XII Jornada de Cromatina i Epigenètica

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XII Annual Chromatin and Epigenetics symposium

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ABSTRACTS
SHORT TALKS

1- The autophagic protein TP53INP2 goes to chromatin

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TP53INP2 is a multifaceted protein that modulates multiple biological processes. It negatively regulates muscle mass and white adipocyte differentiation, and it is a positive regulator of apoptosis and autophagy. Moreover, it is repressed in skeletal muscle and adipose tissue from obese and type 2 diabetic individuals, suggesting its clinical relevance. However, the molecular mechanisms by which TP53INP2 controls the above-mentioned processes remain elusive. To this end, we analyzed the interactome of TP53INP2 using BioID technology. Next, we performed gene ontology enrichment analysis. To our surprise, top enriched gene sets were related to chromatin and epigenetics. In addition, we validated the interaction between TP53INP2 and RING1, an E3 ubiquitin ligase that is part of the polycomb repressive complex 1 (PRC1). Along these lines, we identified other members of the PRC1 in our interactome analysis, including PCGF6, L3MBTL2 and MGA. The PRC1 is a multiprotein complex bound to chromatin that represses transcription. Chromatin purification experiments clearly show that TP53INP2 is indeed bound to chromatin. We report for the first time the binding of an autophagic protein to chromatin.

2- Noncoding regulatory functions in β-cell derived neuroendocrine tumors

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Insulinomas are rare neuroendocrine tumours that arise from pancreatic β-cells. While retaining the ability to produce insulin, insulinomas feature aberrant proliferation and altered hormone secretion resulting in failure to maintain glucose homeostasis. The role of cis-elements and their aberrations to the development of these tumors is currently unexplored. We have now generated insulinoma regulatory maps by profiling gene expression and H3K27ac deposition in a large set of human pancreatic neuroendocrine tumors. We observed widespread chromatin activation of ~8,500 H3K27ac enriched sites in the tumoral tissue but not in untransformed human pancreatic islets. These regions are mostly distal to gene TSS, evolutionarily conserved and contribute to the transcriptomic aberrations of nearby genes. Of note, we show that ~20% of the differential H3K27ac activated regions are H3K27me3 repressed in unaffected β-cells (mean z-score 27.96), suggesting that tumoral transition is coupled with derepression of β-cell polycomb targeted domains. By coupling epigenetic profiling with whole genome sequencing we now aim in uncovering genetic variation implicated in deregulation of noncoding functions Our epigenomic profiling provides a compendium of aberrant cis-regulatory elements that alter β-cell function and fate in their progression to pancreatic neuroendocrine tumors and a framework to identify coding and noncoding driver mutations.

3- Mantle Cell Lymphoma-associated translocations are related to global genome topology disruption.

Authors: Anna Oncins, Kimberly Quililan*, Roser Zaurin, Houyem Toukabri, José Ramón Hernández-Mora, François Serra and Renée Beekman.*
(*) These authors contributed equally to the work.

Mantle cell lymphoma (MCL) is an aggressive non-Hodgkin lymphoma subtype, characterized by the t(11;14) translocation. This translocation leads to overexpression of cyclin D1 (CCND1), a proto-oncogene regulating cell cycle. Yet the upregulation of CCND1 is not enough to drive lymphoma development. Here, we show that beyond CCND1 overexpression, MCL patients carrying the t(11;14) translocation bear global genome topology changes. Among others, translocation partners 11 and 14 show a profound opposite change in their interchromosomal interactions in MCL, pointing towards a potential swap of their territory positioning. Additionally, we observed allele-specific genome topology changes, indicating particular alterations of the translocation products, e.g. derivate chromosomes. The consequences of these effects on lymphoma formation remain to be elucidated.

4- Association of epigenetic markers in peripheral blood and suicidal attempts in bipolar disorder

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Background

Suicidal behavior (SB) is a serious public health problem involving approximately one million annual deaths worldwide, with 90% of them being related to mental disorders such as bipolar or psychotic disorders (Plans et al., 2019). SB is determined by multiple factors emerging because of the interaction of socio-demographic, clinical, neurocognitive, environmental, and genetic factors and new evidence points to an important role of epigenetic factors. The aim of the present study is to compare genome-wide methylation patterns at a peripheral level between bipolar patients with and without suicide attempts (SA).

Methods

A sample of 79 BD patients assessed following DSM-IV-TR criteria (APA, 2000) was recruited from the Bipolar Disorder Program of the Hospital Clinic of Barcelona and Mental Health Services in Oviedo. All patients gave their written informed consent and approval from each institution's ethics committees was obtained. SB was assessed using the Columbia Suicide Severity Rating Scale (C-SSRS) (Al-Halabí et al., 2016; Posner et al., 2011). Individuals presenting one or more SA were categorized in the suicide attempters (SAtt) group (N=44; 75% females) and individuals presenting no SA were classified in the non-SAtt group (N=35; 65.7% females). Peripheral-blood DNA extractions were done using nucleic acid isolation technology (Chemagic, PerkinElmer). Genome-wide DNA methylation was assessed using the Infinium MethylationEPIC BeadChip (Illumina). R program was used to analyze methylation status between SAtt and non- SAtt groups. Raw Illumina microarray data were processed with ChAMP (Tian et al., 2017) by following quality control standards (e.g., filtering of probes with low detection p-value ($p>0.01$), probes with <3 beads in at least 5% per probe, etc.). Normalization and batch correction were done using BMIQ and Combat, respectively. Blood-cell type proportions were calculated using refbase. Differentially methylated positions (DMPs) and regions (DMRs) between SAtt and non-SAtt groups were calculated using Limma and DMRcate, respectively (Peters et al., 2015; Ritchie et al., 2015). Blood cell counts, sex, smoking status (smoking score) (Bollepalli et al., 2019), methylation PCs (Barfield et al., 2014), and age were used as covariates. For both, DMPs and DMRs, Benjamini-Hochberg multiple-testing correction was used to correct the false discovery rate (FDR) and a p-value of 0.05 was considered for significance.

Results

A total of eight CpG sites were significantly differentially methylated between SAtt and non-SAtt groups (p-value<5.62E-7; FDR adjusted p-value<0.05). A total of one DMRs was found between SAtt and non-SAtt groups (FDR adjusted p-value= 4.92E-16). From all the DMPs differentially methylated between SAtt and non-SAtt groups, three of them mapped to the: *LAMC1*, *MSX2*, and *FAM20A* genes. Lastly, the DMR observed in our sample mapped to several genes: *VWA5B2*, *MIR1224*, and *ALG3*.

Discussion

We found eight DMPs and one DMR between SAtt and non-SAtt groups. Two of the genes we identified have been previously associated with lithium treatment, a mood stabilizer with antisuicidal properties. A recent study showed that high dose of lithium treatment affects *LAMC1* expression in hippocampal progenitor cells. In the same study, *LAMC1* was highlighted as a central gene since interacts with most genes also differentially expressed after a high dose of lithium treatment (Palmos et al., 2021). Another recent study found *VWA5B2* gene was differentially methylated between excellent responders and non-responders to lithium in bipolar disorder patients (Marie-Claire et al., 2021). Our results show DNA methylation differences in peripheral blood between SAtt and non-SAtt bipolar patients pointing to the importance of considering epigenetic markers when studying SB. These results should be further studied for a better understanding of SB and ultimately help in suicide prevention. Moreover, epigenetic marks are potential biomarkers in suicide, and they could become attractive therapeutic targets due to their reversibility and important in regulating gene expression.

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5- *FKBP5* methylation patterns: cortical thickness and clinical correlates in schizophrenia

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Schizophrenia (SZ) is a mental disorder that arises from subtle deviations in brain development and maturation due to genetic and environmental insults. The *FKBP5* gene has been associated with SZ and the sensitivity to SZ environmental risk factors. Furthermore, different methylation and expression levels of *FKBP5* have been described in SZ versus health status, and its methylation has been linked to neuroanatomical changes in other psychiatric disorders, but has not been assessed in schizophrenia.

We aimed to: i) Explore methylation case-control differences in the *FKBP5* gene to identify diagnostic-specific methylation patterns. ii) Analyse the methylation effect on cortical thickness case-control differences. iii) Analyse the methylation effect on clinical schizophrenia phenotypes.

From a sample of 35 SZ patients and 35 HC (matched by sex, age and estimated IQ), methylation levels along 3 CpG islands (CGIs) at the *FKBP5* gene were analysed in DNA extracted from mouth mucosa samples (Agena Bioscience EpiTYPER). Cortical thickness was assessed from MRI scans, and whole-brain analysis was performed to identify case-control differences (FreeSurfer). Linear and logistic regression analyses adjusted for sex, age, intracranial volume (and treatment for patients) were performed.

Regarding the first objective, 4 CpG sites located in CGI1 and CGI3 were differentially methylated in SZ with respect to HC. Concerning the second objective, methylation at 3 CpG sites along CGI1 had a diagnostic-dependent effect on several of the observed case-control cortical thickness differences. Lastly, among patients, 5 CpG units in CGI3 had an impact on negative symptomatology (PANSS-N).

Our results represent the first case-control approach in schizophrenia to analyse the effect of *FKBP5* methylation status on cortical thickness and clinical phenotypes. We identify specific CpG sites in two CGIs associated with the risk for the disorder, while others have a modifier effect on structural and clinical phenotypes.

6- Deciphering correlations between DNA methylation, nucleosome positioning, gene expression and 3D chromatin structure using *Saccharomyces cerevisiae.*

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The extreme complexity of epigenetic regulation in higher organisms makes the determination of a causal function of DNA methylation complicated. We investigated the structural effect of DNA methylation in a simpler model system, budding yeast (*Saccharomyces cerevisiae*), a biological system in which methylation and all the methylation-related machinery are absent thus making it a perfect system to study the intrinsic role of DNA methylation on DNA structural and functional properties. With this aim, we expressed the murine DNA Methyl Transferases (DNMT) 1, 3a, 3b and 3L in *S. cerevisiae*, and performed Bisulfite Sequencing (WGBS), RNase-seq, MNase-seq and HI-C analysis to determine the correlation between DNA methylation, gene expression, nucleosome positioning and the three-dimensional folding of chromosomes. We demonstrated that, even in the absence of any directing machinery, methylation occurred in a reproducible pattern reminiscent of that seen in mammals, with methylation concentrated at linkers and depleted at nucleosomes. Moreover, we also revealed that high levels of DNA methylation affects gene expression in a complex manner, altering specific pathways in the cell. Quite interestingly, Hi-C experiments revealed significant changes in global chromatin structure with a noticeable increase in DNA condensation. Taken together, these results demonstrate that methylation intrinsically modulates chromatin structure and function even in the absence of the cellular machinery evolved to recognize and process the methylation signal.

7- MacroH2A1.2 and macroH2A2 modulate the response of cancer cells to innate immune signals involving changes in chromatin architecture and enhancer activation

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MacroH2A histone variants have a poorly understood function in gene regulation. In particular, it is not clear which cellular responses are macroH2A-sensitive and how macroH2As influence gene transcription at the molecular level. Most proliferative solid cancer cells express two macroH2A proteins, macroH2A1.2 and macroH2A2. Here, we report that macroH2A1.2 and macroH2A2 modulate the transcriptional ground-state of cancer cells and how they respond to inflammatory signals central to innate immunity. The transcriptional analysis of cell culture and xenograft experiments cells showed that depletion of macroH2A1.2 and macroH2A2 in hepatoblastoma cells increased gene activation in response to interferon γ but overall dampened the response to tumor necrosis factor α. Correlation studies in a cohort of hepatoblastoma samples and the large pan-cancer cohort of TCGA data set suggest that the function of macroH2A in response to innate immune signals might be more general. When focusing on the genomic loci of tumor necrosis factor α-responsive genes, we found that removal of macroH2A1.2 and macroH2A2 altered the three-dimensional chromatin architecture coinciding or preceding changes in the basal activity of distal enhancers.

Taken together, our results suggest that macroH2A1.2 and macroH2A2 have a role in regulating the response of cancer cells to innate immune signals on the level of chromatin. They are doing so in an unprecedented, bidirectional manner having a largely positive role in tumor necrosis factor α signalling and a repressive role in interferon γ signalling. The consequences of this regulation are yet to be understood but that are likely relevant for the interaction of cancers with their microenvironment.

8- Single-cell multi-omic analysis reveals defective genome activation and epigenetic reprogramming associated with human pre-implantation embryo arrest

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The terminally differentiated gametes unite at fertilization to form a totipotent zygote that can differentiate into all cells and tissues of the developing embryo. In mammals, the zygote undergoes several cleavage divisions to form a blastocyst. During this pre-implantation stage, maternally stored material promotes both the erasure of the sperm and oocyte epigenetic profiles and is responsible for concomitant genome activation. Here we have utilized single-cell methylome & transcriptome sequencing (scM&T-seq), a multi-omic method that allows for simultaneous quantification of both mRNA expression and DNA methylation, in human oocytes and a developmental series of human embryos. We fully characterize embryonic genome activation, maternal transcript degradation and map key epigenetic reprogramming events in developmentally high-quality embryos. By comparing these signatures with early embryos that have undergone spontaneous cleavage-stage arrest, as determined by time-lapse imaging, we identify embryos that fail to appropriately activate their genomes or undergo epigenetic reprogramming. Our results indicate that a failure to successfully accomplish these essential milestones impedes the developmental potential of pre-implantation embryos and is likely to have important implications, similar to aneuploidy, for the success of assisted reproductive cycles.

ABSTRACTS
POSTERS

1- Evolutionary implications of 3D chromatin remodeling in the germ line

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The spatial folding of chromosomes and their organization in the nucleus has profound regulatory impacts on gene expression and genome architecture, whose evolutionary consequences are far from being understood. Here we explore the evolutionary plasticity of the 3D chromatin remodelling in the germ line given its pivotal role in the transmission of genetic information. Using a comprehensive integrative computational analysis, we (i) reconstruct ancestral rodent genomes analyzing whole-genome sequences of 14 rodent species representatives of the major phylogroups, (ii) detect lineage-specific chromosome rearrangements and (iii) identify the dynamics of the structural and epigenetic properties of evolutionary breakpoint regions throughout mouse spermatogenesis by applying integrative computational analyses. Our results show that evolutionary breakpoint regions are devoid of programmed meiotic DSBs and meiotic cohesins in primary spermatocytes but associated with functional long-range interaction regions and sites of DNA damage in post-meiotic cells. Moreover, we detect the presence of long-range interactions in spermatids that recapitulate ancestral chromosomal configurations. Overall, we propose a model, which integrates evolutionary genome reshuffling with DNA damage response mechanisms and the dynamic spatial genome organization of germ cells.

2- Transcriptional profiles reveal sources of cellular stress in patients with undiagnosed rare diseases

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Patients with undiagnosed rare diseases that have tested negative for most common genes related to their pathology enter the next step in the diagnosis pipeline consisting of whole exome sequencing (WES), whole genome sequencing (WGS) and RNA-seq, most commonly in blood. RNA-seq contributes to identify mutated genes by providing information on aberrant gene expression, altered splicing, coding variants and monoallelic expression. In addition, altered transcriptional profiles can be used to identify sources of cellular stress that contribute to patients' pathology. This information can be used to validate variants of unknow significance (VUS), identify markers for drug screenings and suggest potential drugs to alleviate the symptoms of the diseases.

3- A genome-wide CRISPR screen to identify novel pathways involved in reprogramming and X-chromosome reactivation

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Species with genetic sex determination present differences in chromosome composition between the sexes. In mammals, male cells contain one X and one Y chromosome, while female cells have two X chromosomes. In order to achieve an equal sex-linked gene dosage, female cells silence one X chromosome in a process called X-chromosome inactivation (XCI). The silent state of the inactive X chromosome is stably maintained in somatic cells. However, XCI is reversed during development by a process called X-chromosome reactivation (XCR), which takes place in the pluripotent epiblast of the blastocyst and in the germ cell lineage. In the laboratory, XCR can also be studied using in vitro systems, like reprogramming of female differentiated somatic cells to induced pluripotent stem cells (iPSCs). Although previous studies have undertaken candidate approaches, the regulatory network involved in XCR during reprogramming has not been fully investigated. Therefore, we performed an unbiased genome-wide pooled CRISPR-screening approach to identify factors and pathways involved in XCR. To this end, taking advantage of an X-chromosome reporter cell line, we reprogrammed mouse neural precursor cells (NPCs) into iPSCs, producing inducible knock-outs of all the protein-coding genes in the mouse genome during this process. By a gRNA-abundance analysis, we were able to identify known and novel pathways that are linked to pluripotency and XCR. Using this approach, we identified the interferon g pathway to be involved in pluripotent stem cell reprogramming and XCR. The activation of this pathway during the early phase of reprogramming resulted in a decrease in colony number, in addition to a more efficient XCR in the pluripotent colonies. Here we will present stage-specific perturbation experiments and allele-specific RNA-Seq analysis to further delineate the mechanistic function of the interferon g pathway, which we demonstrate to feature in iPSC reprogramming and XCR.

4- Reprogramming of epigenetic marks during early development across vertebrates

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Epigenetics concerns mechanisms modifying gene expression independent of changes in DNA sequence. Three epigenetic mechanisms include DNA methylation, histone modifications and non-coding RNAs. The first two act at transcriptional level, influencing chromatin accessibility and architecture, whereas non-coding RNAs have role in post-transcriptional regulation. DNA methylation, associated with gene expression silencing, has important roles in vertebrates, being associated with phenomena such as genome imprinting and reprogramming. This implies changes of inherited epigenetic marks during embryo development, since they can be transmitted throughout mitotic and meiotic divisions, influencing development of a new individual. Reprogramming is well studied in mammals, characterized by a first wave of demethylation during cleavage stages, followed by a second wave allowing reprogramming of future primordial germ cells (PGCs). In fish, information is available only for a few species. In zebrafish (*Danio rerio*) reprogramming was detected in oocyte-derived DNA to resemble the paternal methylome, allowing zygotic genome activation (ZGA). Since future PGCs inherit from the germplasm the signals necessary for their specification, prior demethylation is not required. In medaka (*Oryzias latipes*), strategies similar to mammals seem to be adopted, since in both early embryo and PGCs, methylation reprogramming occurs. While zebrafish lacks a global demethylation, medaka resemble mice, although in the former the major wave already completes after the first cell cycle, whereas in mouse occurs gradually, during some cleavage stages. In *Kryptolebias marmoratus*, a self-fertilizing hermaphroditic vertebrate, epigenome reprogramming is longer than in other vertebrates, and global demethylation is achieved at a late embryonic stage. This scarce data suggest that reprogramming could be species-specific in fish. Our goal is to determine the situation in European sea bass (*Dicentrarchus labrax)*, given its phylogenetic distance from the other species, and to understand the epigenetic inheritance of this marine teleost to devise breeding strategies able to maintain certain traits in farmed sea bass.

5- The role of C/EBPa isoforms in acute myeloid leukemia

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C/EBPa is a transcription factor that has a key role in hematopoietic differentiation and is one of the most frequently mutated transcription factors in acute myeloid leukemia (AML). The most common type of mutation affects the N-terminal region and results in the exclusive translation of a shorter isoform, known as C/EBPa^{p30}. While expression of this isoform is sufficient for commitment to a myeloid fate, its exclusive production results in the pathogenic expansion of myeloid blasts. Intriguingly, both the full-length and the short isoform share the same DNA-binding and dimerization domains. However, the different transcriptional programs controlled by the two isoforms and the mechanisms that dictate their binding profile remain largely unknown. Consequently, how N-terminal C/EBPa mutations are linked to AML progression is still not understood. To explore these questions, I have generated HSC cell lines that exclusively express the pathogenic C/EBPa^{p30} isoform. Transcriptomic profiling of these cells has revealed a severe downregulation of inflammatory pathways, in agreement with our analysis of large-scale AML datasets and with previously published data of C/EBPa knock-out mice. I am currently generating genome-wide binding profiles of both isoforms as well as functional assays to understand the impact of C/EBPa^{p30}-driven gene expression on hematopoietic differentiation.

6- A non-bisulphite conversion-based method, scEM&T-seq for dual single-cell methylome and transcriptome profiling

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Here we report a novel integration of DNA methylation and gene expression profiling of single cells that bypasses the reliance upon bisulphite conversion. To date, several single-cell multi-omics approaches have been described that enable characterisation of cellular heterogeneity and the description of rare cell types. We have developed a novel method for profiling genome-wide DNA methylation and the transcriptome from the same single cells in samples isolated from the human uterus. Our scEM&T-seq (single-cell Enzymatic Methyl and Transcriptome sequencing) protocol combines Smart-seq2 with NEB's EM-seq technology, with mRNA and genomic DNA physically separated following the G&T-seq protocol. We have shown for the first time that EM-seq works efficiently on single-cells and low input samples. The partially automated plate protocol, excluding flow sorting of cells and sequencing, takes three days to generate the dual libraries for up to 192 samples (two 96-well plates). Here we present our initial findings from the HCA-2020 HUTER project.

7- *PIK3R1* and *G0S2* are human placenta-specific imprinted genes associated with germline inherited maternal DNA methylation.

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Genomic imprinting is the parent-of-origin monoallelic expression of genes that result from complex epigenetic interactions. At its simplest level, monoalleic expression is achieved by decorating gene promoters with 5-methylcytosine (5mC), resulting in differentially methylated regions (DMRs). Often the allelic methylation is inherited from the germline, where there is a bias towards oocyte-derived methylation surviving reprogramming in the pre-implantation embryo. There is complex interplay between the DMRs and additional epigenetic signals as highlighted by the fact euchromatic histone modifications, including H3K4m3, are often observed on the unmethylated allele, while the methylated allele is enriched with heterochromatic marks such as H3K9me3.

Imprinting is widespread in the human placenta. We have recently performed a whole genome scan for novel imprinted germline DMRs (gDMRs) that are present in the placenta by comparing methylomes of gametes, blastocysts and various somatic tissues. We observe that unlike conventional imprinting, for which methylation at gDMRs is observed in all tissues, placenta-specific imprinting is associated with transient gDMRs, present only in the pre-implantation embryo and placenta. This reveals hundreds of potential oocyte-derived gDMRs that survive only in the placenta, many of which orchestrate paternal expression. Fascinatingly, placenta-specific-DMRs can be polymorphic, with some samples being devoid of allelic methylation throughout gestation. Here we have reinvestigated the list of partially methylated interval previously described to be consistent with monoallelic methylation and characterised two novel imprinted genes, *PIK3R1* and *G0S2*, both of which display polymorphic imprinting and investigate their potential role in placenta-associated pregnancy complications.

8- Planarian stem cell differentiation requires the acetyltransferase Smed-cbp-3

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Cell state transitions are associated with epigenetic changes that alter chromatin structure and gene expression. As a stem cell differentiates, genes associated with self-renewal are downregulated, while lineage-specific genes are activated. CBP (CREB- binding protein)/p300 proteins belong to a conserved gene family which functions as transcriptional co-activators regulating gene expression by acetylating histone and non-histone proteins, producing epigenetic marks and changing chromatin architecture. CBP/p300 shows an important role in a wide range of cellular processes, such as stem cell proliferation, differentiation and cell death. In our laboratory we use planarians as a model to investigate stem cell-based regeneration. These flatworms are able to regenerate any part of their whole body thanks to the presence of pluripotent stem cells called neoblasts. But how the differentiation of the neoblasts in all cellular lineages occurs still remains unclear. We have identified several CBP homologues in planarians. Our functional analyses have allowed us to identify an important role of some of them on planarian stem cell maintenance and differentiation. In particular, our results indicate that planarian stem cells accumulate in the absence of Smed-cbp-3 due to its inability to pursue differentiation. Currently, we are investigating the role of Smed-cbp-3 on neoblasts turnover, lineage specification, early response and cell cycle. These analyses will allow us to better determine the function of Smed-cbp-3 in neoblast biology and investigate the epigenetic regulation of planarian stem cells.

9- Polyphosphate degradation by NUDT3-Zn+2 regulates the architecture of nuclear speckles in human cells

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Inorganic polyphosphate (polyP) is a polyanionic molecule formed by hundreds of phosphate residues present in all organisms. In mammals, polyP is involved in crucial physiological processes, including coagulation, inflammation, and cellular stress response.

However, even after decades of research, cellular sub-localization of polyP and the enzymatic activities responsible for its metabolism are still unknown.

Recently, we have identified in NUDT3, a protein from the NUDIX family, as the enzyme responsible for *in vivo* polyP degradation in human cells. We have showed that NUDT3 shifts its substrate specificity depending on the cation; specifically, NUDT3 is active on inositol pyrophosphates or on polyP depending on the presence of Mg^{2+} or Zn^{2+}, respectively.

Moreover, we have demonstrated that polyP is mainly localized in the nucleus. Also, it shows a specific co-localization with SON and SRRM2, two of the main components of nuclear speckles (NS). Interestingly, different expression levels of NUDT3 modulate the nuclear amount of polyP and the architecture of NS accordingly.

The proteins of NS are involved in several nuclear processes like splicing, transcription or chromatin remodelling, suggesting that NUDT3 and polyP could play a role in regulation of gene expression in human cells through the stabilization of NS.

10- The landscape of accessible chromatin in mouse liver regeneration

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Regeneration is the ability of an organism to rebuild parts of the body that have been lost or damaged. The capacity to regenerate differs greatly among species, tissues and life stages. Recent studies of regenerating tissues in model organisms have revealed that chromatin structure, specific enhancers and transcriptional networks are regulated in a context-specific manner to control key gene expression programs. A deeper understanding of the gene regulatory networks of regeneration pathways might ultimately enable their targeted reactivation as a means to treat human injuries and degenerative diseases. Liver is a unique organ in displaying a reparative and regenerative response after damage or partial hepatectomy, when all the cell types must proliferate to re-establish the liver mass. To analyze the relationship between genome structure and changes in global patterns of gene expression during mouse liver regeneration, RNA-seq and ATAC-seq data has been obtained at 6h, 24h and 48h after 2/3 partial hepatectomy (PH). We have identified unique accessible regions in regenerating samples, which may correspond to regeneration-specific enhancers. We have determined that most of the regions detected exclusively during regeneration are timepoint-specific. Gene ontology (GO) analysis of target genes suggest that *de novo* accessible regions at 6h regulate expression of genes related to wound healing and inflammatory response. At 24h, we find enrichment of genes involved in metabolism and signal transduction. Finally, GO analysis indicates that unique open regions at 48h control the transcription of genes with a role in cell proliferation and growth.

11- Immune stimulation on primary ovary cell culture of zebrafish

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Zebrafish is a species widely used as model in both aquaculture and clinical research, thus, fish wellbeing during rearing is of the utmost importance. It is known that infections may alter the epigenome in some immune-targeted tissues, consequently altering fish physiology. Previous research showed a link between DNA methylation of two immune-related genes (*IL1β* and *Casp9)* to the sexual dimorphism in zebrafish gonads. However, to date, the influence of the immune response in the gonadal epigenome is not well documented. The aim of this study is to determine the alteration of the epigenome in ovarian cells in zebrafish. Here, using a new method of zebrafish ovarian cell culture, the influence of an immune stimulation by lipopolysaccharide (LPS) on the survival of the cells has been measured. LPS was added to primary culture at concentrations ranging from 12.5 to 500 µg/mL for 24 hours and flowcytometry was used to analyse and determine cell survival. Furthermore, the gene expression of three immune- (*IL1β*, *Casp9*, *IL6*) and three epigenetic- (*dnmt1*, *dnmt3*, and *tet1*) related genes have been determined. Results showed that LPS had no clear effect on cell morphology or survival rate with the highest concentration. Gene expression data showed that the immune-related genes *IL1β* and *Casp9* increased significantly with a fold change of 8.6 and 1.7, respectively, indicating that an immune response has been initiated with LPS in the cell culture. However, no changes were observed in *IL6*. Furthermore, *dnmt1* increased with a fold change of 1.7, although not significantly. Additionally, *dnmt3* and *tet1* were not differentially expressed. These findings suggest that LPS elicits an immune response but has little effect on the epigenetic mechanisms. In order to deeply study the epigenetic reprograming due to LPS on the fish ovarian cells, more research is conducted to determine the alterations at microRNA level.

12- Investigating the function of the linker domain of macroH2A in chromatin regulation with novel genome-editing approaches

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MacroH2A histone variants are tripartite proteins containing a histone-fold and a globular macrodomain connected by an unstructured linker region. Knock-down of macroH2A drastically modifies nuclear organisation and chromatin conformation causing changes in gene expression. The size and quantity of heterochromatin loci are also affected, which has been shown to be mediated by the poorly characterized linker domain. Specifically, the linker is intrinsically disordered and lysine-arginine rich, resembling the C-terminal tail of H1 histones and the hinge domain of HP1 proteins, functional domains related to phase separation and compaction of DNA. In vitro, the linker causes macroH2A protein aggregation, stabilises DNA at the nucleosome entry-exit site and binds RNA according to a high-throughput study. We aim to characterise the molecular function and regulation of the linker in the context of chromatin organisation and epigenetic regulation. For this we will determine macroH2As protein and nucleotide binding capacities, their relation to chromatin regulation and the domain requirements of these functions.

Typical knock-down and subsequent rescue studies of full length macroH2As do not compensate for the changes to nuclear architecture or gene expression. To overcome this limitation, we are pursuing the development of novel cellular models to better characterise the function of the protein and dissect the contribution of its different domains. In particular, we are using CRISPR/Cas9 to knock-in macroH2A mutants into the endogenous macroH2A locus leading to the simultaneous loss of function of the endogenous gene and replacement by a mutant protein form. These mutants will inform us of the importance of the sequence, conservation, and chemistry of the linker in various aspects of chromatin biology. We will combine several techniques interrogate the function of the different macroH2A domains in chromatin regulation, including chromatin immunoprecipitation, transcriptomics, mass spectrometry, RNA immunoprecipitation and chromosome conformation capture. Additionally, we are complementing these methods with a degron system for the rapid and inducible degradation of macroH2A which will allow us to include a temporal dimension to the study and differentiate early direct effects after loss of function of the protein from indirect or late events.

Taken together, we are implementing novel experimental approaches to overcome major limitations of previous studies and provide new insight into the function of macroH2A proteins in chromatin regulation and the particular role of its unstructured linker domain.

13- β cells regulatory functions and Type 1 Diabetes

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Type 1 Diabetes (T1D) is an autoimmune disease that develops from a complex interaction between genetic background and environmental factors. The disease develops as an autoimmune attack, provoking local inflammation of pancreatic islets (insulitis) and progressive loss of insulin-producing β cells[1]. During early insulitis, inflammatory mediators contribute to the functional suppression and apoptosis of β cells. Insulitis is complex and includes recurrent release of inflammatory mediators, which impacts the cells' gene regulatory networks[2]. However, the molecular mechanisms underlying β cell death in T1D pathogenesis are still not completely clear, limiting the development of new therapies to treat or prevent T1D. Recent studies point to the possibility that β cells actively contribute to their own demise[3,4] possibly mediated by the impact that the inflammatory environment has on the pancreatic β-cell chromatin landscape[5].

We hypothesize that by exploiting β cell-specific regulatory networks critical to the β cell survival in a cellular context that mimics the first phases of T1D, we can identify candidate genes that can, in turn, serve as potential biomarkers or drug targets.

In here, we present a novel *in vitro* model of early insulitis based on the co-culture of primary CD4 T lymphocytes with human pancreatic β cells. Preliminary results show that the activation of lymphocytes induces a transcriptional response in the β cells that include induction immune and stress response genes as well as the loss of critical β cell processes. By applying different techniques including ATAC-seq, Cut&Tag and RNA-seq in bulk and at a single cell resolution, further work is currently focus on assessing if transcriptional changes are coupled with chromatin structural changes affecting the tissue specific regulatory landscape.

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14- Understanding the role of cohesin, CTCF and GATA1 mutations in myeloid leukemia of Down syndrome

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Down syndrome children have a 500-fold increased risk of developing acute megakaryoblastic leukemia. Currently, the main treatment option is standard chemotherapy. Nonetheless, a substantial number of patients are refractory to it, or relapse and have a poor prognosis. Moreover, Down syndrome patients are more prone to suffer from toxicity to chemotherapy. Therefore, there is an unmet clinical need for new treatment options. At the genomic level, Down syndrome-associated acute megakaryoblastic leukemia is characterized by the pervasive acquisition of mutations in the GATA1 transcription factor and by an exceptionally high frequency of mutations in CTCF and in the cohesin complex, both involved in the maintenance of genome 3D structure. This disease represents an ideal model to understand the sequential acquisition of mutations in transcriptional regulators typical of many leukemia subtypes. Based on our recent findings linking cohesin to malignant hematopoietic development, and considering that CTCF works together with cohesin, we are studying the molecular basis of GATA1, CTCF and cohesin mutations in Down syndrome leukemia. Through a combination of genome-wide techniques and *in vivo* approaches, we are (i) analyzing the contribution of CTCF, cohesin and GATA1 to megakaryocytic differentiation, (ii) evaluating the cooperation between these mutations and trisomy 21, and (iii) investigating the functional properties of *in vivo* mutated cells. We expect that this project will not only shed new light on the mechanisms of cohesin and CTCF functions but will also uncover novel vulnerabilities and help the development of new therapeutic strategies.

15- Differential genomic distribution of human histone H1 variants

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Histone H1 binds to the linker DNA at the nucleosome, participating in the formation of higher-order chromatin structures. Human somatic cells may contain up to seven members of the histone H1 family contributing to the regulation of nuclear processes, apparently with certain subtype specificities. We have explored the functional role of histone H1 variants by shRNA-mediated knock-down of single or multiple H1s. In T47D breast cancer cells, the combined knock-down of H1.2 and H1.4 subtypes (multi-H1 KD) has a strong deleterious effect: coordinately deregulates many genes, promotes the appearance of accessibility sites genome-wide and triggers an interferon response via activation of heterochromatic repeats. Besides, multi-H1 KD translated into more de-compacted chromatin structures at the scale of topologically associating domains (TADs). Profiling of endogenous H1 variants in these cells revealed coexistence in the genome in two large groups depending on the local GC content: H1.2, H1.5 and H1.0 were abundant at low GC regions while H1.4 and H1X preferentially co-localized at high GC regions. Interestingly, above-mentioned chromatin changes upon multi-H1 KD occurred with only slight H1 variant redistributions across the genome. Imaging experiments of H1 variants also support differential genomic patterns revealed by ChIP-Seq data and variant-specific association to particular chromatin environments, such as lamina-associated domains (LADs) or the nucleolus.

16- Aurora Kinase B inhibitors: a search for new combinatorial strategies to target Ewing Sarcoma

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Ewing Sarcoma (EwS) is the second most common malignant neoplasm appearing in the bone and soft tissues that predominantly affects adolescents and young adults. EwS is an exclusive human disease cytogenetically characterized by a chromosomal translocation most commonly affecting chromosomes 11 and 22, t(11;22)(q24;q12). The resulting fusion protein, EWSR1-FLI1, has the ability to act as an aberrant transcription factor leading both to gene activation and repression of a large set of genes involved in the tumorigenic phenotype of EwS. Our group has recently demonstrated that the Polycomb subunit with E3 ubiquitin ligase, RING1B, co-localizes genome–wide with EWSR1-FLI1 at active enhancers having a key role in EwS tumorigenesis *in vivo*, mainly by regulating enhancers and recruiting the fusion oncogene to key targets such as *NKX2-2*, *SOX-2* and *IGF-1*.

Aurora Kinases are conserved serine/threonine kinases with high expression in many different tumors that play multiple roles in cell division and maintenance of genomic stability. We have demonstrated that EwS cell lines are specifically and highly vulnerable to inhibition by Aurora Kinase B (AURKB) inhibitors. In the present study we described the contribution of AURKB in the activation of RING1B-EWSR1-FLI1 enhancers necessary for EwS tumorigenesis. Furthermore, enhancer disruption with BET inhibitors, which is efficient in EwS cell lines, synergize with AURKB inhibitors and we proposed the combination between AURKB and BET inhibitors as a novel strategy to target EwS.

17- Profiling the insulinoma genetic landscape

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Insulinoma are rare neoplasms arising from insulin-producing β cells of the pancreas. Their incidence in the general population is of approximately one to four per million per year and their major genetic and epigenetic driver alterations are still unknown. We here collected a dataset of 28 insulinomas to characterize their genetic landscape by whole genome sequencing (WGS). We first defined the tumor mutational burden, described their mutational signatures and identified recurrent coding mutations. Next, we interrogated the cis-regulatory landscape in search of non-coding somatic mutations and describe examples of mutated regulatory regions that may provide insight into the mechanisms of malignant transformation. Overall our findings elucidate on the impact of somatic genomic variants to the loss of β -cell fate and the development of insulinomas.

18- Low input promoter capture Hi-C: a method to decipher the molecular mechanisms underlying non-coding alterations

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Long-range interactions between regulatory elements and promoters are key in gene transcriptional control but challenging to study due to the lack of low input methods. Here we introduce low input capture Hi-C (liCHi-C), a costeffective, customizable method to map and robustly compare promoter interactomes at high resolution in rare cell populations. As a proof of it broaden applicability, we use liCHi-C to in vivo study normal and malignant human hematopoietic hierarchy. We demonstrate that the dynamic promoter architecture foreshadows developmental trajectories meanwhile orchestrates transcriptional decisions along cell commitment. Simultaneously, liCHi-C enables the identification of new disease-relevant cell types, genes and gene pathways potentially deregulated by non-coding alterations at distal regulatory elements. Besides, we show liCHi-C ability to genome-wide uncover structural variants, resolved their breakpoints and propose pathogenic effects, including the formation of new regulatory landscape. Therefore, liCHi-C allows the study of previously unmeasurable cell types to ultimately illuminate disease etiopathogenesis.

19- Epigenome editing uncovers methylation-expression causality in leukemic cell fate model

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Aberrant DNA methylation is considered a hallmark of acute myeloid leukemia (AML), in which epigenetic enzymes are frequently mutated. It is broadly accepted that promoter CpG island hypermethylation leads to gene repression of tumor suppressor genes in a wide-range of cancers. However, the causal relationship between DNA methylation and gene expression and its relevance in cancer onset has not been fully elucidated.

To address this goal, we employ a recently developed epigenome editing tool to modify the DNA methylation status of specific CpG sites in a highly efficient cellular model of human leukemic B cell conversion into non-tumorigenic macrophages. For this, we use a catalytically dead Cas9 (dCas9) nuclease fused with DNA methylation editors *TET1* or *DNMT3A* for targeted erasure or establishment of DNA methylation, respectively. Interestingly, *interleukin-1 receptor antagonist* (*IL1RN)* promoter demethylation is associated with the reactivation of this gene during B cell conversion. Consequently, we design and insert single guide RNAs (sgRNAs) in the genome of human B leukemic cells to site-specifically modify the DNA methylation landscape in the *IL1RN* locus. Moreover, we set up a multiplex sgRNA expression system that allows more efficient DNA methylation editing. Our results suggest that continuous dCas9-DNMT3A recruitment at *IL1RN* promoter abrogates gene activation during B to macrophage conversion while dCas9-TET1-driven *IL1RN* promoter demethylation leads to gene reactivation. Thus, causality in the interplay between DNA methylation and gene expression events in leukemia onset and development can now be tackled through CRISPR/Cas9 epigenome editing tools.

20- RAD21L depletion alters the 3D genome architecture in the male germ line

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During meiotic prophase I, cohesin complexes participate in synapsis and recombination of homologous chromosomes by keeping the two sister chromatids together and holding chromatin loops to the synaptonemal complex (SC). RAD21L is a meiotic-specific cohesin subunit essential for synapsis and male fertility but its implications on the spatial folding of chromosomes during meiosis remain unclear. Here, we study the impact of RAD21L depletion on the 3D genome architecture in the male germ line by combining fluorescence activated cell sorting (FACS) and the chromosome conformation capture technique (Hi-C). We demonstrate that the loss of RAD21L prevents proper chromatin condensation during meiosis, with changes in the inter- and intra-chromosomal interactions ratio and the A/B compartmentalization in pre-meiotic (spermatogonia) and meiotic (primary spermatocytes) cells. We detected defects in the bouquet formation and an increase in telomeric interactions between heterologous chromosomes in primary spermatocytes, resembling telomere aberrations detected in other cohesin deficient models. Overall, our results show how the three-dimensional genomic structure is affected in the absence of the meiotic cohesin subunit RAD21L during mouse spermatogenesis.

21- scFLEA-ChIP: deciphering epigenetic signatures at single cell level

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All pluricellular organisms develop from a single totipotent cell. In the course of development, cells differentiate and get committed to distinct cell fates to, ultimately, form specialized tissues. Through these processes, cells accumulate epigenetic changes, such as DNA methylation and histone marks, leading to high epigenetic diversity within cell populations. Uncovering the epigenetic changes occurring along dynamic processes at the single cell resolution will foster the understanding of the establishment of cell lineages during development and differentiation, as well as other processes, such as cancer progression, and will allow to decipher how cellular heterogeneity contributes to different outcomes along these processes. However, the lack of a reproducible and endorsed protocol to identify protein-DNA interactions at single-cell level represents an important constraint in the field. Aiming to uncover cell heterogeneity from the epigenomic perspective and to provide the scientific community with an affordable single-cell ChIP-Seq protocol, we have recently developed single-cellFLEA-ChIP, a methodology to identify protein-DNA interactions at the single cell level. scFLEA-ChIP can be adopted by any Molecular Biology lab as it does not require the usage of specific devices or complex steps. It relies on cell sorting technologies to isolate and barcode individual cells prior to immunoprecipitation, reducing to the minimum cell remnants and undesired nucleic acids in suspension that could jeopardize the identification of single cell profiles. The scFLEA-ChIP also incorporates an innovative approach to efficiently amplify ultra-low input libraries that prevents the generation of thousands of a few PCR duplicates, avoiding bias derived from successive *in-vitro* transcription and retrotranscription rounds. Using this method, we have performed H3K4me3 scFLEAChIP assays from as few as 1,500 culture cells, obtaining chromatin profiles comparable to bulk ChIP-Seq experiments performed with a similar number of cells. We are currently applying this technology to delineate the epigenetic profile of individual cells from differentiation and cancer models to uncover cell heterogeneity and to decipher the relationship between different cell subpopulations.