent anthropometric measurements/fasting blood tests and adipose tissue biopsy pre- and post-lifestyle (6 months) intervention. RNA and DNA were extracted from adipose tissue and the relative expression of genes known to be involved in T2DM were determined using a LightCycler 480 (Roche).

Results. We found a significant reduction in the expression of caveolin-1 (CAV1) following lifestyle intervention ($t = 2.26$, $p = 0.037$). Subsequently the DNA was bisulfite converted and 12 CpG sites of CAV1 were pyrosequenced based on known transcription factor binding sites within the promoter region. Three sites showed evidence of significantly greater methylation after the intervention when compared to baseline (CpG3: $p = 0.017$, 54% higher; CpG9: $p = 0.05$, 22% higher; CpG11: $p = 0.027$, 42% higher).

Conclusions. CAV1 is an essential constituent of adipocyte caveolae and binds the β-subunit of the insulin receptor. Our findings suggest a role for CAV1 in modulating adipocyte function as a consequence of lost weight and exercise. These results may provide insights into new therapeutic targets for diabetes prevention.

LOSS OF THE IMPRINTED GENE NEURONATIN AFFECTS BOTH FOOD INTAKE AND ENERGY EXPENDITURE

Poster presented by: Irene Cimino, **Institute of Metabolic Science, University of Cambridge**

Neuronatin (Nnat) is an imprinted gene, expressed only from the paternal allele, and found within the brain, the pituitary gland, and a number of peripheral tissues such as adipose tissue and pancreas. A full understanding of its specific function remains elusive.

We have previously shown that Nnat expression within the hypothalamic paraventricular nucleus is leptin-regulated and that mice carrying a paternally inherited null Nnat allele (Nnat $^{+/-p}$) display an unusual body weight distribution, with some phenotypically identical to wild type (WT) mice while others develop obesity. We have undertaken detailed metabolic phenotyping of Nnat $^{+/-p}$ mice to further understand the role of Nnat in energy homeostasis.

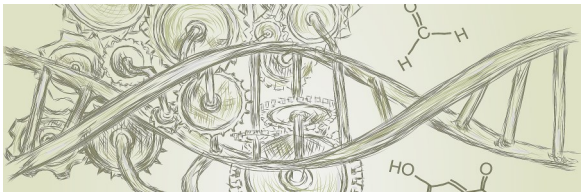
In keeping with previous findings, on normal chow 15-20% of Nnat $^{+/-p}$ mice develop obesity by 12 weeks. Nnat $^{+/-p}$ mice are hyperphagic compared to WT, but intriguingly a proportion of Nnat-deficient mice that are weigh-identical to WT also show an increase in energy expenditure. Unsupervised multivariate analysis of energy phenotype data excluding body weight and genotype reveals 3 distinct clusters; WT, “lean Nnat $^{+/-p}$” and “obese Nnat $^{+/-p}$”. When fed a 45% HFD, the between-genotype difference in body weight distribution persists with a proportion of Nnat $^{+/-p}$ also remaining leaner than WT.

These data indicate Nnat may play a crucial role in neuronal populations that control food intake and energy expenditure. Ongoing transcriptomic analysis of laser captured material from Nnat $^{+/-p}$ mice and functional analysis of hypothalamic Nnat +ve cells aims to further characterize the site of these actions.

IMPACT OF MATERNAL METABOLISM ON EPIGENETIC MECHANISMS INVOLVED IN FOETAL PROGRAMMING OF ENDOTHELIAL DYSFUNCTION AND ATHERSCLEROSIS

Poster presented by: Julia Carrasco Zanini Sánchez, **University of Cambridge**

Disruptions in the one-carbon metabolism, either through nutritional deficiencies or genetic variants, can result in altered DNA methylation, which has been suggested as a mechanism underlying developmental programming. In this study we evaluated the impact of altered one-carbon metabolism during pregnancy on mechanisms involved in



programming of endothelial dysfunction and atherosclerosis.

Pregnant women underwent an oral glucose tolerance test (OGTT) at 24 to 28 weeks of gestation and one-carbon metabolism biomarkers and SNPs were evaluated. After delivery, human umbilical vein endothelial cells (HUVECs) were obtained, to analyse DNA methylation levels by Pyrosequencing, as well as expression of candidate genes.

A weighted genetic risk score (GRS) was generated from one-carbon metabolism SNPs; which in interaction with low maternal folic acid supplementation doses, was significantly associated to elevated maternal serum homocysteine and 2-hour post-load glucose, as well as with HUVEC DNA hypomethylation at a regulatory region -2007 to -1731 bp upstream TNFα transcription start site. This hypomethylation was inversely correlated with TNFα expression. 2-hour post-load glucose was identified as the mediator of the association between the GRS and DNA methylation at the TNFα regulatory region. Finally, in HUVECs exposed to these adverse conditions, ICAM1 adhesion molecule was overexpressed upon endothelial activation. Neither TNFα DNA hypomethylation nor ICAM1 overexpression could be reversed by folate supplementation in HUVEC cultures.

Our findings suggest altered maternal one-carbon metabolism can impose an adverse intrauterine environment which results in epigenetic and functional changes in HUVECs, associated to programming of endothelial dysfunction and atherosclerosis.

LINK OF PLASMA VITAMIN C AND EPIGENETIC DNA MODIFICATIONS IN PATIENTS WITH ACUTE LEUKEMIA AND MYELODYSPLATIC SYNDROME

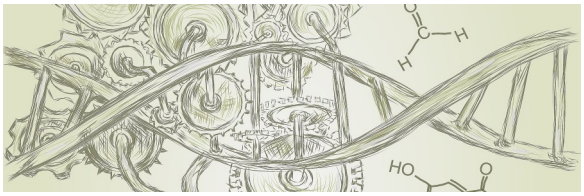
Poster presented by: Maciej Gawroński, **Nicolaus Copernicus University in Torun - Ludwik Rydygier Collegium Medicum in Bydgoszcz**

Rationale. Epigenetic mechanisms such as DNA methylation and active DNA demethylation play a crucial role in gene expression regulation, cell proliferation and differentiation. Their impairment and subsequent changes in epigenetic make-up are considered as one of the early stages of carcinogenesis. Mutations in genes involved in active DNA demethylation, especially the loss-of-function mutations in TET2, are frequently found in hematological malignancies. Moreover TET1 has contrasting roles in myeloid and lymphoid transformation.

Methods. We have analyzed levels of epigenetic DNA modifications arising from TETs activity, namely 5-hydroxymethylcytosine – derivative of 5-methylcytosine, 5-formylcytosine and 5-carboxycytosine, by using stable isotope dilution 2D-UPLC-MS/MS method in DNA isolated form peripheral blood nuclear cells of patients with acute leukemia and myelodysplastic syndrome. We have also determined patients plasma concentration of vitamin C by UPLC-UV method. In various studies vitamin C enhanced TET proteins activity, therefore in order to determine its effect on leukemic cells epigenome, we have cultured K562 cells with various concentrations of ascorbic acid.

Results. Our study showed significantly lower levels of plasma vitamin C concentrations, as well as lower levels of 5-hydroxymethylcytosine and other 5-methylcytosine derivatives in the DNA of afflicted individuals when compared with control group. In contrast, in K562 cells supplemented with ascorbate we have observed dose-dependent increase of 5-hydroxymethylcysosine and 5-formylcytosine levels in cellular DNA.

Conclusions. Our results suggest that aberrant patterns of epigenetic modifications observed in patients with hematological malignancies may not only be result of impaired TETs activity, but also vitamin C deficiencies.



L-ASCORBATE RISES 5-HYDROXYMETHYLURACIL LEVEL IN DROSOPHILA S2 CELL LINE

Poster presented by: Marta Starczak, **Collegium Medicum UMK**

Rationale. The presence of 5-methylcytosine (5-mCyt) in the *Drosophila* genome has been subject to long-standing debates. It was demonstrated that cytosine DNA methylation could be detected and quantified in all developmental stages of the fruit fly. Another epigenetic modification is 5-hydroxymethylcytosine (5-hmCyt), product of 5-methylcytosine oxidation by TET enzymes. *Drosophila melanogaster* also has a single ortholog of the TET family proteins (dTet) and 5-hmCyt was detected in adult flies. Some evidence from experimental studies suggests that TET may be also involved in synthesis of 5-hydroxymethyluracil (5-hmUra), a compound with epigenetic function. Furthermore vitamin C (ascorbic acid) was shown to enhance the catalytic activity of TET proteins *in vitro* and *in vivo*.

Methods. Drosophila S2 cells were cultured in control medium and in medium containing 1mM ascorbic acid (AA) for 24h, 48h, 72h, 120h and 192h. Cellular DNA was isolated by modified phenol method. Obtained genetic material was enzymatically hydrolysed to deoxynucleosides, spiked with stable-isotope labeled internal standards and analyzed using 2D-UPLC-MS/MS method. Intracellular and medium concentrations of ascorbate was determined by UPLC-UV method.

Results. In S2 cells we were able to detect uracil, 5-hmUra and 8-oxoGua. Incubation of S2 cells with ascorbic acid caused time-dependent increase of 5-hmdU, dU and 8-oxodG levels. We also analysed changes in ascorbate concentrations in treated medium and cells. Concentration of ascorbic acid in control cells was below detection limit of our method.

Conclusions. Our findings suggest that some portion of 5-hmUra observed in *Drosophila* may be a product of enzymatic activity of dTET proteins.

EFFECT OF BARIATRIC SURGERY ON THE TRANSCRIPTOME AND EPIGENOME OF MYELOID CELLS

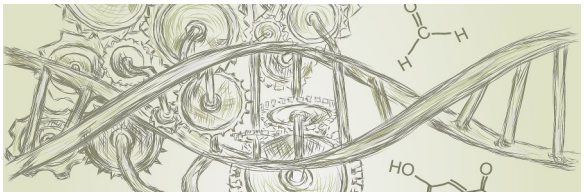
Poster presented by: Mattia Frontini, **Department of Haematology, University of Cambridge**

Rationale. The incidence of obesity and its comorbidities is placing an ever increasing financial burden on healthcare providers. The most recent proposals call for lowering the BMI limit for eligibility for bariatric surgery, thus increasing the number of individuals undergoing the procedure. Here we examine the effect of sudden weight loss on some of the key cells in cardiovascular disease and thrombosis.

Methods. Monocytes, macrophages, neutrophils and platelets were isolated from patients before and after bariatric surgery and subjected to total RNA-sequencing.

Results. Bariatric surgery modifies the transcriptome of all the tested cells but to different extent, with 139 genes differentially expressed in macrophages, 208 in monocytes, 1048 in neutrophils and 1249 in platelets. Across all cell types we observed changes in expression levels of ribosomal subunits and genes involved in ribosome biogenesis. In macrophages and neutrophils we observed the downregulation of genes involved in phagocytosis. In neutrophils and platelets we also observed the downregulation of genes involved in their activation.

Conclusions. Bariatric surgery causes profound changes in the whole organism, here we report the first analysis of the transcriptional changes occurring after surgery in the primary blood cell types involved in cardiovascular disease and thrombus formation. The downregulation of genes involved in neutrophils and platelets activation suggests that at least part of the reduction of the risk of cardiovascular disease could be ascribed to these changes. It remains to be determined if the same type of changes are obtained with other types of interventions.



ABSTRACTS

INVESTIGATING THE EFFECT OF MATERNAL FOLIC ACID INTAKE ON TRANSCRIPTOME AND EPIGENOME OF NEURAL TUBE DEFECT SUSCEPTIBLE MICE

Poster presented by: Miho Ishida, **UCL Great Ormond Street Hospital Institute of Child Health**

Rationale. Neural tube defects (NTDs) are severe birth defects affecting approximately 1/1000 pregnancies. It is well established that up to 70% of potential NTD cases can be prevented by the maternal periconceptional intake of folic acid supplements. Although the prevalence of NTDs has significantly reduced in many countries fortifying food products with folic acid, the underlying molecular mechanisms involved still remain elusive. An important question is the potential effect of folate supplementation for non-NTD risk individuals. One well-described function of folate metabolism is to provide one-carbon (methyl) groups to be used in DNA methylation, possibly contributing to the regulation of key developmental gene expression. The current study investigated this hypothesis.

Methods. *Pax3* mutant mice, which have a high prevalence of NTD, and their WT littermates were maintained on three different diets designed to deliver deficient, normal and excess folic acid. The effects of each diet on the transcriptome and DNA methylation in the cranial region of resulting mouse embryos at E9.5 were investigated using RNA-seq (n=36) and whole genome bisulfite sequencing (n=24), respectively.

Results. A highly significant effect on differential expression was observed between low and high folate diets in *Pax3* mutant mouse embryos. No effects were detectable in wild type embryos. Correspondence of expression with differential DNA methylation is being analysed.

Conclusions. This study showed that maternal folic acid supplementation had a highly significant effect but only on the genetic background susceptible to NTDs. This finding is potentially very reassuring when embarking on a population-wide food fortification strategy.

REDUCTION OF *WARS2* IS A POTENTIAL MECHANISM FOR REGULATING FAT DISTRIBUTION IN THE WAIST-HIP RATIO -ASSOCIATED *TBX15-WARS2* LOCUS

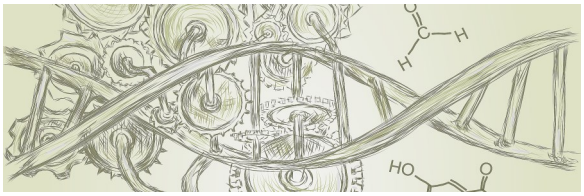
Poster presented by: Milan Mušo, **MRC Harwell Institute**

Rationale. Waist-hip ratio (WHR) is associated with higher risk of diabetes and cardiometabolic disease. Genome-wide association studies in WHR adjusted for BMI have identified 346 loci including *TBX15-WARS2*.[1,2] Expression QTLs for *WARS2* exist in multiple tissues (GTEx) and for *TBX15* in male subcutaneous fat.[3,4] A hypomorphic mouse allele of *Wars2 (Wars2$^{V117L/V117L}$)*, previously characterised by us, showed progressive tissue-specific pathologies including reduced adiposity and browning of subcutaneous inguinal white adipose tissue.[5]

Methods. We used epi-Phylogenetic Module Complexity Analysis (epi-PMCA) and a posterior probability analysis (PPA) to identify candidate causal SNPs within the *WARS2-TBX15* locus and are testing these SNPs in a human white adipose cell line (hWAT). We hypothesised that rs2645294 (in the 3'UTR of *WARS2*), the highest scoring SNP in the PPA, reduces *WARS2* levels through post-transcriptional regulation. To test this, we used a combination of luciferase reporter assays, allele-specific qPCR performed on hWAT nascent RNA or RNA from transcription-inhibited hWAT cells. Parallel studies investigated the body composition and fat depot weights in *Wars2$^{+/-}$* mice.

Conclusions. We report allele-specific expression (ASE) of *WARS2* in rs2645294-heterozygous hWAT cells. Through multiple lines of evidence we show that rs2645294 does not affect RNA stability. The ASE in the hWAT cells is thus likely to be mediated through transcriptional regulation of *WARS2*. No phenotype was observed in the *Wars2$^{+/-}$* mice. Future studies will focus on the more severe *Wars2$^{V117L/V117L}$* allele.

1. Shungin, Nature, 2015. **2**. Pulit, Hum Mol Gen, 2018 **3.** http://gtexportal.org/ **4.** Civelek, AJHG, 2017. **5.** Agnew, Cell Reports, 2018 (under review).



ABSTRACTS

BMI-DEPENDENT LIPID DYSREGULATION IN MULTIPLE SCLEROSIS PATIENTS INDUCES EPIGENETIC CHANGES IN MONOCYTES AND RESULTS IN BRAIN VOLUME REDUCTION

Poster presented by: Patrizia Casaccia, **CUNY Advanced Science Research Center**

The impact of Body-Mass-Index on clinical disability is relevant for a wide number of neurological disorders, but the underlying pathogenic mechanisms remain only partially understood. We studied this question in patients with a diagnosis of Multiple Sclerosis (MS). Immunoprofling revealed higher monocytic counts and plasma lipidomic analysis, identified differentially abundant ceramide species in the overweight/obese MS population, compared to normal BMI. These changes were specific to MS, as they were not detected in healthy controls differing by BMI. DNA methylation analysis of human monocytes sorted from the same patients, using the Illumina 450K chip, revealed increased methylation of negative regulators of proliferation in MS subjects with high BMI, who also showed decreased brain volume by MRI and worsening of disease activity at the two year follow up. Exposure of cultured monocytes to the ceramide combinations detected in high BMI MS patients, recapitulated the increased DNA methylation, decreased transcription of negative regulators of proliferation and increased cell number. Finally, using Experimental Autoimmune Encephalitis as MS model, we detected increased monocytic infiltration and increased CNS damage and neurological deficits in mice fed a high fat diet compared to those on a low fat diet. Overall, this study identifies ceramide-induced changes in DNA methylation as a novel mechanism driving monocytic proliferation, and worse prognosis for overweight/obese MS patients.

A LINK BETWEEN THE MICROBIOTA AND CHROMATIN THROUGH HISTONE ACYLATIONS

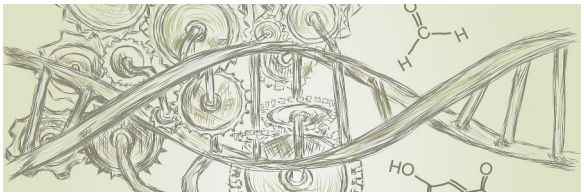
Poster presented by: Rachel Fellows, **Babraham Institute**

Rationale. The intestinal microbiota metabolises dietary fibre to produce short chain fatty acids (SCFA) such as butyrate. SCFAs are chemically similar to histone acylations, including histone acetylation and crotonylation which are abundant in the intestine and are associated with active chromatin. Histone modifications are a critical mechanism to regulate gene expression by specifically recruiting transcription factors and chromatin remodellers. We proposed to alter SCFA concentrations in the gut to investigate the effect on histone acylations.

Methods. We reduced the bacterial load and SCFA concentrations in mice by administering antibiotics, followed by analysis with western blot, ChIP-sequencing and RNA-sequencing. **Results.** We observe a reduction in histone crotonylation and acetylation in the colon on antibiotic treatment. In support of the important role of SCFAs, when colon organoids were treated with butyrate, the abundance of histone crotonylation increased. We also find that when fibre was removed from the diet, histone acylation was reduced indicating that SCFAs are responsible rather than the antibiotic treatment itself.

Interestingly, and in contrast to the colon, specific histone acetylation marks increased in the liver on antibiotic treatment. These findings identify key histone residues that link chromatin of the liver to the presence of microbiota in the gut. We profile histone acetylation in the liver and colon to identify residues linked to changes in gene expression on antibiotics treatment.

Conclusions. These intriguing findings suggest that histone acylations could act as nutrient sensors to couple changes in diet or microbiota composition to that of gene expression and cellular function.



INTRACELLULAR LIPID ACCUMULATION IN HEPATOCYTES LEADS TO DYSREGULATION OF THE TCA CYCLE AND A GLOBAL INCREASE IN 5-HYDROXYMETHYLCYTOSINE

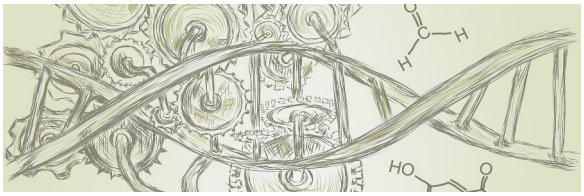
Poster presented by: Matthew Sinton, **University of Edinburgh**

Rationale. Non-alcoholic fatty liver disease (NAFLD) is the most prevalent form of hepatic disease globally. Studies show a close relationship between NAFLD and changes in TCA cycle activity. The TCA metabolites succinate, fumarate, and alpha-ketoglutarate are allosteric regulators of the ten-eleven translocase (TET) enzymes. Our objective was to determine whether steatosis-associated TCA cycle dysregulation is associated with global changes in DNA hydroxymethylation (5hmC) levels, using a stem cell-based *in vitro* model of 'NAFLD in a dish'.
Methods. Human ES cells were differentiated to hepatocyte-like cells (HLCs), before treatment with lactate, pyruvate, and octanoate (LPO). Intracellular lipid accumulation was measured using High Content Analysis microscopy. Global changes in gene transcription were measured by mRNA-seq analysis. HLCs were treated with LPO containing ^{13}C-lactate. Metabolite abundance was measured using combined GC-MS and NMR spectroscopy. Global 5hmC was measured using ultra-performance liquid chromatography.
Results. LPO treatment of HLCs resulted in intracellular lipid accumulation and altered expression of genes associated with glycolysis (increased G6PD $p = 0.017$, and HK1 $p<0.0001$), gluconeogenesis (decreased PCK2 $p = 0.0049$), and lipid droplet formation (increased PLIN2 $p<0.0001$). Metabolomic analysis showed an increased abundance of the TCA cycle metabolites fumarate and malate (69.2%, and 49.1%, respectively). A 42.6% decrease in the ratio of NAD^+:NADH was observed indicating increased redox stress. We observed a concurrent global increase in 5hmC (29%), following LPO treatment.
Conclusions. These data demonstrate that LPO exposure alters glycolysis and gluconeogenesis pathways, and associates with altered TCA cycle activity in HLCs. Increased 5hmC suggests that LPO treatment also impacts on TET activity.

EPIGENETIC REMODELLING IN NATIVE IMMUNE CELLS IN INDIVIDUALS AT HIGH RISK OF CARDIOVASCULAR DISEASE

Poster presented by: Denis Seyres, **Department of Haematology, University of Cambridge**

Rationale. Patients suffering of metabolic syndrome due to obesity or rare monogenic syndromes (lipodystrophy) present specific plasma metabolic signatures and are characterized by a state of chronic inflammation altering gene expression signatures in native immune cells (monocytes, neutrophils, macrophages). In this investigation, we determined the effects of metabolites levels on the regulatory landscape priming of these cells.
Methods. We collected plasma metabolites and clinical biochemistry measurements, histone H3K27ac and gene expression data from lipodystrophy patients, obese individuals and a cohort of blood donors. We used a differential co-abundance network approach to identify patients-specific metabolites. We identified differentially acetylated (DAc) regions and differentially expressed (DE) genes.
Results. We investigated epigenetic priming by comparing the patient cohorts with control. As example, in neutrophils, we observed 1006 DAc regions and 18 DE genes by comparing obese and control individuals. Functional annotation showed enrichment for molecular function related to neutrophils mediated immunity and activation/degranulation. We also found two clusters of metabolites modules containing respectively 115 and 143 species significantly associated with patient cohorts and adiponectin level. Co-enrichment pathway analysis identified a combined enrichment for KEGG pathways essential for innate immune functions.
Conclusions. Our multi-omics approach gave additional insights into the epigenetic changes in innate immune cells caused by chronic low-grade inflammation. In particular, we highlighted the relationship between patients' metabolites and their epigenetic landscape. We observed a profound remodeling of regulatory regions in these cells even in the absence of acute stimuli, akin of the immune training observed in exposure to microbial components.



ABSTRACTS

MITOCHONDRIAL-NUCLEAR COMMUNICATION IN AGEING MESENCHYMAL STEM CELLS

Poster presented by: Peter Tessarz, **Max Planck Institute for Biology of Ageing**

Ageing is accompanied by a progressive loss of cellular and organismal function. On a physiological level, alterations to mitochondrial function and metabolism have been observed in many systems and model organisms. Here, we wanted to investigate mechanistically if and how metabolic changes would impact on chromatin architecture during the ageing process. We used mesenchymal stem cells isolated from the endosteum of bones. They reside in niches that are characterized by low oxygen tension of around 2%, which drives anaerobic glycolysis. We demonstrate that ageing is accompanied by a metabolic switch from glycolysis to beta-oxidation, concomitant with a decrease in chromatin accessibility, as well as proliferation and differentiation capacity. Interestingly, we made similar observations upon shifting these cells to higher oxygen tension. We made use of these phenotypic similarities and asked if there are similar underlying molecular changes, employing the shift to higher oxygen levels as a biochemical tool. This allowed us to dissect the connection between metabolic shifts and chromatin accessibility. In conclusion, we can demonstrate that the rewiring of acetyl-CoA shuttling is important for maintaining open chromatin and differentiation capacity in these cells. In the conference, we will discuss our findings in the context of mitochondrial-nuclear communication.

SECONDHAND SMOKE CAUSES LIVER STEATOSIS THROUGH DEREGULATION OF GENES INVOLVED IN HEPATIC LIPID METABOLISM

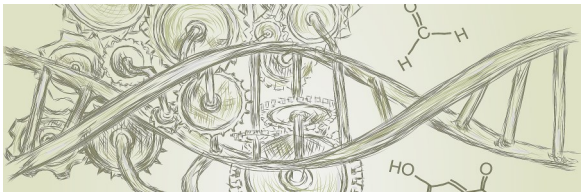
Poster presented by: Stella Tommasi, **University of Southern California**

Rationale. Exposure to secondhand smoke (SHS) has been associated with liver steatosis and non-alcoholic fatty liver disease (NAFLD); however, the role of SHS in the pathogenesis of these diseases remains unknown.

Methods. We sub-chronically exposed standard-diet-fed mice to SHS generated by a microprocessor-controlled smoking machine. Subsequently, we performed histological and molecular analyses on the liver of SHS-exposed mice, immediately after treatment and following one-month recovery in clean air.

Results. Histological analysis revealed significant hepatic fat accumulation in SHS-exposed mice relative to controls, which intensified after one-month-recovery of the animals in clean air. Whole transcriptome microarray analysis showed a unique and persistent transcriptomic response in the liver of SHS-exposed mice, with several hundred aberrant transcripts being detectable both pre- and post-recovery. The lasting transcriptional changes observed in SHS-exposed mice predominantly affect genes and functional networks involved in lipid metabolism and steatosis. Of significance is the SHS-induced upregulation of the regulator of G-protein signaling 16 (*Rgs16*) and lipin 1 (*Lpin1*), two steatogenic genes known to modulate fatty acid oxidation and synthesis. Both *Rgs16* and *Lpin1* are transcriptionally activated by the tumor suppressor *TP53* gene, a preferential target of tobacco smoke carcinogens. Thus, upregulation of the *Rgs16* and *Lpin1* consequent to SHS exposure may provide novel insights into the interplay of carcinogenic assault, p53-dependent response, and metabolic liver disease.

Conclusions. Our findings have significant public health implications as they underscore how environmental carcinogens, such as SHS, in addition to cancer-causing effects, may contribute to metabolic liver disease.



ABSTRACTS

EFFECT OF MATERNAL OBESITY AND PRECONCEPTIONAL WEIGHT LOSS ON FOETO-PLACENTAL GROWTH AND OFFSPRING HEALTH IN MICE: EXPRESSION OF EPIGENETIC MODIFIERS AT THE INTERFACE WITH MATERNAL METABOLISM

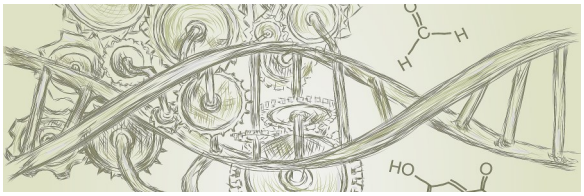
Poster presented by: Anne Gabory, **INRA**

Rationale. According to the 'developmental origins of health and disease' concept, maternal obesity predisposes the offspring to non-communicable diseases. Epigenetic patterns could be affected by maternal weight changes, in turn modifying the expression of key developmental genes with long-lasting consequences. A preconceptional weight loss is widely recommended to obese women. However, its long-term outcomes on the offspring have been poorly assessed.
Methods. We recorded E18.5 mouse development and measured the mRNA expression of epigenetic modifiers and metabolic genes in foetal liver and placenta. Offspring born to 'control', 'obese' or 'weight-loss after diet-induced obesity' mothers were put on a control or high-fat diet, and we tracked their metabolic parameters and olfactory behaviour.
Results. Foetuses from obese dams showed growth restriction and altered mRNA expression of candidate epigenetic modifiers, particularly the histone acetylation pathway. Preconceptional weight loss normalised foetal growth, but did not normalise the mRNA expression of all differentially expressed epigenetic genes. After birth, the offspring's own diet explained most of the variability in metabolic and olfactory phenotypes, but maternal obesity had a sex-specific conditioning effect. Specifically, males born to obese dams were more susceptible to diet-induced obesity. In addition, maternal preconceptional weight loss had positive effects on offspring metabolism, but showed a programming effect on peripheral olfactory sensitivity.
Conclusions. Obesity-induced transcriptional changes may modify the offspring epigenome, leading to growth restriction and an increased sensitivity to diet-induced obesity in adulthood. These results confirm the benefits of maternal preconceptional weight loss but also highlight some possible adverse outcomes.

MITOCHONDRIA, METABOLISM AND CELL FATE: A MITOCHONDRIAL ISOCITRATE DEHYDROGENASE PREVENTS DIRECT REPROGRAMMING OF GERM CELLS TO NEURONS IN *C. ELEGANS*

Poster presented by: Nida ul Fatima, **BIMSB-Max Delbruck Centre for Molecular Medicine**

Direct reprogramming makes use of transcription factors (TFs) that induce the identity of specific cell types. These TFs often are restricted in most cell types by inhibitory mechanisms.In order to identify these barriers in *C. elegans* we are using the zinc-finger TF CHE-1 that is required to induce the glutamatergic ASE neuron fate. Upon ectopic expression of CHE-1 and removal of barrier genes by RNAi,induction of the ASE neuronal fate marker can be seen in a variety of cell types.We identified a candidate barrier gene for reprogramming germ cells into neurons, the NAD+dependent mitochondrial isocitrate dehydrogenase *idha-1*.Upon RNAi knockdown of *idha-1* and ectopic expression of CHE-1,cells in the germline acquire neuron-like morphology and express neuronal fate markers.
It has been shown that mitochondrial dynamics change during differentiation[1].This suggests that disturbing mitochondrial function may feed back to chromatin thus altering gene expression and allowing reprogramming. Interestingly,*idha-1* depletion mediated reprogramming of germ cells to neurons is partially repressed in animals that lack the hypoxia-induced factor HIF-1. HIF-1 has been implicated in regulating iPSC reprogramming, a process that is triggered by changes in the level of the metabolite alpha-ketoglutarate (aKG)[2]. Importantly, aKG levels are regulated by IDHA-1. Moreover,aKG is known to act as a co-factor of histone demethylases and we are currently studying the underlying signaling mechanisms.
References. 1. Bukowiecki R, *et al*. 2014. Mitochondrial function in pluripotent stem cells and cellular reprogramming. Gerontology. 2. Zeng L. *et al.* 2014. Aberrant IDH3α expression promotes malignant tumor growth by inducing HIF-1-mediated metabolic reprogramming and angiogenesis. Oncogene.



SREBP1 DRIVES KRT80-DEPENDENT CYTOSKELETAL CHANGES AND INVASIVE BEHAVIOUR IN ENDOCRINE RESISTANT ERα BREAST CANCER

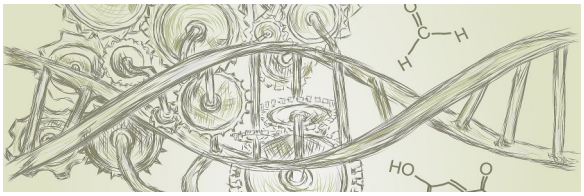
Poster presented by: Ylenia Perone, **Imperial College London**

Rationale. About one third of ERα breast cancer patients progress to invasive metastatic disease despite targeted endocrine therapies. The relationship between acquisition of drug resistance and invasive potential is poorly understood.
Methods. MCF7, T47D cell line and derived resistant clones were used for this study. 3D organoids invasion assay, immunofluorescence, confocal microscopy, RNA-seq, ChIP-seq, RT-qPCR and immunoblotting were performed. Seventy-five human breast specimens and ten metastatic lymph nodes were selected with the approval of Imperial College Healthcare NHS Trust Tissue Bank. Twenty women with suspected breast cancer were prospectively recruited and radiological exam using shear wave ultrasound was performed.
Results. Cells acquiring resistance to aromatase inhibitors (AI) undergo active cytoskeleton re-organisation via Keratin 80 (KRT80) and F-Actin remodelling. These features directly drive the invasive phenotype. This process is promoted by epigenetic reprogramming at the type II keratin locus leading to KRT80 up-regulation. Reprogramming is dependent on *de novo* Sterol Regulatory Element-Binding Protein 1 (SREBP1) binding to a single enhancer that is activated upon chronic AI treatment. AI-treated patients show KRT80 cytoskeletal re-organisation and an increased number of KRT80 positive cells at relapse. We find that KRT80 activation and redeployment leads to increased F-actin deposition and focal adhesion. KRT80 manipulation directly contributes to changes in cellular stiffness and invasive potential. Shear-wave elasticity imaging of prospective patients show that KRT80 levels correlate with stiffer tumours *in vivo.*
Conclusion. Our data uncover an unpredicted and potentially targetable direct link between epigenetic and cytoskeletal reprogramming promoting cell invasion in response to chronic treatment.

SPECIFIC ISOACCEPTOR AND ISODECODER TRNA GENES EXHIBIT INCREASED DNA METHYLATION WITH AGE

Poster presented by: Richard J Acton, **University of Southampton**

Rationale. Age-related epigenetic changes in DNA methylation (DNAm) are a valuable biomarker of biological ageing and may also give novel insights into age-related disease pathophysiology. tRNAs are crucial in global protein synthesis, as well as core metabolic processes, which deteriorate with age.
Methods. The tRNAome (~572 cytoplasmic tRNA genes) was analysed for age-related DNAm changes in 4,324 MeDIP-seq DNA methylomes (16-82 years). We replicated select findings in age-group pools (50 samples each at ~4, ~28, ~63, & ~77 years) with Fluidigm targeted bisulfite-sequencing (BiS-seq).
Results. The tRNAome is enriched for age-related DNAm changes (empirical $p<1x10^{-3}$). Furthermore, 6 tRNA loci significantly hypermethylate after Blood-cell subtype correction (n=2934, $p<4.34x10^{-9}$). The most robust result, tRNA-iMet-CAT-1-4, is 1 of 8 identical Initiator methionine tRNAs, but only this locus shows DNAm increases with age. tRNA-Ile-AAT-1-1 hypermethylates with age whilst an isodecoder (same anticodon, different body sequence) does not. tRNA-iMet-CAT-1-4 hypermethylation, and tRNA-Ile-AAT-1-1 isodecoder differences were validated with targeted BiS-seq. Furthermore, we examined GDC/TCGA Cancer data (n=733), due to the commonality between ageing and cancer-related DNAm changes. This showed the tRNAome mean methylation was 3.8% higher in cancer than normal tissues ($p<2.2x10^{-16}$, Wilcoxon). Also, in both normal and cancer tissue, other specific tRNA loci change DNAm with age.
Conclusions. Both globally, as well as specific tRNAs increase DNAm with age. We have robustly identified and validated specific age-modified tRNA genes, which survive correction for blood cell-type composition. There are possible extra-translatory roles for tRNA in ageing, including regulatory tRNA fragments, which we are now exploring.



ABSTRACTS

INVESTIGATING THE DYNAMICS OF ADENINE METHYLATION DURING GROWTH USING THE MODEL EUKARYOTE, TETRAHYMENA THERMOPHILA

Poster presented by: Robert Lowe, **Queen Mary University of London**

Adenine methylation (6mA) is the main DNA modification in unicellular eukaryotes and despite being identified for some time, the biological functions of this modification are still poorly understood. To investigate the function of 6mA, we have chosen to profile the genome-wide distribution across two different growth stages in *Tetrahymena thermophila.* During growth, there is a rapid expansion in cellular population as cells utilise an initially nutrient rich media, growing exponentially until nutrient availability becomes limiting. We hypothesised that the adaptation to these changing environments is partly encoded in epigenetic processes. To explore this and to detect 6mA in *Tetrahymena thermophila,* we utilised Oxford Nanopore Technologies minION sequencer. We used an existing methodology[1] to reliably detect 6mA genome wide, revealing a strong correlation with existing published PacBio sequencing[2]. For the first time, we have identified dynamic 6mA across growth and have linked this to dynamic changes in nucleosome positioning. This suggests a potential link between 6mA, DNA accessibility and changes in transcriptional regulation during growth.

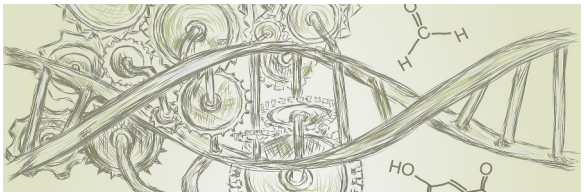
1. McIntyre, A. B. R. *et al.* Nanopore detection of bacterial DNA base modifications. *bioRxiv* (2017). doi:10.1101/127100.

2. Wang, Y., Chen, X., Sheng, Y., Liu, Y. & Gao, S. N6-adenine DNA methylation is associated with the linker DNA of H2A.Z-containing well-positioned nucleosomes in Pol II-transcribed genes in Tetrahymena. *Nucleic Acids Res.* **45**, 11594–11606 (2017).

MACROH2A1.1 - PARP-1 AXIS REGULATES NAD+ METABOLISM AND MUSCLE MATURATION

Poster presented by: Sarah Hurtado-Bagès, **Josep Carreras Leukaemia Research Institute (IJC)**

Throughout evolution, cellular system developed several mechanisms in order to respond to various stress including metabolic stress. Modifying chromatin composition and structure is one of the most potent mechanism involved in such process. At the level of the nucleosome, canonical histones can be exchange by histone variants such as macroH2As. Alternative splicing of macroH2A1 gives rise to macroH2A1.1 and macroH2A1.2 isoforms. We first observed a drastic increase of macroH2A1.1 expression during muscle differentiation in C2C12 cells model. We further demonstrated that macroH2A1.1 depleted mouse C2C12 myotubes show reduced mitochondrial respiratory capacity associated with a reduction of NAD+. Interestingly, macroH2A1.1, but not macroH2A1.2, is able to bind NAD+ derived metabolites such as ADP-ribose. Through its interaction with ADP-ribose, macroH2A1.1 binds and inhibits the activity of Poly ADP-ribose polymerase 1 (PARP-1) enzyme. Yet, PARP-1 mainly known as a cellular stress sensor is one of the major NAD+ consumer in cells. For this reason, we shown that inhibition of PARP1, or addition of NMN, a NAD+ precursor, were sufficient to rescue the mitochondrial phenotype. In conclusion, macroH2A1.1 buffers NAD+ consumption by inhibiting PARP-1 activity and maintains proper mitochondrial activity (Posavec Marjanović, M., Hurtado-Bagès, S., et al., 2017, *NSMB*). Intriguingly, such metabolic phenotype is accompanied by a defect in myotubes fusion and maturation (unpublished data). We are currently investigating how macroH2A1 isoforms manage to regulate muscle fusion in parallel to the metabolic phenotype.



ABSTRACTS

IDH1: LINKING CHROMATIN AND METABOLISM

Poster presented by: Silvia Raineri, **Department of Biochemistry, Oxford University**

Mutations in genes encoding metabolic enzymes often contribute to cancer development and progression by disrupting cell metabolism and altering the epigenetic landscape. An example are the isoforms of Isocitrate Dehydrogenase (IDH1/2), which metabolize Isocitrate to α-Ketoglutarate (α-KG). Interestingly, IDH mutations are common in several types of cancers, occurring in ~80% of oligoblastomas and 20% of acute myeloid leukemias (AML). Gain of function mutations in *IDH1* or *IDH2* result in reduced levels of α-Ketoglutarate and increased formation of D-2-Hydroxyglutarate (2-HG). α-KG is an essential co-factor for certain Histone and DNA demethylases, while 2-HG is a competitive inhibitor. These *IDH1/2* mutations are thought to result in hypermethylated histones and DNA which in turn alters gene expression and drives cancer progression. We aim at defining *IDH1* role in cancer development by investigating the possible alterations in RNA expression, histone modifications and DNA domains formation upon treatment with an IDH1 inhibitor.

EPIGENETICS OF EXCEPTIONAL LONGEVITY

Poster presented by: Simone Ecker, **University College London**

Rationale. Epigenetic modifications are a hallmark of aging. The epigenome changes throughout life and is altered by environmental and lifestyle factors which contribute to increased epigenetic variability with aging ('epigenetic drift'). The best examples of successful human aging are the world's so-called 'Blue Zones': longevity hotspots where people live significantly longer. The largest Blue Zone is the Costa Rican peninsula Nicoya where male life expectancy is among the highest in the world [1].

Methods. We analyzed DNA methylation of blood cells derived from the Costa Rican Longevity and Healthy Aging Study (CRELES) [2], a population-based longitudinal demographic survey including the Blue Zone of Nicoya.

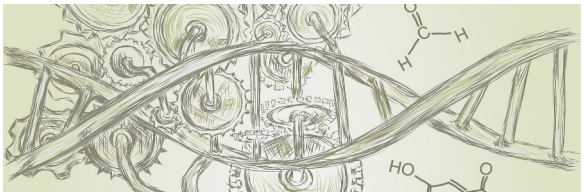
Results. Habitants of the Blue Zone of Nicoya exhibit 'younger' immunological profiles and a generally reduced epigenetic drift [3]. Interestingly, however, we found significantly increased epigenetic variability in genes involved in metabolic and hormone-sensing pathways such as glucose flux, insulin secretion, folic acid receptor binding and lipid metabolism. These highly variable genes also showed important associations with lifestyle and environmental factors. Furthermore, we found significant sex differences in DNA methylation specific to the Blue Zone, which is compelling in light of the fact that the Nicoyan longevity advantage is only present in males.

Conclusions. Epigenetic variability plays an important role in the phenotype of exceptional longevity and is associated with key metabolic pathways. An increased understanding of the interaction between external factors and molecular mechanisms promoting healthy aging will pave the way to develop strategies for personalized disease prevention and ultimately increase human life- and healthspan.

1. Rosero-Bixby L, Dow W, Rehkopf D. The Nicoya region of Costa Rica: a high longevity island for elderly males. Vienna Yearb Popul Res. 2013; 11: 109–36.

2. Rosero-Bixby L, Fernández X, Dow WH. CRELES: Costa Rican Longevity and Healthy Aging Study (Costa Rica Estudio de Longevidad y Envejecimiento Saludable). Ann Arbor, MI: Inter-university Consortium for Political and Social Research. 2005.

3. McEwen LM, Morin AM, Edgar RD, MacIsaac JL, Jones MJ, Dow WH, Rosero-Bixby L, Kobor MS, Rehkopf DH. Differential DNA methylation and lymphocyte proportions in a Costa Rican high longevity region. Epigenetics Chromatin. 2017; 10: 21.



METHIONINE STRESS IN CANCER: UNDERSTANDING A METABOLIC CONNECTION TO EPIGENETIC REMODELING

Poster presented by: Stacey Borrego, **University of California - Irvine**

Rationale. The majority of cancer cells have a unique metabolic demand for methionine that is not observed in normal, non-transformed cells. This "methionine dependent" phenotype describes a strict and irreplaceable requirement for methionine for proliferation. Previously, we identified metabolic effects of methionine stress including a reduction in S-adenosylmethionine abundance and a shift toward a more oxidative cell state. We further define a molecular connection to methionine metabolism in cancer with regulation of chromatin and gene expression programs.

Methods. The study presented focuses on the genetic response to methionine stress in the breast cancer cell line MDA-MB468 and their methionine stress insensitive derivatives MDA-MB468res. These methionine stress resistant lines have a similar proliferation rate and genetic background as the parental cell line; yet reversion to a non-transformed phenotype has resulted in loss of methionine dependence. Alongside our earlier metabolomic studies, we further characterize the response to methionine stress by employing deep sequencing and mass spectroscopy of histone post-translational modifications (PTMs) at both early (30min, 2h) and late (12h, 24h) time points.

Results. While global metabolite and lipid abundances are largely affected by methionine stress early on, more subtle and selective changes are observed in chromatin modifications and gene expression. During a later response many metabolites recover to starting levels; however, changes in gene expression, splicing, and histone PTMs persist and are likely a sign of adaptation to methionine stress.

Conclusions. Our results indicate a connection of methionine stress to epigenetic modifications in a temporal, cancer-specific manner.

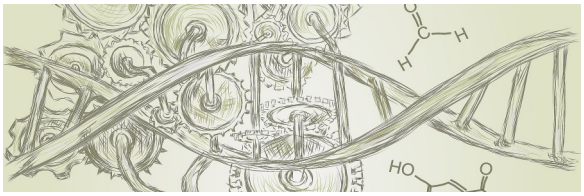
INHERITANCE AND ENVIRONMENTAL MODULATION OF VARIABLY SILENCED REPEAT ELEMENTS

Poster presented by: Tessa M Bertozzi, **Department of Genetics, University of Cambridge**

Generally repressed by epigenetic mechanisms, retrotransposons represent around 40% of the murine genome. At the *Agouti viable yellow* (A^{vy}) locus, an endogenous retrovirus (ERV) of the intracisternal A-particle (IAP) class retrotransposed upstream of the agouti coat-colour locus, providing an alternative promoter that is variably methylated in genetically identical individuals. This results in variable expressivity of coat colour that is inherited across generations. The A^{vy} mouse has been used as both a model for non-genetic inheritance and as a potential epigenetic biosensor of environmental compromise. Given how widespread ERVs are in the mouse genome, we set out to determine the prevalence of this phenomenon.

We conducted a systematic screen using whole-genome bisulfite sequencing (WGBS) datasets and identified a repertoire of variably methylated IAPs (VM-IAPs) possessing A^{vy}-like properties [1]. We find that VM-IAPs are reprogrammed after fertilization and re-constructed as variable loci in the next generation. Only a single locus exhibited evidence of epigenetic inheritance and the effect size was small.[1] Similar to A^{vy}, abnormal folate metabolism shifts VM-IAP methylation levels. In contrast, no epigenetic changes were observed following BPA-exposure or administration of an obesogenic diet. Our catalogue of novel loci will be useful in studying the mechanisms underlying repeat element silencing and reconstruction of epigenetic variability across generations. In addition, VM-IAPs provide a model to explore the epigenetic impact of altered environmental contexts, including metabolic disease.

[1] Kazachenka, A.*, Bertozzi, T.M.*, Sjoberg-Herrera, M.K., Walker, N., Gardner, J., Gunning, R., Pahita, E., Adams, S., Adams, D., Ferguson-Smith, A.C., *Cell* (2018), https://doi.org/10.1016/j.cell.2018.09.043



CREBBP STATUS IS A BIOMARKER FOR PALBOCICLIB RESPONSE IN CANCERS THAT UNDERGO NUTRIENT STRESS

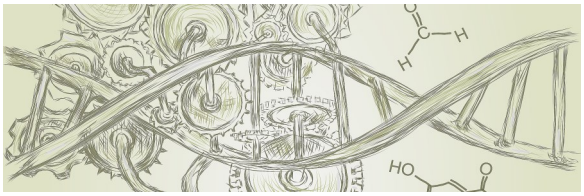
Poster presented by: Barrie Peck, **ICR**

Rationale. The contribution of the majority of frequently mutated genes to tumourigenesis is not fully defined. Many aggressive human cancers, such as triple negative breast cancers (TNBCs), have a poor prognosis and lack tractable biomarkers and targeted therapeutic options.
Methods. Here, we systematically characterize loss-of-function mutations to generate a functional map of novel driver genes in a 3-dimensional (3D) model of breast cancer heterogeneity that more readily recapitulates the unfavourable tumour microenvironment *in vivo* i.e. nutrient stress.
Results. We identified several genes, including CREBBP, FOXA1 and NIPBL, whose silencing provided a 3D specific growth advantage. Indeed, the histone acetyltransferase *CREBBP* was a potent tumour suppressor gene whose silencing provided a 3D-specific growth advantage only under oxygen and nutrient deplete conditions.
CREBBP protein expression was altered in a third of TNBCs as well as several other solid tumours, including bladder, ovarian and squamous lung cancers. In multiple primary tumours and cell models, loss of CREBBP activity resulted in upregulation of the FOXM1 transcriptional network. Strikingly, treatment with a range of CDK4/6 inhibitors (CDK4/6i), that indirectly target FOXM1 activity, selectively impaired growth in both CREBBP-altered spheroids and cell line xenografts from multiple tumour types. Moreover, preliminary evidence shows that CREBBP-altered spheroids are metabolically distinct from their wildtype counterparts.
Conclusions. This study is the first to provide rationale for CREBBP as a biomarker for CDK4/6i response in cancer representing a new treatment paradigm for tumours that harbour *CREBBP* alterations that have limited therapeutic options.

THE LEVEL OF METABOLITES (L-ASCORBIC ACID, 2-KETOGLUTARATE, 2-HYDROXYGLUTARATE) IN PLASMA, TISSUE AND URINE OF PATIENTS WITH INTESTINAL DISEASES IN RELATION TO THE LEVEL OF TET PROTEINS PRODUCTS MEASURED IN THE SAME CLINICAL MATERIAL

Poster presented by: Martyna Modrzejewska, **Nicolaus Copernicus University in Torun - Ludwik Rydygier Collegium Medicum in Bydgoszcz**

Rationale. Molecular changes in DNA methylation pattern, similar to those caused by functional mutations in TET proteins, might have been the result of changes in cellular metabolic state. The oncometabolites (e.g *IDH* mutation product) can inhibit enzymatic oxidation of 5-methylcytosine to 5-hydroxymethylcytosine and further TET products in DNA. **Methods.** The essence of the study was quantitative analysis of plasma/intracellular level of L-ascorbic acid and 2-ketoglutarate; plasma and urinary level of L-, D-2-hydroxyglutarate among patients suffering from colon adenoma/cancer and inflammatory bowel diseases and healthy controls using highly sensitive UPLC-MS/MS, UPLC-UV methods. Moreover, our recent project has delivered data about the level of 5-methylcytosine and its derivatives in DNA of cancer tissues and in urine of the same study groups (obtained with 2D-UPLC-MS/MS method). **Results.** We have observed the lowest 2-ketoglutarate plasma concentration (9.05µM) and simultaneously the highest D-2-hydroxyglutarate plasma (0.23 µM) as well D-2-hydroxyglutarate urinary (6.50 µmol/mmol creatinine) level in inflammatory group of patients. The plasma/urinary level of L-2-hydroxyglutarate is approximately 1.5 fold higher in colorectal cancer patients than control (R=0.60). There is a negative correlation between plasma L-ascorbic acid and urinary level of 5-hydroxymethylcytosine, 5-formylcytosine, 5-carboxycytosine. **Conclusions.** The data shows that sustained inflammation can be associated with significant change in the level of metabolites directly involved in an active DNA demethylation pathway, and can promote tumorigenesis via interfering with formation of epigenetic modifications. The level of individual metabolites can be genetically determined, however measured level of D-2-hydroxyglutarate probably arises from different genetic and/or metabolic alterations rather than *IDH* mutations.



ABSTRACTS

EPIGENOME-WIDE ASSOCIATION STUDY IN CHILDHOOD OBESITY: SEARCHING FOR EARLY MARKERS OF LATE DISEASE RISK

Poster presented by: Pol Castellano-Escuder, **Institut de Recerca Sant Joan de Déu**

Rationale. Childhood obesity is one a major Public Health issue. Overweight/obese children have a high risk of being obese as adults and develop other co-morbidities, including type 2 diabetes, cardiovascular disease and several types of cancer. It has been proposed that epigenetic mechanisms might be involved in mediating long-term metabolic dysfunction.

Methods. Here we analyzed DNA methylation profiles (Infinium MethylationEPIC BeadChip, 850K) in whole blood from 26 obese (zBMI > 2) and 12 control lean pre-pubertal children (zBMI < 1).

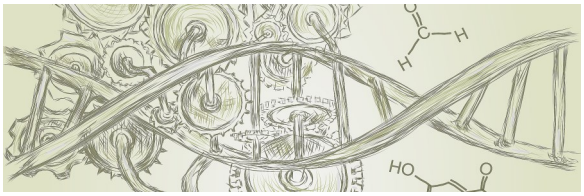
Results. 109 CpG sites appeared differentially methylated between the two groups (methylation change 10% and FDR value <0.05). 5 out of the 109 targets, were located within the *SPATC1L* (Spermatogenesis and Centriole Associated 1 Like) gene. Next, we performed a Two Sample Mendelian Randomization test, and found that 2 of the 5 CpG sites mapping the *SPATC1L* locus are causal for multiple disorders, including childhood obesity, type 2 diabetes, ulcerative colitis and inflammatory bowel disease. *SPATC1L* expression was similar in whole blood of obese and lean subjects. However, the contiguous genes *COL6A2* and *COL6A3* (Collagen Type VI Alpha 2 and 3 Chains), which are strongly associated to obesity, were differentially expressed between the two groups.

Discussion. Childhood obesity is associated to a small change in DNA methylation (109 CpG sites). In our dataset, only 2 CpG sites appeared to play a causative role in the development of the disease. We hypothesize that these CpG sites might mediate disease risk by modulating the expression of physically close genes.

NUCLEOSOMAL POSITIONING IS PREDICTIVE OF CHROMATIN STATE

Poster presented by: Chris Clarkson, **University of Essex**

The genome of a eukaryotic cell is stored inside the nucleus in a highly condensed form called chromatin. The basic unit of chromatin is the nucleosome, where the histone octamer is wrapped by 147 DNA base pairs (bp) [1]. The positioning of nucleosomes on the DNA is an area of active research. The determinants of cell type-specific nucleosome positioning are still poorly understood [2]. Even less understood is the relation between nucleosome positioning patterns and local transcription rates. The effects on gene activity of multiple histone modifications and chromatin protein enrichments (collectively termed ‘chromatin-states’) have been studied extensively using machine learning [3,4]. On the other hand, the direct effects of nucleosome positioning patterns on gene expression have not been resolved. Here I present an attempt to address this challenge using a hypothesis that nucleosome organisation is predictive of the chromatin states. Following binning the genome into different chromatin states and applying deep learning it was possible to find associations between nucleosome positioning patterns and local chromatin conformation for regions as small as 3,000 bp.



MTHFD1 LINKS REDOX HOMEOSTASIS AND DNA METHYLATION IN *ARABIDOPSIS*

Poster presented by: Martin Groth, **Helmholtz Zentrum Muenchen**

Rationale. DNA methylation in plants is the most stable of the known epigenetic marks. However, recent genome-wide studies have shown substantial dynamics in DNA methylation that are associated with environmental stress. Stress-induced DNA methylation changes not only lead to reactivation of transposable elements (TEs), the primary targets of transcriptional gene silencing, but can also affect the expression of genes, which contain TEs in their regulatory regions. This is relevant because dynamic DNA methylation contributes to priming and acclimation in plants.

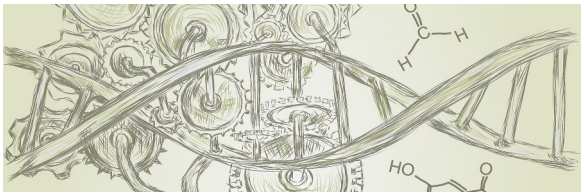
Methods and Results. In an effort to identify new mechanisms controlling DNA methylation and/or transcriptional gene silencing, we performed a forward genetic screen based on a DNA methylation-sensitive GFP reporter line (SDCpro-GFP). One of the identified mutants contained the causative mutation in a gene that encodes a nucleocytoplasmic protein involved in folate metabolism (MTHFD1)(1). Further analyses showed that the mutation of MTHFD1 leads to metabolic inhibition of DNA and histone methylation. Strikingly, methylome, transcriptome, and metabolic profiling indicated that DNA methylation and transcriptional gene silencing depend on MTHFD1-mediated redox homeostasis, whereas concomitant production of folate intermediates by MTHFD1 is not required for DNA methylation.

Conclusion. As redox regulation is central to stress responses, our results provide new links between stress-induced DNA methylation dynamics and metabolic regulation.

THE ROLE OF macroH2A1.2 HISTONE VARIANT IN CHROMATIN METABOLITE SENSING

Poster presented by: Iva Guberovic, **Josep Carreras Leukaemia Research Institute**

Rationale: Within a diverse group of H2A variants, macroH2A histone variants differ the most from their canonical counterpart. They have a particular structure in which a globular macrodomain is coupled to a histone domain. Crystal structures show a distinct metabolite-binding pocket in the macrodomains of all three members of macroH2A family. However, the ligand is detected only for macroH2A1.1, which binds ADP-ribose and indirectly affects the function of mitochondria. Interestingly, the other two isoforms remain to be orphans. Our goal is to detect the ligand of alternatively spliced macroH2A1.2 isoform, and describe its implication in chromatin metabolite sensing.
Methods: Origin and evolutionary conservation of macroH2A was analysed using phylogenetic and molecular evolution approaches. Human Metabolome Database (HMDB) was used in the molecular docking analysis for the prediction of macroH2A1.2 ligands. The binding of candidate compounds was tested *in vitro* using saturation transfer difference NMR.
Results: Molecular evolution analysis shows high conservation of macroH2A1.2 histone variant, especially with respect to its binding pocket. From the list of candidate ligands, we detected a first ever ligand of macroH2A1.2.
Conclusions: Our preliminary results show strong evolutionary conservation of macroH2A1.2. This indicates that macroH2A1.2 might have a binding pocket-related physiological role, and could therefore be implicated in chromatin metabolite sensing. Additionally, we have detected the first ever ligand of macroH2A1.2, a plant metabolite which will be used as a tool compound in the future studies. The physiological role of macroH2A1.2 with respect to its metabolite binding remains to be elucidated.



Exhibitor poster

A MULTI– OMICS TOOLBOX TO DEFINE THE EPIGENETIC PROFILE IN CANCER DEISEASE AREAS

S.Chlamydas,G. Martovetsky, A. Blattler, T. Yen, P. Labhart, B. Egan

Active Motif, Inc. Carlsbad CA, USA.

Epigenetic profiling is critical for understanding the underlying mechanisms involved in cell fate decisions, cellular response to treatment and disease. Unfortunately, no single assay can provide a comprehensive view of the epigenetic state of your cells of interest. Here, we present four different services offered by Active Motif that, when performed in unison, are able to provide a comprehensive understanding of epigenetic determinants that are involved in your model system. Active Motif's comprehensive service offering includes: 1) ATAC-seq (Assay for Transposase-Accessible Chromatin using sequencing), which interrogates chromatin accessibility changes in your cells or tissues of interest; 2) Mod Spec™, which quantifies histone post-translational modifications by mass spectrometry, making it possible to measure global changes of greater than 80 different histone modification states in a single assay; 3) RRBS (Reduced Representation Bisulfite Sequencing) which provides single base-pair resolution methylation status at a at over 75% of CpG islands and over 50% of promoters; 4) RIME (Rapid Immunoprecipitation Mass Spectrometry of Endogenous Proteins) which can elucidate physically-interacting co-regulators that may be required in establishing the functional specificity of your target protein. Together, these assays will provide you with multi-dimensional mechanistic insight into the factors and pathways involved in the response of your model system.