Societat Catalana de BIOLOGIA

VII Jornada de Cromatina i Epigenètica

Organitzada per la Secció de Cromatina i Epigenètica de la Societat Catalana de Biologia (SCB)

amb el Barcelona Chromatin Club (BCC)

Societat Catalana de Biologia 1912-2012

BCC

Resums de les comunicacions

VII Annual Chromatin and Epigenetics symposium

Organized by the Chromatin and Epigenetics section of the Catalan Society of Biology (SCB)
—Albert Jordan—

and the Barcelona Chromatin Club (BCC)
—Sonia Forcales, Tanya Vavouri—

March 24, 2017

IEC: carrer del Carme, 47, Barcelona
Prat de La Riba hall

Sponsored by:

Institut d'Estudis Catalans

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LabClinics

Durviz

PROGRAM

8.20-8.50 ***Registration and documentation pickup***
8:50 ***Opening***

Session I. *Chair:* ***Albert Jordan***

9:00-9:20 **15min +5**
Alfred Cortés (CRESIB)
Adaptation to changes in the environment in malaria parasites: epigenetic variation, and what else?

9:20-9:40 **15min +5**
Thomas Graf (CRG)
Moving out of pluripotency

9:40-10:00 **15min +5**
Laia de Nadal (UPF)
A single-cell genetic screen uncovers the histone requirements in response to stress

10:00-10:20 **15min +5**
Oriol Bachs (IDIBAPS, UB)
Role of the cyclin-dependent kinase inhibitor p27(Kip1) in the regulation of transcription

10:20-10:40 **15min +5**
Ángel Barco (Inst. Neurociencias, IN-UMH-CSIC, Alicante)
Epigenetic etiology of intellectual disability syndromes

10:40-10:50 **9 x 1min**
Speed poster presentations

10:50-11:20 ***Coffee break and poster session***

Session II. *Chair:* ***Luciano di Croce***

11:20-11:40 **15min +5**
Luciano di Croce (CRG)
Gene regulation dynamics mediated by Polycomb complexes

11:40-12:00 **15min +5**
Marian Martínez Balbas (IBMB-CSIC)
H3K27me3 demethylase, JMJD3, activates neuronal enhancers

12:00-12:20 **15min +5**
Maribel Parra (PEBC-IDIBELL)
Gene silencing mechanims during B cell development

12:20-12:40 **15min +5**
Alicia Roque (UAB)
Complex Evolutionary History of the Mammalian Histone H1.1–H1.5 Gene Family

12:40-13:00 **15min +5**
Alejandro Vaquero (PEBC-IDIBELL)
Role of SIRT7 in stress response

13:00-14:30 ***Lunch and poster session***

Session III-BCC7. Epigenetic inheritance
Chair: ***Tanya Vavouri***

14:30-15:00 **25min +5**
Vardham Rakyan (Blizard Inst., London, UK)
The role of epigenetic mechanisms in the developmental origins of mammalian phenotypes

15:00-15:20 **15min +5**
Ben Lehner (CRG)
Long lasting trans-generational epigenetic transmission of environmental information in an animal

15:20-15:35 **10min +5**
Adelheid Lempradl (Max Planck Inst. Immunobiol. and Epigenetics, Germany)
Involvement of the piRNA pathway in intergenerational inheritance

15:35-15:55 **15min +5**
Tanya Vavouri (IJC & PMPPC)
5-Aza-2-deoxycytidine mediated activation of repeats and inactivation of enhancers

15:55-16:00 Sponsor' talk **5min +2**
Sarantis Chlamydas (Active Motif)
TBA

16:00-16:20 ***Coffee break and poster session***

Session IV. *Chair:* ***Miquel A. Peinado & Marcus Buschbeck***

16:20 Short Talk 1 **7min +3**
Ricky S. Joshi (IBMB-CSIC)
Epimutations as a novel cause of congenital disorders

16:30 Short Talk 2 **7min +3**
Robert Albero (IDIBAPS-UB)

Cyclin d1 oncogenic overexpression leads to a global transcriptional downregulation in malignant lymphoid cells

16:40 Short Talk 3 **7min +3**
Alberto de la Iglesia (IDIBAPS-UB)
Characterization of histone specific H4K5 butyrylation pattern during human spermatogenesis

16:50 Short Talk 4 **7min +3**
Mireia Ramos (IGTP)
The impact of pro-inflammatory cytokines on the regulatory landscape of the pancreatic beta-cells

17:00 Short Talk 5 **7min +3**
Elena Vizcaya (UB)
Chromatin landscape and transcriptional program of regeneration

17:10 Short Talk 6 **7min +3**
Roser Vilarrasa (UB)
Activation and de novo 3D looping of a distant enhancer element leads to SOX11 oncogene expression in mantle cell lymphoma

17:20 Short Talk 7 **7min +3**
Estela Dámaso (ICO-IDIBELL)
Primary constitutional *MLH1* epimutations: a focal epigenetic event

17:30 Short Talk 8 **7min +3**
Mireia Jordà (PMPPC-IGTP)
A DNA methylation signature associated with metastatic thyroid cancer

17:40-18:00 **15min +5**
Bernhard Payer (CRG)
X-Chromosome reactivation: An Epigenetic Hallmark of Pluripotency and Germ Cell Development

18:00-18:20 **15min +5**
Roderic Guigó (CRG)
Transcription without histone modifications. The paradox of correlations

18:20-18:40 **15min +5**
Marcus Buschbeck (IJC)
A histone variant links chromatin state and metabolic activity

18.45 Meet together for a beer

Secretaries of SCB:

Mariàngels Gallego and Maite Sánchez
Societat Catalana de Biologia
C/ Maria Aurèlia Capmany, 14-16, 08001 Barcelona.
Tel. 933 248 584; E-mail: *scb@iec.cat*

Organized by:

Albert Jordan Vallès
Coordinator of the Chromatin and Epigenetics section of the SCB
Dept. Molecular Genomics, Institut de Biologia Molecular de Barcelona (IBMB-CSIC), Parc Científic de Barcelona
A/e: *albert.jordan@ibmb.csic.es*

Coorganized by:

Sònia Forcales and Tanya Vavouri
Coordinators of the Barcelona Chromatin Club
Programa de Medicina Predictiva i Personalitzada del Càncer (PMPPC)
A/e: *bcc@igtp.cat*

POSTERS

1. Vanesa Izquierdo Cadenas (UB)
Influence of diet during the development of samp8 mice: epigenetic and cognitive changes

2. Andrea Izquierdo Bouldstridge (IBMB-CSIC)
Histone H1 depletion triggers an interferon response in cancer cells

3. Irene Miguel Escalada (IDIBAPS-UB)
Unravelling the 3D chromatin topology of human pancreatic islets

4. Anna Casas Lamesa (IBMB-CSIC, IRB)
Linker histone H1 prevents R-loop accumulation and genome instability in heterochromatin

5. Ada Soler Ventura (IDIBAPS-UB)
Identification of the human sperm protamine post-translational modifications code through LC-MS/MS.

6. Noelia Díaz (Max Planck Institute for Molecular Biomedicine)
Characterisation of the zebrafish 3d genome

7. Dafni Anastasiai (ICM-CSIC)
Age-related DNA methylation changes in somatic and gonadal tissues of teleost fish (European sea bass)

8. Paula Climent (IBMB-CSIC, IRB)
Effects of the expression of the *Drosophila* embryonic linker histone dBigH1 on the epigenetic state of chromatin

9. Albert Carbonell (IBMB-CSIC, IRB)
The germline linker histone dBigH1 and the translational regulator Bam form a repressor loop essential for male germ stem cell lineage differentiation.

10. Luz Jubierre (VHIR)
BRG1: The Dr. Jekyll & Ms. Hide of cancer.

11.Carlos Jiménez (VHIR)
Targeting ZRF1 as a new epigenetic therapy strategy for neuroblastoma.

ABSTRACTS

Involvement of the piRNA pathway in intergenerational inheritance

Adelheid Lempradl

Max Planck Institute of Immunobiology and Epigenetics, Freiburg, Germany

Given the rise in obesity and the parallel increase in comorbidities like diabetes, cardiovascular disease, stroke and cancer, it is exceedingly important to begin a systematic examination into heritable effects of diet and other environmental factors. We established a *Drosophila* model of paternal-diet-induced *I*nter-*G*enerational *M*etabolic *R*eprogramming (IGMR). Intriguingly, as little as two days of dietary intervention in fathers can stably reprogram offspring physiology, lifelong, confirming the responsiveness of mature sperm to physiological cues. Paternal sugar leads to H3K9/K27me3 dependent alterations that affect expression of metabolic genes in two distinct germline and zygotic windows. Novel findings provide evidence towards the involvement of the piRNA pathway in IGMR giving direction to the most difficult question challenging the field: What is the nature of the intergenerational signal? Mutant analysis, used to genetically dissect the gene regulatory network responsible for the initiation, establishment and/or transfer of IGMR, helped identify specific piRNA pathway members to be involved in either the generation of the signal and/or the establishment of the phenotype in the offspring. Small RNA sequencing revealed diet induced chromatin specific changes in the small RNA profiles of both mature sperm and early offspring embryos.

Long lasting trans-generational epigenetic transmission of environmental information in an animal

Ben Lehner (CRG)

A fundamental question in genetics is the extent to which environmentally-acquired information can be transmitted from an animal to its descendants. I will present an example of trans-generational epigenetic inheritance where a temperature-induced change in gene expression lasts for >10 generations in C. elegans. Inheritance is primarily in cis and is associated with a specific heterochromatin modification before the onset of transcription in the embryo. Transmission occurs through both oocytes and sperm. Long-lasting chromatin-associated epigenetic memory of environmental change therefore occurs in an animal.

Short talk 1

Epimutations as a novel cause of congenital disorders

Ricky S. Joshi[1]

[1] Icahn School of Medicine at Mount Sinai, New York, NY, USA

Despite the application of arrays, exome- and whole-genome sequencing in patients with multiple congenital anomalies and intellectual disability (ID/MCA), causative mutations can typically be identified in only a minority of cases. We therefore hypothesized that some cases of ID/MCA are instead caused by underlying epigenetic aberrations that dysregulate normal genome function, and that such events would be missed by conventional sequence-based mutation screening. We therefore performed genome-wide DNA methylation profiling in 500 individuals with ID/MCA, all of whom were negative for causative mutations by array CGH, exome- and/or whole genome sequencing. To identify disease-associated epimutations, we screened methylation profiles in disease cases for differentially methylated regions (DMRs) absent in 4,859 controls, identifying 173 epimutations in 118 of the individuals tested (28% of the cohort). Validation studies indicate an 83% true positive rate, and showed that all epimutations represent large methylation changes occurring specifically on one allele. This included seven recurrent DMRs, two of which (*FMR1*, *MEG3*) already have known disease associations, thus validating our method for detecting pathogenic events. Other recurrent changes include hypo-methylation of the imprinted locus *NAA60*|*ZNF597*, likely representing a novel imprinting disorder, and hypo-methylation at the promoter of *MOV10L1*, a gene with an embryonic heart-specific isoform that interacts with the master cardiac transcription factor NKX2.5, in two patients with congenital heart defects. We also identified five patients each carrying an average of 16 *de novo* autosomal DMRs suggesting a global epigenetic disturbance in these cases, and one female with a likely abnormality of X chromosome inactivation characterized by aberrant methylation at dozens of X-linked gene promoters.

We performed a variety of other studies in different cohorts to investigate the nature of epimutations and conclude: (i) The presence of an epimutation is frequently associated with extreme outlier and mono-allelic gene expression, indicating functional consequences comparable to loss-of-function sequence mutations; (ii) Epimutations are generally conserved across multiple different tissues within an individual, validating the use of peripheral blood DNA for the study of ID/MCA; (iii) While a subset of epimutations occur secondary to *cis*-linked regulatory mutations, many others are sporadic *de novo* events that are frequently reset between generations by epigenetic reprogramming during embryogenesis.

Overall we conclude that pathogenic epimutations likely underlie 5-10% of patients with ID/MCA who are refractory to conventional mutation screening approaches, and propose that epigenome profiling represents a promising new avenue for the diagnosis of human disease.

Short talk 2

CYCLIN D1 ONCOGENIC OVEREXPRESSION LEADS TO A GLOBAL TRANSCRIPTIONAL DOWNREGULATION IN MALIGNANT LYMPHOID CELLS

Robert Albero [1], Anna Enjuanes [2], Santiago Demajo[1] , Giancarlo Castellano[3], Magda Pinyol[2], Noelia Garcia [1], Cristina Capdevila[1], Helena Suarez[2], Shimada M[4], Kenosuke Karube[4], Silvia Bea[1,5] , Ignacio Martin-Subero[1,5] , Elias Campo[1,4,5] , Pedro Jares [5]

[1]*Lymphoid Neoplasm Program, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.*

[2]*Genomics Unit, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Barcelona, Spain.*

[3]*Core Biologia Molecular, Hospital Clínic, Barcelona, Spain*

[4]*f Laboratory of Anatomic Pathology, Hospital Clinic of Barcelona, Universiy of Barcelona, Barcelona, Spain.*

[5]*Centro de Investigación Biomédica en Red de Cáncer (CIBERONC)*

E-mail: ralbero@clinic.ub.es

Cyclin D1 is an oncogene frequently overexpressed in human cancers, such as breast cancer, head and neck, colon carcinoma or hematological neoplasm. Among them, mantle cell lymphoma and multiple myeloma present deregulated cyclin D1 expression due to a t(11;14) translocation. Cyclin D1 plays a dual function as cell cycle and transcriptional regulator, although the latter is widely unexplored. In this study, we investigate the transcriptional role of cyclin D1 in lymphoid tumor cells.

Chromatin immunoprecipitation (ChIP) followed sequencing was performed in four established MCL cell lines. RNA-Sequencing (RNA-Seq) and histone ChIP-Seq data were correlated with genomic intervals displaying cyclin D1 binding. Transcriptional downregulation was studied in lymphoblastic cyclin D1 inducible overxpressing models, through cytometric and spectophotometric RNA quantification. RNA Pol II ChIP-Seq was also performed in inducible cell lines in order to study Pol II occupancy upon cyclin D1 expression.

Endogenous cyclin D1 showed widespread binding to active promoters and its overexpression was responsible for a global transcriptional downmodulation. Cyclin D1, instead of showing specific gene activation, seems to globally decrease cell transcription. Total transcriptional reduction correlated with cyclin D1 quantification. Moreover, mantle cell lymphoma and multiple myeloma cell lines displayed an inverse relation of transcription and cyclin D1 quantity. This transcriptional effect was associated with an increased RNA polymerase II pausing in promoters due to cyclin D1 overexpression. This mechanism places the transcriptional machinery in the centre of the oncogenic functions of cyclin D1 in human cancers.

Short talk 3

Characterization of histone specific H4K5 butyrylation pattern during human spermatogenesis

Alberto de la Iglesia[1]*, Ferran Barrachina[1]*, Afsaneh Goudarzi[2], Sophie Rousseaux[2], Saadi Khochbin[2], Carme Mallofré[3], Leonardo Rodriguez-Carunchio[3], Josep Lluís Ballescà[4], Rafael Oliva[1]

1. Molecular biology of Reproduction and Development Research Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Faculty of Medicine, University of Barcelona, Barcelona, Spain, and Biochemistry and Molecular Genetics Service, Hospital Clínic, Barcelona, Spain (roliva@ub.edu)
2. CNRS UMR 5309; Inserm, U1209; Université Grenoble Alpes; Institute for Advanced Biosciences, Grenoble, F-38700 France
3. Department of Pathology, University of Barcelona. Hospital Clínic, Barcelona, Spain
4. Clinic Institute of Gynaecology, Obstetrics and Neonatology, Hospital Clínic, Barcelona, Spain

*Equal contribution

Histone post-translational modifications (PTMs) are epigenetic modifications shown to play a crucial role in chromatin remodeling during the male gametogenic process. The recent finding of histone PTMs acylations other than the well-known lysine acetylation (Kac), such as lysine butyrylation (Kbu), has broadened the field of epigenetic regulation of gene expression during spermatogenesis. Kbu stimulates transcription and is known to directly compete with Kac, being specifically relevant in H4K5, residue in which the acetylation-dependent nucleohistone to nucleoprotamine transition is triggered. Given the importance of butyrylation in chromatin remodeling throughout spermatogenesis, the aim of our study is to characterize for the first time the pattern of H4K5bu during the different stages of the human spermatogenic process and to compare it to patients with altered spermatogenesis. To address this issue, immunohistochemistry (IHC) and immunofluorescence (IF) assays were performed in human testicular biopsies and sperm cells, respectively. IHC results in normal spermatogenesis revealed high levels of H4K5bu around the stages of elongated spermatids, a similar behavior than H4K5ac (previously described). This observation supports the hypothesis that in human, as well as previously shown in mouse, a dynamic H4K5bu-H4K5ac competing mechanism would lead to the control of histone-to-protamine transition in male gametogenic cells. Furthermore, IHC in patients with altered spermatogenesis (hypospermatogenesis, spermatogenic arrest or testicular cancer) evidences different deregulations of the described pattern, suggesting that this histone-specific PTM could be involved in the suitable development of spermatogenesis. Briefly, our findings contribute to nourish the knowledge of this non-acetyl acylation during human spermatogenesis supporting a novel competing mechanism of chromatin remodeling required for the histone-to-protamine transition process. Supported by Ministerio de Economía y Competitividad, Fondos FEDER, ISCIII (PI16/00346, PI13/00699), Fundación Salud 2000 (SERONO 13-015) and EUGIN-UB (EU-REP 2014), to RO.

Short talk 4

The impact of pro-inflammatory cytokines on the regulatory landscape of the pancreatic beta-cells.

Ramos-Rodríguez M.[1] Raurell-Vila H.[1] Juan-Mateu J.[2], Eizirik DL.[2], Pasquali L.[1,3,4]

1 Endocrine Regulatory Genomics, Program of Predictive and Personalized Medicine of Cancer (PMPPC), Germans Trias i Pujol University Hospital and Research Institute, Badalona, Spain.

2 Medical Faculty ULB Center for Diabetes Research and Welbio, Université Libre de Bruxelles (ULB), Brussels, Belgium.

3 Josep Carreras Leukaemia Research Institute, Badalona, Spain.

4 CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), Barcelona, Spain.

Type 1 Diabetes (T1D) is a chronic autoimmune disease that develops as a consequence of a combination of genetic predisposition and environmental factors. Combined these events trigger an aggressive autoimmune assault against the pancreatic β-cells provoking local inflammation of pancreatic islets (insulitis) and progressive loss of β-cells due to apoptosis.

During early insulitis, inflammation contributes to both the primary induction and secondary amplification of the immune assault with inflammatory mediators contributing to the functional suppression and apoptosis of β-cells. In this context a "dialogue" between the invading immune cells and the target β-cells, mediated by cytokines/chemokines released by both cell populations, is established and subsequently maintained by putative immunogenic signals delivered by dying or "altered" β-cells. Although several advances allowed a partial understanding of the disease physiopathogenesis, the precise mechanisms by which autoimmunity is triggered and aggravated in T1D remain to be clarified.

We have now mapped cis-regulatory networks in pancreatic β-cells exposed to pro-inflammatory cytokines to identify critical molecular pathways central to the activation and modulation of β-cell survival. By applying genome wide epigenomic analysis we find that exposure to proinflammatory cytokines cause profound changes in the pancreatic β-cells transcriptome and regulatory landscape. We exploit such changes to unmask regulatory networks involved in the pathogenesis of the disease and to uncover genes that could serve as potential biomarkers to monitor the disease progression. To our knowledge these experiments represent a novel approach that will open unexplored paths in the context of the molecular mechanisms underlying T1D.

Short talk 5

CHROMATIN LANDSCAPE AND TRANSCRIPTIONAL PROGRAM OF REGENERATION

Elena Vizcaya[1], Cecilia Klein[2], Carlos Camillieri[1], Florenci Serras[1], Roderic Guigo[2], Montserrat Corominas[1]

[1]Universitat de Barcelona, Genetics, Microbiology and Statistics, Institute of Biomedicine (IBUB), Barcelona, Spain
[2]Centre for Genomic Regulation (CRG), Universitat Pompeu Fabra, Bioinformatics and Genomics, Barcelona, Spain

Regeneration is the ability to renew or reconstruct missing parts. A variety of mechanisms have been proposed to explain regeneration, ranging from stem cells to tissue remodeling, however, a central question is the identification of specific regulatory regions capable to trigger regeneration. *Drosophila* imaginal discs are epithelia that activate a regenerative response upon cell death. We used these epithelia to unveil the transcriptional program as well as the regulatory elements and reorganization of genome architecture responsible for tissue regeneration. Engineered flies allowed us to conditionally induce apoptosis, controlling the time of cell death and the zone to be removed. We report here genome-wide chromatin analyses (ATAC-Seq) and RNA-Seq (protein coding and long non coding RNAs) at different time points of regeneration after cell death induction. By integrating the maps of cis-regulatory elements and transcriptomic analyses we have constructed the gene regulatory network, with the aim to identify which regulatory regions are fundamental during the process. Comparison between control discs and discs immediately after cell death shows more regions of accessible chromatin, which correlates with higher number of up-regulated genes detected at the same time point. Both ATAC and RNA profiles tend to be more similar in late regeneration, when the recovery process is almost completed. Based on their presence or absence in control discs we have divided enhancers in developmental or damage-specific and, depending on their position relative to the TSS, classified them in proximal (5kb from the TSS) or distal (more than 5KB from the TSS). Distal enhancers have been linked to clusters of genes that show similar expression profiles, and both can be associated based on their position inside the same Topologically Associated Domains (TADs), meanwhile proximal enhancers tend to fall inside the cluster of co-regulated genes. We have also validated several damage-induced enhancers using reporter fly lines after inducing apoptosis as well as after physical injury. Moreover, Chromatin Conformation Capture analysis (3C) has been used to confirm interacting chromatin loops between regulated genes with distal and proximal enhancers. Our work provides a frame to understand gene expression regulation after damage and confirms that specific regeneration enhancers exist.

Short talk 6

Activation and De Novo 3D Looping of a Distant Enhancer Element Leads to SOX11 Oncogene Expression in Mantle Cell Lymphoma

Authors: Roser Vilarrasa-Blasi1,14, Ana C. Queirós1,14, Renée Beekman2,14, Nuria Russiñol1,2, Giancarlo Castellano2, Julie Blanc3, François Serra3, Sílvia Beà2, Marta Kulis1, Núria Verdaguer-Dot1,2, Anna Enjuanes4, Anke Bergmann5,6, Itziar Salaverría2, Harmen J. G. van de Werken7,8,9, Avik Datta10, Paul Flicek10, Joost Martens11, Hendrik G. Stunnenberg11, Marta Gut3, Marc Marti-Renom3, Ivo G. Gut3, Reiner Siebert5, Elías Campo2,12,13 & José I. Martín-Subero1,2

1Departament de Fonaments Clínics, Universitat de Barcelona, Facultat de Medicina, 08036 Barcelona, Spain; 2Institut d'Investigacions Biomèdiques August Pi I Sunyer (IDIBAPS), 08036 Barcelona, Spain; 3Centre Nacional d'Anàlisi Genòmica-Centre de Regulació Genòmica (CNAG-CRG), 08028 Barcelona Spain; 4Unitat de Genòmica, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), 08036 Barcelona, Spain; 5Institute of Human Genetics, Christian-Albrechts University, 24105 Kiel, Germany; 6Department of Pediatrics, Christian-Albrechts University & University Hospital Schleswig-Holstein, 24105 Kiel, Germany; 7Department of Cell Biology, Erasmus MC, 3015 CN Rotterdam, Netherlands; 8Cancer Computational Biology Center, Erasmus MC, 3015 CN Rotterdam, Netherlands; 9Department of Urology, Erasmus MC, 3015 CE Rotterdam, Netherlands; 10European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Hinxton, CB10 1SD, United Kingdom; 11Radboud Institute for Molecular Life Sciences, 6525 GA Nijmegen, Netherlands; 12Hospital Clínic, Universitat de Barcelona, 08036 Barcelona, Spain; 13Departament d'Anatomia Patològica, Hospital Clínic and Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Universitat de Barcelona, 08036 Barcelona, Spain

14Co-first author

Presenter email: rvilarrasa@clinic.ub.es

Three-dimensional chromatin structure plays an important role in regulating gene expression by bringing remote enhancers in physical proximity with their target genes. Recent reports suggest that oncogene deregulation in cancer can be mediated by the activation of distant enhancers. The oncogene SOX11 is a transcription factor (TF) upregulated in the majority of mantle cell lymphomas (MCLs), but such alteration is not related to genetic aberrations or DNA methylation changes at the gene promoter.

We previously identified a distant putative SOX11 regulatory element showing enhancer activity and 3D contacts with the SOX11 gene in a SOX11-positive MCL cell line, but not in a SOX11-negative MCL cell line. Here, to deepen into this phenomenon, we applied HiC-sequencing on these MCL cell lines and observed that this DNA looping was not associated with a shift in topologically associated domain (TAD) boundaries. We then performed ATAC-seq, ChIP-seq for different histone modifications and chromatin state modeling in normal B cells and samples from MCL patients. We observed that the SOX11 promoter is poised in normal naive and memory B cells and SOX11-negative MCLs. In SOX11-positive MCL samples, we identified a strong enhancer activity and two specific chromatin accessible regions. By 4Csequencing we confirmed high 3D contacts between SOX11 locus and the distant enhancer in two other SOX11-positive MCL cell lines and in a SOX11-positive primary MCL case, but not in a SOX11-negative primary MCL case. Interestingly, no 3D contacts were observed in normal B cells, indicating the absence of primed looping at these regions. These results are being currently validated by 3D FISH at the single cell level.

In summary, we provide evidence indicating that aberrant upregulation of the SOX11 oncogene in MCL is associated with de novo activation of a distant enhancer element which interacts at a 3D level with the SOX11 gene.

Short talk 7

PRIMARY CONSTITUTIONAL *MLH1* EPIMUTATIONS: A FOCAL EPIGENETIC EVENT

Estela Dámaso[1], Adela Castillejo[2], María del Mar Arias[3], Matilde Navarro[1], Jesús del Valle[1], Olga Campos[1], Anna Fernández[1,] Fernando Setien[4], Fatima Marín[1], Daniela Turchetti[5], Juan de Dios García-Díaz[6], Maurizio Genuardi[7], Daniel Rueda[8], Ángel Alonso[3], Jose Luis Soto[2], Megan Hitchins[9], Marta Pineda[1] and Gabriel Capellá[1].

(1) Hereditary Cancer Program, Catalan Institute of Oncology - Bellvitge Biomedical Research Institute (ICO-IDIBELL), CIBERONC, L'Hospitalet de Llobregat, Barcelona, Spain;
(2)Hereditary Cancer Program Valencian Region, Molecular Genetics Laboratory, Elche University Hospital, Elche, Alicante, Spain;
(3) Genetics Service, Complejo Hospitalario de Navarra,Pamplona, Navarra, Spain;
(4) Cancer Epigenetics and Biology Program (PEBC), Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain;
(5) Dipartimento di Scienze Mediche e Chirurgiche, Alma Mater Studiorum - Università di Bologna, Bologna, Italy;
(6) Unidad de Genética Clínica, Servicio de Medicina Interna, Hospital Universitario Príncipe de Asturias, Alcalá de Henares, Madrid, Spain;
(7) Genetics Medicine Institute, Catholic University of the Sacred Heart, Rome, Italy;
(8) Hereditary Cancer Genetic Diagnostic Laboratory. Doce de Octubre University Hospital, Madrid, Spain;
(9) Department of Medicine, Division of of Oncology, Stanford University,Stanford, California, United States.

Introduction: Constitutional *MLH1* epimutations are an alternative cause for Lynch syndrome, characterized by monoallelic methylation of the *MLH1* promoter throughout normal tissues, resulting in allele-specific loss of *MLH1* expression. While secondary epimutations are caused by an adjacent genetic alteration and are dominantly transmitted, primary epimutations typically arise *de novo* and are reversible between generations. The aim of this study was to perform a molecular characterization of constitutional MLH1 epimutations aiming at the elucidation of their causal mechanism.

Patients and methods: Twelve carriers of *MLH1* constitutional epimutation (4 of them previously unreported) were recruited. Global methylome analysis was performed using Infinium 450K array (Illumina) in blood DNA from all 12 *MLH1* epimutation carriers, 61 Lynch syndrome patients (19 *MLH1*-mutated) and 42 healthy controls. A variety of sequencing techniques, SNUPE and customized CGH arrays were used to further characterize *MLH1* epimutation carriers. Inheritance pattern was determined by MS-MLPA and haplotype analyses.

Results: The *EPM2AIP1-MLH1* CpG island was the sole differentially methylated region (FDR<0.05) in *MLH1* epimutation carriers compared to Lynch syndrome cases or healthy controls, pointing to it as the candidate region to search for the causal mechanism for epimutations. No germline point mutations or structural variants were identified *in-cis* on the methylation-associated allele in 10 epimutation carriers, suggesting these are cases of primary epimutation. In one of these patients, the promoter variant c.-234_-236del was identified in-*trans*. Small deletions (range size 15-20 Kb) outside the *EMP2AIP1-MLH1* CpG island were found in 2 patients (phase unknown). In 5 patients heterozygous at rs1799977 the transcriptional silencing of the methylated allele was evidenced. Intergenerational erasure of the epimutation was demonstrated in two families.

Conclusions: Suspected primary constitutional *MLH1* epimutations arise as a focal epigenetic event covering the *EPM2AIP1-MLH1* CpG island and are not associated with cis-acting genetic variants. Refined molecular characterization is needed to elucidate the mechanistic basis of *MLH1* constitutional epimutations and their heritability/reversibility.

Short talk 8

A DNA methylation signature associated with metastatic thyroid cancer

Helena Rodríguez, Núria Villalmanzo, Carles Zafón, Carmela Iglesias, Esther N. Klein Hesselink, Bettien M. van Hemel, Joan Gil, Cristina Montero, Jordi Reverter, Dídac Mauricio, Manel Puig, Mercedes Robledo, Thera Links, Mireia Jordà

1. Helena Rodríguez: Program for Predictive and Personalized Medicine of Cancer, Germans Trias i Pujol Research Institute (PMPPC-IGTP), Badalona 08916, Spain; helen.helena1994@gmail.com
2. Núria Villalmanzo: Program for Predictive and Personalized Medicine of Cancer, Germans Trias i Pujol Research Institute (PMPPC-IGTP), Badalona 08916, Spain; nvillalmanzo@igtp.cat
3. Carles Zafón: University Hospital Vall d'Hebron and Autonomous University of Barcelona, Diabetes and Metabolism Research Unit (VHIR) and Department of Endocrinology, Barcelona 08035, Spain; czafon@vhebron.net
4. Carmela Iglesias: University Hospital Vall d'Hebron, Department of Pathology, Barcelona 08035, Spain; ciglesias@vhebron.net
5. Esther N. Klein Hesselink: University of Groningen and University Medical Center Groningen, Department of Endocrinology, 9700 RB Groningen, The Netherlands; e.klein01@umcg.nl
6. Bettien M. van Hemel: University of Groningen and University Medical Center Groningen, Department of Pathology, 9700 RB Groningen, The Netherlands; b.m.van.hemel@umcg.nl
7. Joan Gil: Program for Predictive and Personalized Medicine of Cancer, Germans Trias i Pujol Research Institute (PMPPC-IGTP), Badalona 08916, Spain; jgil@igtp.cat
8. Cristina Montero: Hereditary Endocrine Cancer Group, Spanish National Cancer Research Centre (CNIO), Madrid 28029, Spain; cmontero@cnio.es
9. Jordi Reverter: Germans Trias i Pujol Research Institute and University Hospital, Department of Endocrinology and Nutrition, Badalona 08916, Spain; reverter.germanstrias@gencat.cat
10. Dídac Mauricio: Germans Trias i Pujol Research Institute and University Hospital, Department of Endocrinology and Nutrition, Badalona 08916, Spain; dmauricio.germanstrias@gencat.cat
11. Manel Puig: Germans Trias i Pujol Research Institute and University Hospital, Department of Endocrinology and Nutrition, Badalona 08916, Spain; mpuigd@igtp.cat
12. Mercedes Robledo: Hereditary Endocrine Cancer Group, Spanish National Cancer Research Centre (CNIO), Madrid 28029, Spain
13. Thera Links: University of Groningen and University Medical Center Groningen, Department of Endocrinology, 9700 RB Groningen, The Netherlands; t.p.links@umcg.nl
14. Mireia Jordà: Program for Predictive and Personalized Medicine of Cancer, Germans Trias i Pujol Research Institute (PMPPC-IGTP), Badalona 08916, Spain; mjorda@igtp.cat

Thyroid cancer is the most common endocrine malignancy and the majority of patients have an excellent prognosis. However, around 20% show a poor outcome. To date, risk stratification is mainly based on clinicopathological features and there are still no effective methods to determine which patients will eventually develop local or distant recurrence. A better understanding of the molecular pathogenesis of the disease will be key.

As in most cancers, in thyroid cancer not only genetic alterations but also epigenetic deregulation play a major role. However, the involvement of DNA methylation in the progression of thyroid cancer is completely unknown. Thus, the aim of this study was to investigate whereas DNA methylation plays a role in the metastatic process of thyroid cancer and can be used to predict the metastatic risk from primary tumors.

Here we performed genome-wide DNA methylation profiling using the Illumina Infinium HumanMethylationEPIC platform on a formalin-fixed paraffin-embedded samples series composed of 68 primary thyroid tumors (30 non-metastatic and 38 metastatic), 18 paired metastases and 15 adjacent normal tissues.

According to previous studies, including ours, we found a close relationship between the methylation profile and the histology and mutational status. Moreover, metastatic tumors were associated with a higher frequency of DNA methylation alterations, and their methylomes were similar to those of the matched metastatic tissues. Most interestingly, we identified a 156 CpG-signature associated with metastatic tumors independently of histology and mutation. These differentially methylated sites were enriched in hypomethylations and over half were located in promoters and enhancers, suggesting their role in gene expression regulation. Further studies are required to investigate their functional implications.

In conclusion, this comprehensive study provides novel insights into the role of DNA methylation in metastatic thyroid cancer as well as potential biomarkers for risk stratification and recurrence.

Poster 1

INFLUENCE OF DIET DURING THE DEVELOPMENT OF SAMP8 MICE: EPIGENETIC AND COGNITIVE CHANGES

Vanesa Izquierdo Cadenas, Veronica Palomera-Avalos, Christian Griñan y Mercè Pallàs.

Department of Pharmacology, Toxicology and Therapeutic Chemistry, Faculty of Pharmacy. University of Barcelona

The SAMP8 mouse is one of the nine strains of mice with accelerated senescence (SAM) most used for the study of neurodegenerative diseases, since, has certain characteristics indicative of accelerated aging, such as: loss of hair, changes a cognitive level, increased oxidative stress and impaired precursor protein β-amyloid and tau phosphorylated. Therefore, the objective was to investigate whether a diet rich in resveratrol can potentially make changes both cognitive level as epigenetic in this model. To study these changes different behavioral tests were performed to analyze the cognitive status of the animal and subsequently different molecular tests. The results show that mice whose mothers were fed resveratrol have a cognitive improvement in tests such as NORT and MWM, and that this improvement correlated with improved markers such as oxidative stress and neuroinflammation in hippocampal tissue. There have also been changes at the epigenetic level (DNA methylation, histone acetylation...). So we can conclude that resveratrol consumption by mothers produce epigenetic level changes and these changes to the next generation are transmitted improving producing different molecular mechanisms helping to cognitive improvement SAMP8 mouse

Poster 2

Histone H1 depletion triggers an interferon response in cancer cells

Andrea Izquierdo-Bouldstridge[1,#], Alberto Bustillos[1,#], Carles Bonet-Costa[1], Albert Jordan[1]

[1] Institut de Biologia Molecular de Barcelona (IBMB-CSIC), Barcelona

[#] These two authors contributed equally to this work.

Seven linker histone H1 variants exist in human somatic cells (H1.1 to H1.5 being expressed in a replication-dependent manner, whereas H1.0 and H1X are replication-independent), with distinct prevalence depending on the cell type and along differentiation. H1 bind to linker DNA contributing to higher order chromatin compaction. In addition, H1 seems to be actively involved in the regulation of gene expression. It is not well known whether the different variants have specific roles, are distributed differentially along the genome, or regulate specific promoters. We explored this by inducible shRNA-mediated knock-down of each of the H1 variants. Thus, specific phenotypes are observed in breast cancer cells depleted of individual histone H1 variants. Moreover, knock-down of each H1 variant alters expression of a different, reduced subset of genes, with more genes being repressed than activated, suggesting a local positive role of H1 on gene expression control. Although H1.2 and H1.4 knock-downs decrease proliferation similarly by arresting cells in G1, H1.2 is the only variant essential for cells to grow in anchorage-independent conditions, being the H1 that alters the most the proliferative and metastatic properties of cancer cells. Combined depletion of H1.2 and H1.4 has a strong deleterious effect in the cancer cells examined, and induces a strong interferon (IFN) response with up-regulation of many IFN-stimulated genes (ISGs), which is not seen in individual H1 knock-downs. Although H1 participates to repress ISG promoters, its activation upon H1 KD is mainly generated by the activation of the IFN response through cytosolic nucleic acids receptors, IFN synthesis and JAK-STAT pathway activation. The IFN response may be triggered by the expression of noncoding RNA generated from heterochromatic repeats or endogenous retroviruses upon H1 KD. In conclusion, redundant H1-mediated silencing of heterochromatin is important to maintain genome stability and to avoid an unspecific growth-inhibiting IFN response.

Moreover, using variant-specific antibodies to H1 and HA-tagged recombinant H1 variants expressed in breast cancer cells, we have investigated the distribution of six H1 variants in promoters and genome-wide. H1 is depleted from promoters depending on its transcriptional status. We show that histone H1 variants are not distributed uniformly along the genome and there are differences between variants, H1.2 being the one showing the most specific pattern and strongest correlation with low gene expression. We conclude that H1 variants are not distributed evenly along the genome and may participate with some specificity in chromatin organization and gene regulation.

Poster 3

Unravelling the 3D chromatin topology of human pancreatic islets

Irene Miguel-Escalada[1,2,*], Joan Ponsa[3], Delphine Rolando[3], Inês Cebola[3], Silvia Bonas[1,2], Jorge Ferrer[1,2,3,*].

[1] Genomic Programming of Beta-cells Laboratory, Institut d'Investigacions August Pi i Sunyer (IDIBAPS), 08036 Barcelona, Spain.
[2] CIBER de Diabetes y Enfermedades Metabólicas Asociadas (CIBERDEM), 08036 Barcelona, Spain.
[3] Department of Medicine, Imperial College London, London W12 0NN, United Kingdom.
*miguel@clinic.cat; j.ferrer@imperial.ac.uk

The human genome contains thousands of regulatory elements that are located very far away from their target genes. The recent advent of chromosome conformation capture techniques has shown that enhancer-promoter contacts happen through looping in the 3D space and multiple lines of evidence suggest suggests that alteration of this chromatin topology can lead to ectopic gene expression and disease. There is very little knowledge about the genome-wide interactions occurring in human pancreatic islets and the impact of chromatin topology on a pathogenic condition, such as Type-2 diabetes. We set out to characterize the 3D regulatory landscape of human pancreatic islets, explore what is the linkage between physical contacts and functional interactions, as well as investigate whether this 3D maps can be used to identify the transcriptional targets of type-two diabetes-associated variants.

De-identified human pancreatic islets from organ donors received from European islet isolation centres were fixed and processed to generate ChIP-Seq libraries of CTCF, SMCA1 and MED1 architectural proteins and Hi-C libraries. A sequence capture approach was used to enrich Hi-C libraries for ≈26,000 annotated coding and non-coding promoters, allowing us to identify significant 3D regulatory interactions genome wide.

We used high-resolution Promoter Capture Hi-C and ChIP-Seq of proteins involved in genomic architecture to investigate all enhancer-promoter interactions occurring in de-identified human pancreatic islets from organ donors. This approach allowed us to characterize the genomic elements contacting with promoters and identify key topological proteins mediating the contacts. We illustrate how this type of dataset can be applied to help identify endogenous targets of T2D-loci.

We identify a network of enhancer-promoter interactions in pancreatic islets, which highlights the complexity of the regulome controlling gene expression and demonstrate the utility of 3D datasets to identify transcriptional targets of disease-associated variants, aiding the understanding of molecular mechanisms underlying T2D.

Poster 4

TITLE: Linker histone H1 prevents R-loop accumulation and genome instability in heterochromatin.

AUTHORS: Casas-Lamesa, A.*[1,2], Bayona-Feliu, A. *[1,2], Bernués, J., Azorín, F.

AFFILIATIONS:
[1]Institute for Research in Biomedicine, IRB Barcelona. The Barcelona Institute of Science and Technology.
[2]Institute of Molecular Biology of Barcelona, IBMB, CSIC.

ABSTRACT: Linker histone H1 plays a particular prominent role in the structure of chromatin, stabilizing and folding the nucleofilament into higher-order structures. However, little is known about the biology of histone H1. Here, we show that in spite of *Drosophila* linker histone H1 (dH1) is uniformly distributed along the genome, its depletion preferentially affects expression of heterochromatic elements and induces DNA damage and genome instability in heterochromatin. These defects are due to the accumulation of R-loops (DNA:RNA hybrids) in heterochromatin, which are formed at G1-phase and induce damage during DNA replication. Furthermore, no such effects are observed upon HP1a depletion, which also upregulates heterochromatin expression, indicating that dH1 specifically prevents R-loops accumulation in heterochromatin. Altogether, our results unveil a novel role of histone H1 in preventing R-loop-induced replication stress in heterochromatin and reinforce its essential contribution in the maintenance of genome integrity.

*Equal contribution.

Poster 5

Identification of the human sperm protamine post-translational modifications code through LC-MS/MS.

Ada Soler-Ventura[1*], Meritxell Jodar[1], Ferran Barrachina[1], Josep Maria Estanyol[2], Judit Castillo[1], Josep Lluís Ballescà[3], Rafael Oliva[1]

[1]Molecular biology of Reproduction and Development Research Group, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Faculty of Medicine, University of Barcelona, Barcelona, Spain, and Biochemistry and Molecular Genetics Service, Hospital Clinic, Barcelona, Spain. [2]Proteomics Unit, Scientific Technical Services, University of Barcelona, Barcelona, Spain.[3]Clinic Institute of Gynecology, Obstetrics and Neonatology, Clinic Hospital, Barcelona, Spain.
*E-mail: ada.soler.ventura@gmail.com

Protamine 1 (P1) and protamine 2 (P2) family components are the most abundant sperm nuclear proteins packing and structuring the majority of the paternal DNA in mammals. Recently, the use of liquid chromatography followed by tandem mass-spectrometry (LC-MS/MS) has identified post-translational modifications (PTMs) in both protamines from human and mice sperm pools. Although little is known about protamine PTMs, it is suggested the existence of a protamine code similar to the well-known histone code with a potential role in infertility and early embryogenesis. The aim of this ongoing study is to identify the human protamine PTMs in controls using LC-MS/MS. A combination of two LC-MS/MS strategies has been selected to study the PTMs present in the small arginine-rich protamines. The first strategy is based on intact protein top-down LC-MS/MS (exclusion criteria set to 20 ppm) followed by amino acid sequencing to localize the protamine modified residues. The second strategy is based on protamine digestion followed by peptide-based bottom-up LC-MS/MS and confirmation of the modified residues through its comparison with synthetic peptides. Preliminary results from the set-up of intact protein top-down LC-MS/MS has enabled to detect intact proteins potentially corresponding to native P1, P1 mono-phosphorylated and P1 di-phosphorylated in the proteins extracted from a single sperm sample from one male. Sequencing of P1 phosphorylations will reveal the unique or uncommitted positions of the modified residues. In order to reduce the complexity of the intact protein in the top-down LC-MS/MS approach, we have also determined the best conditions for proteinase K digestion, instead of standard trypsin digestion. Once the detection of the LC-MS/MS protamine PTMs becomes standardized it will have the potential to shed new light into the relationships between the protamine PTMs profile and the pathogenesis of male infertility and embryogenesis, as well as its potential epigenetic role. Supported by Ministerio de Economía y Competitividad, Fondos FEDER, ISCIII (PI16/00346, PI13/00699), Fundación Salud 2000 (SERONO 13-015) and EUGIN-UB (EU-REP 2014), to RO.

Poster 6

CHARACTERISATION OF THE ZEBRAFISH 3D GENOME

Noelia Díaz[1], Benjamín Hernández-Rodríguez[1], Kai Kruse[1], Juan M. Vaquerizas[1]

1. Max Planck Institute for Molecular Biomedicine, Röntgenstraße 20, 48149 Münster, Germany.

Presenter e-mail: noelia.diaz@mpi-muenster.mpg.de

The hierarchical organisation of chromatin within the interphase nucleus plays a key role in regulating gene expression. In species ranging from human to worm, the genome adapts a specific topology into compartments and topologically associating domains. This domain-based organization of the genome seems to be strongly conserved across species, even if the main actors differ. In Zebrafish, the 3D structure and conformational changes of its genome have remained elusive - in part due to its genome duplication event and the presence of ohnologous genes scattered throughout its genome. Here, we present the first set of genome-wide chromatin conformation capture (Hi-C) studies of the zebrafish genome at a 10kb resolution. We were able to characterize the structural features of chromatin topology using interaction maps obtained at 48 hours post fertilization. We show how chromatin in fish embryos has a similar organization as other higher eukaryotes with topological domains readily apparent at the megabase scale. Our results provide a first insight into the three dimensional organization of chromatin in zebrafish and open the door to further studies on conformational aspects of zebrafish chromatin.

Poster 7

Age-related DNA methylation changes in somatic and gonadal tissues of teleost fish (European sea bass)

Dafni Anastasiadi and Francesc Piferrer

Institute of Marine Sciences (ICM-CSIC).

Passeig Marítim, 37–49, 08003 Barcelona

dafanast@icm.csic.es

In animals, age-related changes in DNA methylation occur. In general, there is global genomic hypomethylation accompanied by CpG-specific hypermethylation as a result of intrinsic and extrinsic influences. Mammalian epigenetic clocks have been suggested and used that estimate the biological age independently of the tissue tested. In fish, ageing studies based on epigenetic changes are scarce, while in fisheries and fish population studies there is a need for accurate estimation of age for which epigenetic biomarkers can be candidates. Here, we suggest CpG loci exhibiting decreasing or increasing methylation with age as potential piscine biomarkers of age. We used individuals from three different age classes comprising the reproductively immature and the senescent phase of a marine teleost fish, the European sea bass, to inquire the methylation status of the regulatory regions of important genes. For this inquiry, we successfully developed a low-cost high-resolution PCR-based NGS protocol applied in 22 genes and 36 samples. We found gene-specific ranges of DNA methylation that were affected by tissue identity and age. Furthermore, we fitted the elastic net regularization paths for generalized linear model in order to identify the minimum CpG number sufficient to predict the fish age. A minimum of 10 CpGs, out of 301 tested in total (3.3%), distributed in 6 genes, were enough to explain the age of the fish. Importantly, CpGs with clear increasing or decreasing tendencies in DNA methylation with age were detected and the youngest fish mean methylation matched with ~1 year old fish from an independent experiment. The sum of the suggested CpGs can serve as a guide to focus the efforts for the development of an accurate intertissue piscine epigenetic clock.

Supported by MINECO grant AGL2013-41047-R "Epifarm" to FP.

Poster 8

Effects of the expression of the *Drosophila* embryonic linker histone dBigH1 on the epigenetic state of chromatin

Climent-Cantó P., Carbonell A., Azorín F.

Institute of Molecular Biology of Barcelona, CSIC

Institute for Research in Biomedicine, IRB Barcelona. The Barcelona Institute for Science and Technology

Baldiri Reixac, 10. 08028 Barcelona. Spain

e-mail: paula.climent@irbbarcelona.org

Abstract

Linker histone H1 is one of the main components of chromatin. Histone H1 binds to the nucleosome core particle at the exit/entry sites of linker DNA. It stabilizes the nucleosome and facilitates the folding of the chromatin fiber to form higher-order structures. Histones H1 are a highly heterogeneous group of proteins and several embryonic and germline-specific H1 variants have been found in metazoans. However, the complexity of these proteins is reduced in *Drosophila melanogaster* since only one somatic dH1 variant and a second embryonic one, dBigH1, have been described. dBigH1 is expressed in the early embryo during precellular stages, when dH1 is absent and the zygotic genome is silent. Upon cellularization, when activation of the zygotic genome (ZGA) occurs, dBigH1 is replaced by somatic dH1 in all cells except in primordial germ cells. Previous studies have shown that dBigH1 is essential for proper ZGA since a dBigH1 loss-of-function mutant shows premature ZGA. However, how dBigH1 represses transcription is not known. Here, we show the effect in somatic dH1 and different epigenetic marks upon overexpression of dBigH1 in *Drosophila* S2 cells.

Poster 9

The germline linker histone dBigH1 and the translational regulator Bam form a repressor loop essential for male germ stem cell lineage differentiation.

Albert Carbonell[1,2], Salvador Pérez-Montero[1,2], Paula Climent-Cantó[1,2], Oscar Reina[2] and Fernando Azorín[1,2,*]

[1]Institute of Molecular Biology of Barcelona, CSIC

[2]Institute for Research in Biomedicine, IRB Barcelona. The Barcelona Institute for Science and Technology.

Drosophila spermatogenesis constitutes a paradigmatic system to study maintenance, proliferation and differentiation of adult stem cell lineages. Each *Drosophila* testis contains 6-12 germ stem cells that divide asymmetrically to produce gonialblast cells that undergo four transit-amplifying (TA) spermatogonial divisions before entering spermatocyte differentiation. Mechanisms governing these crucial transitions are not fully understood. Here, we report the essential role of the germline linker histone dBigH1 during early spermatogenesis. ChIPseq analyses suggest that dBigH1 is a general silencing factor that represses Bam, a key regulator of spermatogonia proliferation that is silenced in spermatocytes. Reciprocally, Bam represses dBigH1 during TA-divisions. This double-repressor mechanism switches dBigH1/Bam expression from off/on in spermatogonia to on/off in spermatocytes, regulating progression into spermatocyte differentiation. dBigH1 is also required for GSCs maintenance and differentiation. These results show the critical importance of germline H1s for male GSC lineage differentiation, unveiling a novel regulatory interaction that couples transcriptional and translational repression.

Poster 10

BRG1: The Dr. Jekyll & Ms. Hide of cancer

Luz Jubierre[1], Carlos Jimenez[1], Dan Hassond[2], Zhen Sun[2], Zulekha Qadeer[2], Aroa Soriano[1], Montserrat Sánchez-Céspedes[3], Josep Roma[1], Soledad Gallego[1], José Sánchez de Toledo[1], Ana Santa María[4], Emily Bernstein[2], Miguel Segura[1].

[1] Laboratory of Translational Research in Childhood Cancer. Vall d'Hebron Research Institute (VHIR), Universidad autónoma de Barcelona, Barcelona, Spain.

[2] Department of Oncological Sciences, Icahn School of Medicine at Mount Sinai, New York, USA; Department of Dermatology, Icahn School of Medicine at Mount Sinai, New York, USA.

[3] Genes and Cancer Group, Cancer Epigenetics and Biology Program-PEBC (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain.

[4] Biomedical Research in Gynecology. Vall d'Hebron Research Institute (VHIR), Universidad autónoma de Barcelona, Barcelona, Spain.

Adress: Vall d'Hebron Institut de Recerca, Passeig Vall d'Hebron 119-129, edificio Collserola, Laboratorio 207, 08035 Barcelona, Spain. Email: luz.jubierre@vhir.org

Relapsed or metastatic tumors are responsible for the majority of cancer-related deaths. These tumors usually acquire multi-drug resistance, raising the need for alternative treatments. Owing to the diverse mechanisms responsible for tumor chemoresistance, we propose to target epigenetic factors that control multiple pathways to bypass therapy resistance. Genomic analyses have found frequent mutations in chromatin-remodeling enzymes, of which the SWI/SNF complex shows a similar mutation frequency to other well-known tumor suppressors. The SWI/SNF complex is a large multiproteic structure which couples ATP hydrolysis with unraveling of the chromatin structure, thereby facilitating access of transcription factors to their target DNA sequences. ATPase function is carried out by either the BRG1 or the BRM enzyme which, through interaction with different proteins, will confer specific gene expression outputs. BRG1 is frequently mutated or silenced in some malignancies and therefore considered as a tumor suppressor; however, there are several tumors where BRG1 is over-expressed and driving the expression of pro-oncogenic genes, thereby suggesting a context-dependent oncogenic or tumor-suppressor role. Our group recently found BRG1 to be essential for the maintenance and growth of chemoresistant neuroblastoma (NB), while having tumor suppressor functions in a particular subset of tumors. We will take advantage of the differential behavior that BRG1 displays in different NB subtypes to unveil the function of BRG1 by characterizing the interactome of BRG1 in the two different contexts, the correlation with its genomic occupancy and the expression of its downstream effectors.

Poster 11

Targeting ZRF1 as a new epigenetic therapy strategy for neuroblastoma

Authors: Carlos Jiménez[1], Luz Jubierre[1], Aroa Soriano[1], Josep Roma[1], Josep Sánchez de Toledo[1], Soledad Gallego[1], Luciano Di Croce[2], Miguel F. Segura[1].

[1]Group of Translational Research on Child and Adolescent Cancer, Vall d'Hebron Institut of Research, UAB, Barcelona, Spain; [2]Center for Genomic Regulation, Barcelona, Spain.

Address: Vall d'Hebron Institut de Recerca, Passeig Vall d'Hebron 119-129 08035 Barcelona, Collserola Building, Laboratory 207. carlos.jimenez@vhir.org

ZRF1, an epigenetic regulator that displaces Polycomb repressing complexes from gene promoters, has been proved to be essential for the maintenance of neural progenitor's identity and self-renewal. Given the embryonal origin of neuroblastoma, a pediatric solid tumor of the peripheral nervous system, our hypothesis is that ZRF1 maintains neuroblastoma cells in a progenitor state, resulting in an undifferentiated state and high proliferative capacity. The main objective of this work is to determine the contribution of ZRF1 to the malignant phenotype of neuroblastoma.

ZRF1 expression levels in human samples were analyzed by gene expression arrays (GSE45547, n = 649). Correlations between gene expression and clinical parameters were calculated using t-test and Chi-square test. ZRF1 protein levels were assessed by Western Blot. Knockdown experiments were performed using two shRNAs against ZRF1, followed by proliferation and colony formation experiments. Expression data revealed a correlation between high expression levels of ZRF1 and poor event-free survival, whereas significant higher levels were found in advanced stages and MYCN amplified tumors. Moreover, ZRF1 was found to be highly expressed in all neuroblastoma cell lines tested. Functional experiments showed a significant decrease in proliferation ratio and colony formation when knockdown of ZRF1 was performed in different neuroblastoma cell lines.

Altogether, ZRF1 expression was shown to be related to poor prognosis and aggressiveness in human samples, and essential for neuroblastoma cells viability *in vitro*. These results reveal an important role of ZRF1 in neuroblastoma progression and maintenance. Further studies will try to assess the gene network that ZRF1 controls, and to validate this protein or its immediate downstream effectors as new therapeutic targets in neuroblastoma.