Epi Besançon

10th & 11th May 2022
Besançon, Kursaal Theater

EDITO

Thank you for attending this 6th edition of the EpiMeetings. After EpiNantes-2013, EpiNantes-2015, EpiBrest-2016, EpiNantes-2018, e-EpiMeeting-2021, it is a great pleasure for us to welcome you in Besançon under the leadership of our two hosts: Paul Peixoto and Eric Hervouet.

After the 5th edition turned into a virtual edition due to the sanitary context, it is with a great enthusiasm that we host you in this theater to live together this 6th edition of our EpiMeetings around the themes of epigenetics and epitranscriptomics.

With a rich program of presentations and posters covering both fundamental and translational aspects of epigenetics, we hope that this meeting will be an opportunity to discuss our current and future projects, and to establish future collaborations between our laboratories.

Thank you to our institutional and industrial partners which, despite the circumstances, remained faithful to us and also those which trusted us this year for the first time.

We are looking forward to meeting you all in a casual atmosphere of researchers who are passionate about the science of epigenetics and epitranscriptomics.

We wish you a pleasant EpiMeeting!

The organizers.
Pierre-François Cartron, Eric Hervouet, Paul Peixoto & Aurélien Sérandour
with the support of Gwénola Cartron (Cancéropole Grand Ouest)

The scientific organizers

Pierre-François CARTRON obtained his PhD from Ecole Pratique des Hautes Etudes (EPHE) Paris La Sorbonne from his work on the structure-function interaction of the pro-apoptotic protein Bax in glioblastoma multiforme. He joined the Memorial Sloan Kettering Cancer Center (New York) in the EC Holland's lab to study the role of DNA methylation dysregulation occurring during gliomagenesis (initiation, progression and relapse). Since 2005 he is permanent researcher at INSERM CRCI2NA. He develops research aiming to characterize the epigenetic and epitranscriptomic mechanisms in order to derive innovative molecules selectively directed against epigenetic and epitranscriptomic players. In cancer area, he focuses its researches on the deciphering of DNA and miRNA methylation patterns to develop biomarkers and therapeutic options for individualized and precision medecine. He has authored 79 peer-reviewed publications.

Aurelien SERANDOUR is Associate Professor at Nantes University – Ecole Centrale de Nantes and researcher at INSERM. He received his PhD in October 2011 in cell and molecular biology from the University of Rennes 1 where he worked on epigenetics dynamics during neural cell differentiation. He then joined Jason Carroll's laboratory at the University of Cambridge as a Postdoctoral Fellow to discover and characterize new protein partners of the Estrogen Receptor in breast cancer. In February 2015 he was awarded with the Marie Curie Postdoctoral Fellowship and joined the laboratories of Daniel Panne (EMBL Grenoble) and Kyung-Min Noh (EMBL Heidelberg) to characterize a transcription factor involved in cognition. In October 2016 he joined Ecole Centrale de Nantes as a tenure Associate Professor and was awarded with the chaire Inserm-Centrale Nantes for 5 years. He is applying epigenomics approaches to study human pathologies. He has authored 24 peer-reviewed publications.

Paul PEIXOTO obtained his Ph.D. at Université de Lille II in 2008, in the Antitumoral Research Laboratory (directed by Christian Bailly) in which he focused his research in the modulation of DNA binding activity of transcription factors using small synthetic compounds. He performed his postdoc in the Metastasis Research Laboraty in Liège (directed by Pr Vincent Castronovo), he exlored the role of HDACs class II in cancer. Since 2015 he is Professor associated at the Université de Franche-Comté and he is co-responsible of the EpiGenExp platform, INSERM UMR1098. His group is working in the epigenetic regulation of EMT, and the identification of new epigenetic cancer biomarkers. Paul PEIXOTO is Associate Professor at Bourgogne Franche Comté University at Besançon and researcher at INSERM RIGHT.

EpiBesancon 2022

Program

Tuesday May 10th – Wednesday May 11th, 2022

Kursaal Theater

DAY ONE, MAY 10TH, 2022

8:45 Welcoming and registration

EPITRANSCRIPTOMICS AND NON-CODING RNAs

9:15 - **Ciro Isidoro,** ***Non-coding RNA epigenetic regulation of autophagy in cancer*****, Novara, Italy**

9:45 - **Francesco Fazi,** ***Non-coding RNA and N6-Methyladenosine (m6A) -dependent networks in cancer cell behavior*****, Roma Italy**

10:15 Q&R

10:30 Meet the speakers at the coffee break

11:05 - **Petr Svoboda,** ***Small RNA-mediated innate and acquired genome immunity in rodents*****, Prague, Czech Republic**

11:35 Q&R

11:55 - **Angela Garding,** ***Deciphering the Epigenetic Landscape using AFA Technology*****, Covaris**
- Christophe Fleury, ***Simultaneous profiling of the epigenome and transcriptome at a single cell level,*** **10xGenomics**

12:15 Lunch and poster session

EPIGENETICS AND REGULATION OF IMMUNITY

13:45 - **Olivier Joffre,** ***Epigenetic control of T helper cell programming*****, Toulouse, France**

14:15 - **Marek Mraz,** ***Impact of microenvironmental interactions on the biology of B cells malignancies*****, Brno, Czech Republic**

14:45 - **Elina Zueva,** ***Transposon-host interaction in immune cells*****, Paris, France**

15:15 Q&R

15:30 - **Marie Hautin-Robert,** ***$IDH2^{R140Q}$ mutation induces expression and splicing changes in key myeloproliferation pathways in a cellular model of myeloproliferative neoplasms*****, Brest, France**

15:40 - **Emilie Elvira-Matelot,** ***The NF-KB pathway controls H3K9ME3 levels at intronic LINE-1 elements and hematopoietic stem cell gene expression in cis,*** **Villejuif, France**

15:50 Q&R

16:05 - **Clément Proux, *New advances in chromatin research,* Active Motif**
- Andrea Hita Ardiaca, Solving the multi-alignment challenge for interrogating non-coding RNA-seq, Diagenode

16:25 Meet the speakers at the coffee break

KEYNOTE:

17:00 - Duncan Odom, *How unrepaired DNA lesions shape genome evolution*, Heidelberg, Germany 17:50 Q&R

Gala Dinner ("only for people who are registered for the dinner")

DAY TWO, MAY 11TH, 2022

EPITRANSCRIPTOMICS AND NON-CODING RNAs (PART 2)

9:00 - **Carmen Jeronimo, *Non-coding RNAs in liquid biopsy as urological cancer biomarkers,* Porto, Portugal**

9:30 Q&R

EPIGENETICS IN PHYSIOPATHOLOGY

9:40 - **Jérôme Eeckhoute, *Control of cellular identity in liver pathophysiology*, Lille, France**

10:10 Q&R

10:20 Meet the speakers at the coffee break

EPIGENETIC MECANISMS IN CANCER (PART 1) kindly sponsorised by La Ligue Contre le Cancer

10:35 - **Anaïs Bardet, *Interplay between transcription factors and DNA methylation in cancer*, Strasbourg, France**

11:05 - **Peter Mulligan, *Regulation of cancer hallmarks by epigenetic reader proteins*, Lyon, France**

11:35 - **Marie-Alice Durand, *EZH2, a putative therapeutic target in Merkel cell carcinoma ?,* Tours, France**

11:45 - **Alexis Overs, *Specific DNA methylation profile in colorectal cancer*, Besançon, France**

11:55 - **Jules Durand, *Identification of novel partner proteins of EZH2 and KDM6B in Epithelial to Mesenchymal Transition,* Besançon, France**

12:05 Q&R

12:20 Lunch and poster session

EPIGENETIC MECANISMS IN CANCER (PART 2) kindly sponsorised by La Fondation pour le Recherche sur le Cancer

14:00 - **Hisham Mohammed, *Transcriptional and epigenetic cell states and its impact on disease progression in breast cancer,* Portland, USA**

14:30 - **Gilles Salbert, *TET2- mediated epigenetic reprogramming of breast cancer cells*, Rennes, France**

15:00 Q&R

15:15 - **Robel A. Tesfaye, *Super-enhancer induced ABC transporter abundance drives resistance to doxorubicin in HOS-MNNG cells,* Nantes, France**

15:25 - **Fabien Guidez, *Modulation of the activity of the stem cell epigenetic regulator PLZF through pollutant exposition,* Dijon, France**

15:35 Q&R

15:45 AWARDS (three best short talks of a young researcher and the two best poster communications)

15:50 Concluding remarks

Pr Ciro Isidoro D.Sc., M.D., UNIVERSITÀ del PIEMONTE ORIENTALE, Laboratory of Molecular Pathology and NanoBioImaging, Department of Health Sciences, Novara, Italy

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Non-coding RNA epigenetic regulation of autophagy in cancer

Alessandra Ferraresi[1], Andrea Esposito[1], Letizia Vallino[1], Amreen Salwa[1], Chiara Vidoni[1], Danny N Dhanasekaran[2], **Ciro Isidoro[1]**

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Autophagy is a catabolic process devoted to the degradation of non-functional cellular components and macromolecules within the lysosomes. The autophagy process begins with the recognition and sequestration of the *cargo* within the forming autophagosome, proceeds with the autophagosome-lysosome fusion and ends with the full degradation of the *cargo* and translocation in the cytosol of the elementary substrates reutilized for new synthesis. Altogether, 34 ATGs have been identified as part of the *core* autophagic machinery. Genetic alterations involving these genes lead to autophagy dysregulation and altered cellular homeostasis, predisposing to cancer. For instance, monoallelic deletion of the pivotal gene BECLIN1 compromises the protective function of autophagy and increases spontaneous tumors. Additionally, the autophagy pathway is regulated also at epigenetic level by a variety of non-coding RNAs. Here we present novel non-coding RNAs involved in the epigenetic regulation of autophagy that impact on cancer behavior such as proliferation, migration, and dormancy. We also present data showing how polyphenol nutraceuticals can modulate the expression of such non-coding RNAs. This knowledge may open new avenues for the personalized cure of cancer using epigenetic modulators capable of controlling autophagy in cancer cells.

Pr Francesco Fazi, PhD, Associate Professor of Histology & Human Embryology, Junior Research Fellow - Sapienza School for Advanced Studies (SSAS), Dept. of Anatomical, Histological, Forensic & Orthopaedic Sciences, , Section of Histology & Medical Embryology
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Non-coding RNA and N6-Methyladenosine (m6A) -dependent networks in cancer cell behavior

RNA chemical modifications in coding and non-coding RNAs are known from decades. They are generally installed by specific enzymes and, in some cases, they can be read and erased by other specific proteins. The majority of them occurs in transfer RNA (tRNA) and ribosomal RNA (rRNA) while a minority of them occurs in messenger RNAs (mRNA) and long non-coding RNAs (lncRNAs). In all cases RNA modifications may play important role in RNA folding, stability and function. The impact of RNA chemical modifications on gene expression regulation and the reversible nature of some of these modifications led to the birth of the word epitranscriptomics, in analogy with the changes that occur on DNA and histones. Among more than 100 different modifications identified so far, most of the epitranscriptomics studies focused on the N6-methyladenosine (m6A), which is the more abundant internal modification in protein coding RNAs. controlling several pathways of gene expression at the basis of the cell fate determination.
Even if most of the m^6A studies focused on its direct role on mRNA function, recent evidences showed that m^6A can also regulate the synthesis and function of ncRNAs and, on the contrary, ncRNAs can also influence the function of m^6A modification in mRNAs, highlighting a novel interplay between m6A and non-coding RNAs functional activity.
Of note, N^6-methyladenosine (m6A) is emerging as a relevant RNA modification involved also in the biogenesis and function of circular RNAs (circRNAs), which are a novel class of covalently closed single stranded RNAs. Interestingly, a molecular interaction between m6A modifiers and the proteotoxic stress response, whose induction is emerging as a relevant anticancer therapy in Acute Myeloid Leukemia (AML), has been recently described. Since the proteasome inhibition, leading to the imbalance in protein homeostasis, is strictly linked to the stress response induction, we decided to investigate the role of Bortezomib (Btz) on m^6A enzymes expression and in particular its impact on the modulation of m6A-dependent circRNAs expression. Our results highlighted that treatment of AML cells with Btz induced m^6A enzymes downregulation at translation level. Moreover, we evidenced that this modulation is mediated by ROS generation and activation of the oxidative stress response. Indeed, the administration of the reducing agent N-acetylcysteine inhibits Btz-mediated m^6A enzymes downregulation. Moreover, we identified Btz-dependent modulation of m^6A-modified circRNAs that could be involved in the proteotoxic stress-dependent induction of cell death. Our studies could be relevant to identify specific m^6A-dependent circRNAs to be exploited to improve Btz effectiveness in AML.

Dr Petr Svoboda, Laboratory of Epigenetic Regulations, Institute of Molecular Genetics of the Czech Academy of Sciences, Czech Republic

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Small RNA-mediated innate and acquired genome immunity in rodents

Small RNA pathways, which are found in most eukaryotes, play many different roles. Mammals utilize three small RNA pathways: PIWI-associated small RNA (piRNA) pathway, RNA interference (RNAi), and microRNA (miRNA) pathway. All three pathways co-exist in mouse oocytes. The piRNA pathway is a germline adaptive defense system able to learn to recognize invading mobile elements and suppress them in a sequence-specific manner. Mechanistically, it employs entirely different protein factors than the other two pathways and is non-essential in the mouse female germline. In contrast, it is essential in the hamster female germline, which makes it an interesting counterpart to RNAi in rodent oocytes. RNAi is an ancient eukaryotic defense mechanism against viruses and mobile elements. It is a sequence-specific innate defense system, which recognizes long double-stranded RNA, cuts it into ~22nt short interfering RNAs (siRNA), which guide elimination of RNAs with complementary sequences. In mammals, RNAi became a vestigial pathway because mammalian Dicer, an enzyme making miRNAs and siRNAs, is structurally adapted to make miRNAs. However, RNAi became re-activated in rodent oocytes because a long terminal repeat (LTR) insertion in the common ancestor of mice and hamsters gave rise to a truncated oocyte-specific Dicer isoform, which is efficiently producing siRNAs. This isoform became dominant and essential in mouse oocytes where RNAi acquired function in gene regulation and retrotransposon repression. This apparently did not happen in the lineage leading to hamsters, which shows remarkable divergence of RNAi and piRNA pathway activities in rodent oocytes.

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Immunobiologie Intégrative des cellules T

Epigenetic control of T helper cell programming

The integrity of the organism is constantly ensured by a tight interplay between conventional (Tconv) and regulatory (Treg) T cells. The development and differentiation of these cells are characterized by a gradual specialization of gene expression that defines hierarchical stages of maturation and commitment. Therefore, the molecular events that control signal-specific gene expression in T cells are critical to generate effective immune responses. Enhancers constitute major components of the regulatory networks that shape cell type-specific responses to environmental cues. In differentiating conventional Th2 cells, we recently showed that transcriptional specificity is largely controlled by a restricted set of endogenous retroviruses (ERV) that regulate cis-regulatory elements or act themselves as enhancers. We further showed that H3K9me3-dependent epigenetic pathways determine the repertoire of cis-regulatory elements, including ERV, amenable to activation. Based on these observations, we are now analyzing the global influence of transposable elements on genetic networks controlling Treg and Tconv identity. In parallel, we are also testing whether the manipulation of H3K9me3-dependent pathways could be interesting to promote protective immune responses or inhibit immunopathologies.

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Impact of microenvironmental interactions on the biology of B cells malignancies

B Cell Receptor (BCR) signalling and B cell-T cell interactions play an important role in the onset and progression of numerous B cell malignancies. The impressive clinical effect of inhibitors targeting BTK and PI3K in chronic lymphocytic leukaemia (CLL), a subset of diffuse large B cell lymphoma cases, and other B lymphomas underscores the dependence of malignant cells on these kinases involved in multiple microenvironmental interactions. The differences in BCR signalling propensity and B-T cell interactions contribute to variable prognosis of “mature” B cell malignancies. It has been shown that non-coding RNAs can affect BCR signalling (in)activation and its crosstalk with T cell interactions. The talk will focus on the roles of microRNAs (miRNAs) in fine-tuning the propensity of BCR signalling and B cell-T cell interactions and also describe the related changes during therapy. The regulation of malignant B cell migration will also be discussed.

This project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement No 802644). Supported by Ministry of Health of the Czech Republic, grant nr. NU20-03-00292 and NU22-03-00117, Czech Science Foundation (20-02566S), MH CZ - DRO (FNBr, 65269705) and MUNI/A/1330/2021

Dr Elina Zueva, Ph.D, Research Fellow, Institut Curie, Paris, France
Epigenetics of T lymphocyte development

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Host-transposon interaction in immune cells

Transposable elements (TEs) are mobile selfish genetic entities that have propagated in genomes via self-replication. Over evolutionary timescales, most of them undergo silencing and accumulate mutations that render immobility. Remnants of their ancestral regulatory sequences, however, may persist and be functionally repurposed for the host's needs. A growing body of evidence suggests that specific TE subfamilies are associated with enhancers in a handful of biological contexts. Owing to their ability to spread, TEs are thought to play a role in the evolution of regulatory networks. Identical copies disseminated across the genome may rapidly rewire novel coordinated transcriptional patterns. We hypothesized that such an acceleration of regulatory evolution may be particularly relevant for the immune system, which needs to rapidly adapt and effectively respond to a variety of mutable stimuli.
Using a combination of various NGS techniques, we surveyed TE content within putative enhancers in lymphocytes. TE subfamilies specifically enriched at enhancers contain regulatory motifs associated with immune functions. Computational prediction of enhancer gene targets suggests that TE-derived enhancers may act as backup enhancers that buffer against the potentially deleterious phenotypic consequences of enhancer loss. To distinguish elements that may be particularly important for immune functions, we compared enhancers between immune and nonimmune tissues. A higher proportion of immune enhancers are TE-derived, consistent with the idea that immune cells are more prone to hijacking TEs as regulatory elements. The higher density of TEs in the genomic loci of immune genes suggests that immune regulatory networks evolve under unique selective pressures that favor TE cooption. Abundant TEs carrying functional motifs may serve as a "reservoir" to accelerate the evolution of immune enhancers and the adaptation of response to repeated infectious challenges.
In perspective, the association between TE-derived enhancers and immune genes may have significant implications for understanding how genes are dysregulated in immune-related diseases. TEs are efficient platforms for epigenetic silencing, and pathological loss of their repression is implicated in autoimmunity and cancer. Our study suggests that epigenetic dysregulation of TE-derived enhancers may result in the inappropriate and untimely activation of immune genes. TEs, therefore, may serve as a “double-edged sword”: they provide regulatory elements for immune genes but are potentially sensitive to reactivation during pathogenesis. Overall, this study is beginning to reveal the “rules of TE cooption” by which TEs contribute as regulatory elements in both health and disease.

Keynote

Dr Duncan Odom, Ph.D, Regulatory Genomics and Cancer Evolution, German Cancer Research Center (DKFZ) in Heidelberg, Germany

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How unrepaired DNA lesions shape genome evolution

Cancers arise through the acquisition of oncogenic mutations and grow by clonal expansion. Here we reveal that most mutagenic DNA lesions are not resolved into a mutated DNA base pair within a single cell cycle. Instead, DNA lesions segregate, unrepaired, into daughter cells for multiple cell generations, resulting in the chromosome-scale phasing of subsequent mutations. We characterize this process in mutagen-induced mouse liver tumours and show that DNA replication across persisting lesions can produce multiple alternative alleles in successive cell divisions, thereby generating both multiallelic and combinatorial genetic diversity. The phasing of lesions enables accurate measurement of strand-biased repair processes, quantification of oncogenic selection and fine mapping of sister-chromatid-exchange events. Finally, we demonstrate that lesion segregation is a unifying property of exogenous mutagens, including UV light and chemotherapy agents in human cells and tumours, which has profound implications for the evolution and adaptation of cancer genomes. (Abstract from Aitken et al Nature, 2020.)

Pr Carmen Jerónimo, Ph.D, Director of the Research Center, Head of Cancer Biology & Epigenetics Group Portuguese Oncology Institute of Porto (IPO Porto) & Invited Full Professor at the Department of Pathology and Molecular Genetics School of Medicine & Biomedical Sciences (ICBAS), University of Porto, Portugal

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Non-coding RNAs in liquid biopsy as urological cancer biomarkers

Reduction of cancer mortality is a major societal goal, in part materialized in the many efforts to develop effective biomarkers for early detection. Indeed, early detection of cancer significantly increases the likelihood of curative treatment, avoiding the need for subsequent therapies, which have side effect and entail more comorbidities. Moreover, the current parameters for cancer patient stratification have been associated with different outcomes. Therefore, new biomarkers that could aid in cancer detection and prognosis, preferably detected by minimally invasive methods, are of major importance and likely to have substantial impact.

Liquid biopsies are promising tools that have been gaining significant attention over the last decade. Among the different classes of biomarkers that can be isolated from biofluids, non-coding RNAs have shown potential to improve cancer diagnosis and patient management.

In my talk, I will focus on the major recent findings of our research team concerning non-coding RNAs as biomarkers for detection and prognostication in urological cancers, as well current challenges for their implementation in the clinics.

Dr Jérôme Eeckhoute, Ph.D, DR Inserm U1011 Molecular analysis of gene regulation in cardiometabolic diseases, University of Lille, France

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Control of cellular identity in liver pathophysiology

Organ development requires cells to acquire specific identities and functions. At the molecular level, this is underlain by acquisition of cell-specific transcriptional regulatory programs chiefly established by a handful of cell-specific transcription factors (TFs). These TFs also cross-regulate their own expression within so called core transcription regulatory circuitry (CRC) which allow for high and robust expression of TFs of the CRC. In this context, defining TFs driving different cell-lineages has been a research area of intense interest. In particular, epigenomic signatures have been leveraged to predict TFs

Once deemed terminally differentiated, it now widely accepted that mature cells present in adult organs retain some levels of phenotypic plasticity. For instance, upon liver injury, hepatocytes transiently and partially dedifferentiate in order to re-enter the cell cycle and replenish the pool of functional hepatocytes. Hepatocytes can also give rise to cholangiocytes in cholestatic diseases. More generally, the response to injury and the regenerative processes involves multi-cellular responses where identity of additional liver cell-types are also altered.

Our laboratory has been interested in defining liver cell identity TFs. Epigenomic-based approaches have been used and compared (super-enhancers, broad histone modification domains) with this goal. How these TFs are organized into CRCs is also further investigated in order to further understand how pathophysiological cellular plasticity of liver cells is controlled at the CRC level. I will present our latest work dedicated to the transcriptional control of hepatocyte and mesenchymal-like hepatic stellate cell identity with regards to liver regeneration, fibrosis and organ failure.

Dr Anaïs Bardet, Ph.D, Research associate, IGBMC - CNRS UMR7104, University of Strasbourg, France

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Interplay between transcription factors and DNA methylation in cancer

Aberrant DNA methylation is a hallmark of cancer cells. However, the mechanisms underlying changes in DNA methylation remain elusive. Transcription factors initially thought to be repressed from binding by DNA methylation, have recently emerged as being able to shape DNA methylation patterns. We integrated the massive amount of data available from The Cancer Genome Atlas to predict transcription factors driving aberrant DNA methylation in 13 cancer types. We identified differentially methylated regions between cancer and matching healthy samples, searched for transcription factor motifs enriched in those regions and selected transcription factors with corresponding changes in gene expression. We predict transcription factors known to be involved in cancer as well as novel candidates to drive hypo-methylated regions such as FOXA1 and GATA3 in breast cancer, FOXA1 and TWIST1 in prostate cancer and NFE2L2 in lung cancer. We also predict transcription factors that lead to hyper-methylated regions upon transcription factor loss such as EGR1 in several cancer types. Finally, we validate that FOXA1 and GATA3 mediate hypo-methylated regions in breast cancer cells. Our work highlights the importance of some transcription factors as upstream regulators shaping DNA methylation patterns in cancer.

Dr Peter Mulligan, Ph.D, Inserm CRCN, Group leader at the Institute NeuroMyoGène in Lyon, France

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Regulation of cancer hallmarks by epigenetic reader proteins

Histone H3 trimethylation on lysine 9 (H3K9me3) is a defining feature of mammalian pericentromeres, loss of which results in genome instability via mechanisms that remain unclear. In previous work, we identified the poorly characterised H3K9me3-reader Chromodomain on Y-like 2 (CDYL2) as a novel regulator of breast cancer cell plasticity, but also noted an apparent growth inhibitory effect of CDYL2 inhibition in cell cultures. Here we show that CDYL2 is recruited to pericentromeres in an H3K9me3-dependent manner and is required for faithful mitosis and genome stability. RNAi inhibition of CDYL2 in MCF-7 breast cancer cells and Hela cervical cancer cells resulted in growth inhibition, apoptosis and mitotic defects. Using a proteomics approach, we identified novel CDYL2-interactors that contribute to its regulation of these cellular processes. Our findings reinforce the notion that CDYL2 is an important regulator of the so-called hallmarks underlying breast cancer progression, impacting genome stability in addition to cellular plasticity.

Dr Hisham Mohammed, Ph.D., Assistant Professor of Molecular and Medical Genetics, School of Medicine Scientist, CEDAR, OHSU Knight Cancer Institute, School of Medicine, Portland, United States

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Transcriptional and epigenetic cell states and its impact on disease progression in breast cancer

Breast cancer is known to be driven by the hormone estrogen. Whilst targeting the hormone and its receptor has proved highly successful, resistance to disease is common. The disease manages to continually evolve and adapt to therapeutic stress in ways other cells cannot. Our work has identified a factor called KLF4, one that is critical for normal stem cells to be 'plastic', being utilized by estrogen driven breast cancer cells to impart cellular heterogeneity. Targeting this factor not only reduces heterogeneity in the system, but also halts estrogen's ability to stimulate cell growth or transcription. Hence, we believe that strategies to target heterogeneity in cancer, along with hormone signaling could be vital in stopping the disease's ability to survive and evolve over time.

Pr Gilles Salbert, , Ph.D, Group leader at the Institut de Génétique et Développement de Rennes, France

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TET2- mediated epigenetic reprogramming of breast cancer cells

Cell-specific gene expression programs are sustained by epigenetic landscapes established by enzymes targeting histones and DNA. Accordingly, genome-wide epigenomic rewiring associates with acquisition of new cellular identities during development. Cancer cells, although maintaining a cell-of-origin epigenomic imprint, acquire specific epigenomic features, some of which are common between different cancer types. One such cancer-associated epigenetic feature is the so-called CpG Island Methylator Phenotype (CIMP) in which hypermethylation of a substantial number of CpG islands (CGIs) that surround transcription start sites (TSSs) associates with low gene expression. Although it is not precisely known what triggers CIMP, a decrease in the activity of Ten Eleven Translocation (TET) enzymes has been documented in various cancers and linked to the occurrence of CIMP in leukemia and colorectal cancer. TETs are 2-oxoglutarate/Fe^{2+}-dependent dioxygenases that trigger active DNA demethylation. Consistent with a role in maintaining an hypomethylated state in CpG-rich regions, TET1 knock-out in mouse embryonic stem cells (mESCs) leads to CGI hypermethylation, suggesting that CIMP could indeed be caused by a reduced TET activity in cancer cells. Here, we genetically engineered MCF-7 cells, a model of low metastatic luminal breast cancer cells, to activate 5mC turnover through TET2 overexpression. Alteration of DNA methylation dynamics led to a decreased tumorigenic potential of cells through both activation and repression of a repertoire of genes that differed in part from the one observed upon treatment with the hypomethylating agent decitabine. In addition to promoting the establishment of an antiviral state, TET2 activated 5mC turnover at thousands of MYC binding motifs and down-regulated a panel of known MYC-repressed genes involved in lysosome biogenesis and function. This extensive cross-talk between TET2 and the oncogenic transcription factor MYC establishes a lysosomal storage disease-like state that contributes to an exacerbated sensitivity to autophagy inducers.

Short talks

Marie Hautin-Robert, , Ph.D student , INSERM U1078 - ASTRE, Brest, France

IDH2^{R140Q} MUTATION INDUCES EXPRESSION AND SPLICING CHANGES IN KEY MYELOPROLIFERATION PATHWAYS IN A CELLULAR MODEL OF MYELOPROLIFERATIVE NEOPLASMS

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Myeloproliferative Neoplasms (MPN) are chronic hematological malignancies characterized by an excessive proliferation of myeloid progenitors, resulting from the acquisition of driver mutations in cytokine signaling pathways. *IDH1/2* additional mutations, which mostly affect DNA and histone methylation, are associated with a high risk of evolution into Acute Leukemia (AL). AL are particularly affected by splicing alterations, even in the absence of spliceosome mutation.
We hypothesize that splicing anomalies involved in MPN leukemic transformation partly result from mutant IDH-induced epigenetic modifications.
UT-7 cells expressing *JAK2*^{V617F} together with *IDH2*^{R140Q} recapitulate major characteristics of MPN primary cells. RNA-seq-based transcriptomic analyses showed expression deregulation of genes involved in immunity, inflammation, signaling, hypoxia and apoptosis pathways, similar to MPN patients and murine models. A global splicing deregulation was also identified with a stronger impact of *IDH2* mutation, mainly affecting cassette exons. In order to decipher mechanisms responsible for these alterations, we tested the implication of DNA methylation changes by inhibiting *TET2* in cells expressing *JAK2*^{V617F}. Of 16 mutant IDH-induced differentially expressed or spliced genes, 10 were similar in TET2-underexpressing cells, 2 were not affected, and 4 were reversed. Among the deregulated cassette exon events, we are focusing on two genes involved in NF-κB and c-Myc signaling pathways.
Our results show that *IDH2* mutation in *JAK2* mutated cells induces numerous transcriptomic and splicing alterations in major pathways involved in leukemogenesis. Characterization of proleukemic pathway gene expression and/or splicing deregulations in response to epigenetic modifications should help for identification of therapeutic targets in post-MPN AL.

Dr Emilie Elvira-Matelot, PhD, INSERM U1287, Cancer Campus Gustave Roussy, France

THE NF-KB PATHWAY CONTROLS H3K9ME3 LEVELS AT INTRONIC LINE-1 ELEMENTS AND HEMATOPOIETIC STEM CELL GENE EXPRESSION IN CIS

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Exposure to ionizing radiations (IR) is an independent risk factor for many disorders characteristic of an accelerated aging. This has become a major public concern in the last decades as the number of long-term survivors of cancer treatments increase.

Hematopoietic stem cells (HSCs) self-renew and give rise to all blood cell lineages. Maintenance of their integrity is crucial throughout life. IR and aging induce similar defects in HSCs: DNA damage accumulation, loss of self-renewal, and a biased differentiation towards the myeloid lineage. These changes are likely contributing to many IR-induced premature aging disorders and to the higher risk of developing secondary myeloid malignancies. **Understanding the molecular mechanisms leading to HSC loss of function upon IR is necessary to modulate its adverse effects. It may also help identifying the first events leading to hematologic malignancies.**
We recently showed that L1Md, the mouse young subfamilies of LINE-1/L1 retroelements, are highly expressed in HSCs, and that their expression is further increased up to 1 month after IR. L1 are major contributors of gene regulatory networks. Notably, repression of genes following derepression of intragenic L1 was reported in cancers. L1 expression is tightly regulated by the heterochromatin mark H3K9me3. However, how L1Md are derepressed upon IR and how they impact HSC gene expression is not known. We hypothesized that L1Md derepression may be due to IR-induced epigenetic defects and may be involved in transcriptomic alterations in HSCs.

Using H3K9me3 ChIP-seq experiments performed in HSCs sorted from mice one month after IR, we show that IR triggers a genome-wide decrease in H3K9me3 that occurs mainly at L1Md. RNA-seq experiments showed that IR strongly affects the HSC transcriptome, leading to a loss of the long-term HSCs and several signaling signatures, including TNF-/NF-B signaling. Surprisingly, we found that gene deregulation is not associated with H3K9me3 changes at gene promoters, but rather with the loss of H3K9me3 at intronic L1Md.

This association is specific for genes whose expression is reduced upon IR, notably HSC genes. We showed that loss of H3K9me3 mark at intronic L1Md upon IR is linked to gene repression *in cis*, both after short time *in vitro* and long time *in vivo*, highlighting the direct effect of IR on HSC heterochromatin and transcriptome, and suggesting that heterochromatin alterations may explain the long-term effect of IR on HSC function.
Loss of H3K9me3 occurs specifically at intronic L1Md harboring NF-B binding site motifs and is associated with the repression of HSC specific genes. It is correlated with reduced expression of NFKB1, which may act as a repressor, upon IR. TNF-treatment before IR rescued all these effects *in vitro* and prevented IR-induced HSC loss of function *in vivo.*

These results show for the first time that IR directly affects HSC heterochromatin. They also show that regulating gene expression through a TNF-/NF-B/H3K9me3/L1Md axis expands the NF-B repertoire to HSC genes. This might be important to maintain of HSCs while allowing expression of immune genes during myeloid regeneration or damage-induced bone marrow ablation.

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EZH2, a putative therapeutic target in Merkel cell carcinoma ?

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Merkel cell carcinoma (MCC) is an aggressive and deadly skin cancer. In 2008, Merkel cell Polyomavirus (MCPyV) integration was evidenced in most MCC and the expression of the two viral oncogenes was identified as the main oncogenic trigger. Recently, EZH2 expression (Enhancer of zest homolog 2), an histone methyl transferase involved in aggressiveness of many solid cancer, was evidenced in MCC tumors. In this context, our working hypothesis is that EZH2 may represent a therapeutic target in MCC.

To verify this hypothesis, we first assessed by immunochemistry EZH2 expression in a cohort of 132 MCC tumors. Firstly, this analysis showed high EZH2 expression levels in MCC (n=95, 72%), correlated with high levels of H3K27me3 (p=0.009), and a more frequent expression in MCPyV-positive MCC tumors than in MCPyV-negative tumors (p=0.026) suggesting a possible induction of EZH2 expression by viral oncogenes. According to this observation, knock-down (shRNA) of viral oncogenes in MCC cell lines (WaGa, PeTa and MKL1) led to EZH2 down-regulation and ectopic expression of viral oncogenes in primary fibroblasts induced EZH2 expression (**Figure 1**). Moreover, using several LT mutants on Rb binding domain, we evidenced that the EZH2 induction in primary fibroblats is dependent of an intact Rb binding domain. Secondly, EZH2 inhibition by RNA interference (shRNA) or by chemical inhibitors (tazemetostat, GSK343 and DZNeP) induced an increase of cell death evidenced using cytotoxicity assay, cell cycle analysis (**Figure 2**) and mixedculture assay. Lastly, EZH2 chemical inhibition *in vivo* is currently evaluated in a xenograft model of MCC.

To conclude, EZH2 is highly expressed in MCPyV positive MCC tumors. Such expression is induced by LT oncogene through pRB sequestration. Chemical inhibition of EZH2 by several inhibitors (tazemetostat, GSK343 and DZNeP) induce cell death in vitro and may constitute a therapeutic target currently evaluated in a preclinical model.

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Specific DNA methylation profile in colorectal cancer

Introduction:
Colorectal cancer (CRC) is a public health issue with over 1,2 million new cases worldwide in 2018. To improve management of these tumors, non-invasive biomarkers for diagnosis and follow are being studied. Thanks to the sensibility improvement of detection techniques, methylation profile of circulating tumor DNA can provide non-invasive biomarkers. The objective of this work is to establish a DNA methylation profile specific of CRC that can be used in circulating tumor DNA.
Material and method:
A bioinformatic analysis was performed on the GEO and TCGA databases to establish a DNA methylation profile of CRC specific to non-tumor colorectal tissue and physiological blood DNA. *Quantitative Methylation Specific PCR* (qMSP) was then performed on CRC and non-tumor colorectal tissues samples to confirm the DNA methylation profile.
Results:
The bioinformatic analysis found a DNA methylation profile composed of 6 CpGs. The methylation of these CpG is 98.5% sensitive and 98.8% specific on the GEO data and 98.4% sensitive and 100% specific on the TCGA data. qMSP was performed for 3 of the CpGs on 19 CRC and 15 non-tumor colorectal tissue samples. The qMSP confirmed the differential methylation for all 3 CpGs ($p < 10^{-4}$) with a combined sensitivity and specificity of 100%.
Conclusion and perspectives:
Bioinformatic analysis of public databases can provide new DNA methylation biomarkers with potential clinical relevance. Following this work, we are exploring the sensitivity and specificity of the DNA methylation profile for clinical diagnosis and follow in the PREDICT-IRFC protocol. This biomarker elaboration method can also be applied to other type of tumor.

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Identification of novel partner proteins of EZH2 and KDM6B in Epithelial to Mesenchymal Transition

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Epithelial to mesenchymal transition (EMT) is a progressive and reversible mechanism that allows cells with an epithelial phenotype to lose it in favor of a mesenchymal one. During EMT, epithelial cells forming a cohesive tissue through cell-cell and cell-matrix junctions progressively dissociate from it and gain migratory properties. This mechanism has many physiological occurrences during embryogenesis and wound healing for example but has also been linked to the formation of metastases in cancer. This switch in phenotype is made possible by a wide reprogramming of gene expression leading to the downregulation of pro-epithelial genes (e.g. E-Cadherin, ZO-1) and upregulation of pro-mesenchymal genes (e.g. N-cadherin, Vimentin), both of which are tightly controlled by epigenetics. In particular, the methylation level of H3K27 (Lysine 27 oh the Histone H3) has been shown to vary significatively on the promoters of key genes during EMT. The methylation of H3K27 is regulated by the methylase EZH2 (Enhancer of Zest Homolog 2) and the demethylase KDM6B (Lysine Demethylase 6 B), which have both been shown to be key regulators of EMT. However, despite having opposite catalytic activities, both enzymes induce EMT when overexpressed in cancerous cells. This could be due to the recruitment of these enzymes towards different sets of genes by partner proteins and/or post-translational regulations of non-histone proteins by EZH2 and KDM6B that would influence EMT. To investigate this, we used RIME (Rapid Immunoprecipitation Mass spectroscopy of Endogenous proteins) to identify proteins interacting with EZH2 or with KDM6B and associated with chromatin while using a TGFβ/TNFα treatment to induce EMT. We identified, in A549 cells (non-small cell lung carcinoma), 93 putative partners of KDM6B and 77 putative partners of EZH2. Amongst these, 22 putative partners of KDM6B and 56 putative partners of EZH2 were specifically identified during EMT induction. Some of these partners are transcription factors or co-regulators as well as proteins known to be involved in EMT. Investigating these proteins and the importance of their interaction with EZH2 and KDM6B during EMT could help better understand the role of EZH2 and KDM6B during EMT as well as unravel new targets for cancer therapies.

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Super-enhancer induced ABC transporter abundance drives resistance to doxorubicin in HOS-MNNG cells.

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E-mails: robel.tesfaye@etu.univ-nantes.fr, robel.tesfaye@hotmail.com**Abstract (500 words max)**

Even though more and more clinical trials of molecules targeting osteosarcoma (OS) markers are emerging, high-dose methotrexate, doxorubicin and cisplatin (MAP) regimen is the most often used treatment especially in children and young adults. This combination of molecules is applied as neo-adjuvant and adjuvant therapy, before and after surgical resection respectively, with higher survival rates compared to surgery alone. However, survival rates drop from 70 % to 30 % among patients with metastases at diagnosis or among poor responders to treatment. The aim of this study is to identify mechanisms driving resistance to doxorubicin (DOX) and propose strategies to circumvent them. As key cell identity defining genes are reported to be induced by highly efficient clusters of enhancers called superenhancers (SEs), we hypothesized targets of SEs specific to resistant cells could lead to the identification of key resistance driving genes. To this end, we tracked epigenetic reprogramming across enhancer and SE profiles in DOX sensitive (Ct) and DOX resistant (DoxR) HOS-MNNG cell lines. We identified genes encoding for members of ABC transporters, specifically *ABCB1* and *ABCB4*, as targets of *de-novo* SEs in resistant cells. Inhibiting the activity of P-glycoprotein (P-gp) – the *ABCB1* encoded protein – appears to restore sensitivity of resistant cells to DOX treatment. Similarly, simultaneous treatment with JQ1, that was reported to preferentially impair the activity of SEs, seems to partially restore sensitivity to DOX. Heterogeneity in transcriptome and accessible chromatin sites was assessed on a single cell level by single-cell Multiome (RNA+ATAC) in control (Ct.), resistant to intermediate doses and highly resistant (DoxR) cells. After integration of the three datasets using Harmony, expression of *ABCB1* seems to be exclusive to and ubiquitous in cells that are highly resistant. On the other hand, cells resistant to intermediate doses do not demonstrate expression of *ABCB1*, suggesting other mechanisms could be in play at early days of resistance acquisition.

Dr Fabien Guidez, CRCN/HDR UMR1231 Inserm/uB/AgroSup Université de Bourgogne, Dijon, France

Modulation of the activity of the stem cell epigenetic regulator PLZF through pollutant exposition

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The promyelocytic leukemia zinc finger protein (PLZF) is a regulator of heterochromatin and is involved in stem cell and progenitor maintenance. PLZF epigenetic activity is directly modulated by acetylation of specific lysine residues and controls its cellular localization by increasing its DNA affinity. Here, we report that environmental pollutants, such as benzene, affected directly the levels of PLZF acetylation and, thus, disturbed the heterochromatin patterning in hematopoietic cells.

Pr Charles De Smet, Institut de Duve, Group leader Genome Alterations, Brussels, Belgium

DECIPHERING THE FULL IMPACT OF GENOME HYPOMETHYLATION ON THE TUMOR TRANSCRIPTOME

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Genome hypomethylation is commonly observed in human tumors, and we have shown that it often causes aberrant de-repression of genes that normally display specific expression in testicular germline cells. Several of these “cancer-germline“ genes encode proteins with oncogenic potential, and are therefore attracting interest for the development of highly-specific anti-cancer drugs. It is anticipated indeed that therapies directed against proteins expressed only in testis and tumor cells will have little side-effects in cancer patients.
Recently, we made the surprising observation that several cancer-germline genes produce long non-coding transcripts that overlap downstream promoters, thereby triggering their hypermethylation. Another consequence of CG gene activation in tumors is therefore the epigenetic repression of neighboring genes, which include tumor suppressor genes. These observations establish an unsuspected connection between DNA hypomethylation and DNA hypermethylation, two opposite, yet co-existing, epigenetic alterations occurring in most human tumors.
The impact of DNA hypomethylation on the tumor transcriptome remains incompletely defined. To determine the full spectrum of gene activations induced by genome hypomethylation in tumors, we performed computational analyses integrating transcriptomic and methylomic data from normal and cancerous cells and tissues, with special focus on lung adenocarcinoma. The results showed that besides the activation of a germline specific gene expression program, DNA hypomethylation in lung tumors is also associated with activation of gene clusters displaying specific expression in somatic tissues, namely in the lower digestive tract, and in stratified epithelia. Interestingly, activation of several of these genes was tightly associated with shorter survival of lung cancer patients.

Sponsor talks

Covaris

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Deciphering the Epigenetic Landscape using AFA Technology

Mapping the epigenetic landscape as well as spatial chromatin organization is essential for understanding gene regulatory mechanisms and holds huge potential for identification of biomarkers and targets for epigenetic therapy. However, existing chromatin preparation workflows introduce significant variability due to partial cell lysis, inconsistent fragment sizes and epitope loss. Reliable sample preparation is key to uncover the regulatory landscape, especially when working with scarce samples or looking into binding kinetics of non-abundant chromatin regulators. We present a fast, easy and scalable workflow which results in high quality sheared chromatin from virtually any sample type including cultured cells, primary cells, organoids, fresh-frozen tissue as well as FFPE (formalin-fixed, paraffin embedded)
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10XGenomics

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Active Motif

Clement Proux
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EpiBesançon 2022 Active Motif - New advances in chromatin research Clément Proux – Key Account Manager – France & South Switzerland

Active Motif is the industry leader in providing solutions for Epigenetics research. This short talk offers an overview of both ATAC-Seq and CUT&Tag methods and capabilities developed at Active Motif. ATAC-Seq is a powerful technique that enables the mapping of accessible, or open, chromatin regions across the genome. The ATAC-Seq assay has been used by many researchers to investigate the role of epigenetics in many biological processes and disease states. CUT&Tag is an invaluable method, following a similar principle as ChIP-Seq, to investigate genomic localization of histone modifications and some non-histone proteins that reveals interactions between proteins and DNA or identifies DNA binding sites for proteins of interest with low amounts of input material. However, ChIP-Seq and sonication remains the gold standard method for mapping transcription factors and a the presentation will introduce the next generation PIXUL multi-sample sonicator that overcome shearing challenge.

Diagenode

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Solving the multi-alignment challenge for interrogating non-coding RNA-seq

Total-RNA sequencing (total-RNA-seq) allows the simultaneous study of both the coding and the non-coding transcriptome. However, transcripts from distinct RNA biotypes vary in length, biogenesis, and function, can overlap in a genomic region, and may be present in the genome with a high copy number. Consequently, reads from total-RNA-seq libraries may cause ambiguous genomic alignments, demanding flexible quantification approaches capable of analyzing these reads. To overcome this challenge, we present Multi-Graph count (MGcount), a flexible quantification framework that firstly, it assigns reads to transcripts in a hierarchical fashion to account for loci length disparity between small-RNA and long-RNA and secondly, it collapses locus where reads systematically multimap into communities. Ultimately, this strategy maximizes the information extracted from total-RNA-seq and improves the interrogation of non-coding RNA

Posters

P01

EPIGENETIC MECHANISMS CONTROLLING CANCER ASSOCIATED FIBROBLASTS COMMITMENT IN CONTEXT OF FOLLICULAR LYMPHOMA

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Follicular Lymphoma (FL) is a pertinent model to study co-evolution between tumor and a supportive microenvironment. There are recurrent mutations found in FL-B cells against key actors of the epigenetic pathways. However, some mutations are not oncogenic per se, but impact crosstalk with a supportive microenvironment which is composed of immune cell and lymphoid stromal cells (LSC). LSCs allow the survival, differentiation and selection of B cells at the germinal centers (GC). Tumor cells are in contact with the LSC network, creating an inflammatory context in which FL tumor B cells could trigger the commitment of LSCs to a CAF phenotype. The role of epigenetic during the commitment and regulation of normal and pathological LSC remains unknown. H3K27me3 is strongly associated with inhibition of gene expression unlike H3K27ac. Interestingly, the binding profile of this mark, specific to a differentiation state, is associated with cellular identity and was recently found modulated in CAFs in the context of gastric cancer. Epigenetic changes in H3K27 are mediated by EZH2, which is found mutated in 10-30% of FL patients and inactivates the targeted gene by methylating H3K27. Conversely, KDM6B demethylates it. Studying the presence of these marks will help define the enhancers activation state. Thus, we hypothesize that epigenetic mechanisms are involved and deregulated during CAFs polarization in FL. Approaches based on the analysis of native cells and on an in-vitro differentiation model of FL-CAFs are used. Our preliminary results showed that epigenetic regulations are involved during normal LSC commitment. We extended this approach to the commitment of LSC towards CAFs. Our results highlight an increased expression of CXCL12 expression after induction of CAF-like phenotype in-vitro, which is partly dependent of KDM6B. Altogether, our results should shed new lights on CAFs commitment regulation in FL and drive the emergence of new therapeutic research axis.

P02

KETOGENIC DIET ADMINISTRATION TO MICE AFTER A HIGH FAT DIET REGIMEN PROMOTES HISTONE β-HYDROXYBUTYRYLATION ASSOCIATED TO WEIGHT LOSS AND IMPROVED METABOLIC FITNESS.

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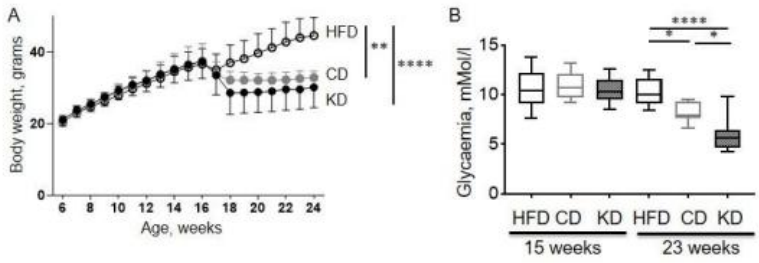
Introduction Ketogenic diets, consisting in the almost complete elimination of dietary carbohydrates, induce the body to rely on ketone bodies as primary energy source. The ketogenic diet (KD), characterized by a very limited carbohydrate content, it used as nutritional treatment for GLUT1-deficiency syndromes and pharmacologically refractory epilepsy. The increase of ketone bodies in the bloodstream at physiological doses confers multiple metabolic, anti-inflammatory and antioxidant benefits. Therefore, the ketogenic diet could be a valuable nutritional option to counter several pathological conditions, including non-alcoholic fatty liver disease, insulin resistance, obesity and osteoarthritis. The objective of this study is to investigate the beneficial impact of the ketogenic diet to promote weight loss and improve metabolic fitness. Methods Males C57Bl6/J mice aged 6 weeks were fed a high fat diet for 10 weeks followed by a 8 weeks dietary switch to a chow diet (CD), ketogenic diet (KD) or continuation on a high fat diet (HFD). Body weight, kidney weight, body fat content and serum glucose and β-hydroxybutyrate (BHB) concentrations were evaluated. In liver and kidney, protein expression and histone post-translational modifications were assessed by western blot, and gene expression by quantitative real-time PCR. Results After the initial HDF feeding, administration for 8 weeks of a KD or CD induced a comparable weight loss and decrease in fat mass, with better glycemic normalization in the KD group. Histone β -hydroxybutyrylation, but not histone acetylation, was increased in the liver and kidney of mice fed the KD and the rate-limiting ketogenic enzyme HMGCS2 was upregulated – at the gene and protein level - in liver and, to an even greater extent, in kidney. KD-induced HMGCS2 overexpression may be dependent on FGF21, whose expression was dramatically increased by KD in liver. Moreover, we observe a general mild pro-inflammatory effect in the liver of the KD group, which is correlated with increased expression of the NLPR3 inflammasome subunit and an increase of pro-inflammatory IL-1β and Socs3. Conclusions/interpretation Over a period of 8 weeks, KD is equally effective than a chow diet in inducing weight loss, but glycemic normalization was superior. Besides acting as a fuel molecule, BHB may exert its metabolic effects through modulation of the epigenome, via histone β-hydroxybutyrylation and transcriptional modulation in liver and kidney of ketogenic genes. These data show that a transitorily administered ketogenic diet may improve the metabolic status after previous exposure to a high fat diet.



(A) Mice body weight throughout the study protocol. Body weight area under the curve was determined from week 18 to week 24 and compared among the three groups by one-way Anova. (B) Glycemia and BHB concentration measured in tail blood at week 23. Glycemia was compared among the three groups by one-way Anova.

P03

Identification of novel partner proteins of EZH2 and KDM6B in Epithelial to Mesenchymal Transition

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Epithelial to mesenchymal transition (EMT) is a progressive and reversible mechanism that allows cells with an epithelial phenotype to lose it in favor of a mesenchymal one. During EMT, epithelial cells forming a cohesive tissue through cell-cell and cell-matrix junctions progressively dissociate from it and gain migratory properties. This mechanism has many physiological occurrences during embryogenesis and wound healing for example but has also been linked to the formation of metastases in cancer. This switch in phenotype is made possible by a wide reprogramming of gene expression leading to the downregulation of pro-epithelial genes (e.g. E-Cadherin, ZO-1) and upregulation of pro-mesenchymal genes (e.g. N-cadherin, Vimentin), both of which are tightly controlled by epigenetics. In particular, the methylation level of H3K27 (Lysine 27 oh the Histone H3) has been shown to vary significatively on the promoters of key genes during EMT. The methylation of H3K27 is regulated by the methylase EZH2 (Enhancer of Zest Homolog 2) and the demethylase KDM6B (Lysine Demethylase 6 B), which have both been shown to be key regulators of EMT. However, despite having opposite catalytic activities, both enzymes induce EMT when overexpressed in cancerous cells. This could be due to the recruitment of these enzymes towards different sets of genes by partner proteins and/or post-translational regulations of non-histone proteins by EZH2 and KDM6B that would influence EMT. To investigate this, we used RIME (Rapid Immunoprecipitation Mass spectroscopy of Endogenous proteins) to identify proteins interacting with EZH2 or with KDM6B and associated with chromatin while using a TGFβ/TNFα treatment to induce EMT. We identified, in A549 cells (non-small cell lung carcinoma), 93 putative partners of KDM6B and 77 putative partners of EZH2. Amongst these, 22 putative partners of KDM6B and 56 putative partners of EZH2 were specifically identified during EMT induction. Some of these partners are transcription factors or co-regulators as well as proteins known to be involved in EMT. Investigating these proteins and the importance of their interaction with EZH2 and KDM6B during EMT could help better understand the role of EZH2 and KDM6B during EMT as well as unravel new targets for cancer therapies.

P04

Sensitive and unbiased detection of clinically actionable gene fusions from FFPE tumor biopsies using the Arima-HiC platform

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Accurate and sensitive identification of gene fusions is critical to understanding disease mechanisms and the development and selection of optimal treatment regimens for cancer patients. Typically, these fusions are detected using low-resolution karyotyping, low throughput and biased FISH assays, or RNA sequencing approaches; however, the accuracy and sensitivity of gene fusion detection can be limited by factors such as low transcript abundance, transcript length, RNA degradation from formalin fixed paraffin embedded (FFPE) tissues, or the limited availability of fresh biopsy samples for RNA extraction. To address these limitations, we developed a novel approach to identifying gene fusions from FFPE samples using the Arima-HiC platform and short-read sequencing. We performed pan-cancer analysis on 12 FFPE adult tumor biopsies, each with gene fusions known to be clinically actionable. With this newly developed Hi-C workflow we identified all the known gene fusions with 100% sensitivity in the samples including those involving ALK, NTRK3, ROS1, FGFR2, and SS18 genes. This approach also revealed that the NTRK3 gene fusion was the result of a more complex rearrangement within chr12, within chr15, and between chr12 and chr15. Additionally, we detected the presence of numerous structural variants per sample in addition to the known gene fusion in each sample. For example in a bile duct tumor, Arima-HiC detected an FGFR2-EEA1 gene fusion, as well as 25 other structural variants genome-wide including in a CPD-LASP1 gene fusion This gene fusion has not been reported to our knowledge, however, LASP1 is a reported 3’ fusion partner with MLL in leukemia. While these findings are not clinically actionable today, the unbiased, accurate, and sensitive detection of structural variants from frozen and FFPE solid tumor biopsies may facilitate further research into their relationship between disease mechanisms and clinical outcomes. Taken together, these findings demonstrate the analytical utility of Arima Hi-C sequencing technology to provide both chromosome-scale and gene-level resolution for the detection of structural variants in tumor biopsies samples. This workflow can provide improved access to critical genomic information from FFPE blocks for the identification of pathognomonic and druggable gene fusion events and other structural variants across tumor types.

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P05

BET inhibitors as treatment of acquired resistance to CDK4/6 inhibitors in breast cancers.

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Although the majority of these targeted therapies have improved breast cancer patient outcome, resistance ultimately develops to these agents. The acquired resistance to CDK4/6 inhibitors illustrates this idea since it develops in almost all cases after 24–28 months when first-line therapy is used.
Several therapeutic strategies were proposed to prevent or abrogate the acquired resistance of CDK4/6 inhibitors. Thus, literature reported that the pharmacological inhibition of CDK2, PDK1 and PI3K in combination with CDK4/6 inhibitors can be used to restore the sensitive of cells to CDK4/6 inhibitors.
To expand the investigations on the combinatorial approaches aiming to restore the sensitive of cells to CDK4/6 inhibitors, we here studied the effect of a panel of epigenetic drugs (epidrugs) since the use of these types of drugs is envisioned to overcome therapy resistance in cancer.
Our results indicated that MCF7 breast cancer cells presenting a resistance to Palbociclib showed a better sensitivity to JQ1 (an epidrug targeting BET proteins such as BRD4) than naïve MCF7 breast cancer cells. Our results associated the gain of JQ1 sensitivity with the BRD4 overexpression in a context of hypomethylation of BRD4 gene. In addition, our BRD4-ChIPseq and RNAseq allowed to identify two therapeutic vulnerabilities that can be targeted to increase the efficacy of palbociclib. In other terms, our study suggest that the Palbociclib resistance could be limited by combining the Palbociclib administration with molecules targeting BRD4 or MAP4K4 or MAPK12 and this when the BRD4 is hypomethylated. Besides, our study also shows that the modification of BRD4 methylation status can be detectable in longitudinal blood samples of Palbociclib-treated patient.
In conclusion our work provides proof of concept that the methylation level of BRD4 could be used as a blood biomarker to identify the right time for the right patient to be treated with the therapies combining Palbociclib with BRD4i or MAP4K4i or MAPK12i in a dynamic manner.

Key words: Breast cancer, BRD4, RNA-seq, CHIP-seq, CDK4/6 resistance, epigenetics.

P06

EPIGENETIC REGULATION OF TRANSCRIPTION AND SPATIAL GENOME ORGANIZATION DURING CHRONIC LUNG DISEASES

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In addition to nucleosomes, chromatin contains non-histone chromatin-associated proteins, of which the highmobility group proteins are the most abundant. Chromatin-mediated regulation of transcription involves DNA methylation and histone modifications. However, the order of events and the precise function of high-mobility group proteins during transcription initiation remain unclear. Here we show that high-mobility group AT-hook 2 protein (HMGA2) induces DNA nicks at the transcription start site, which are required by the histone chaperone FACT complex to incorporate nucleosomes containing the histone variant H2A.X. Further, phosphorylation of H2A.X at S139 (γ-H2AX) is required for repair-mediated DNA demethylation and transcription activation. The relevance of these findings is demonstrated within the context of TGFB1 signalling and idiopathic pulmonary fibrosis, suggesting therapies against this lethal disease. Our data support the concept that chromatin opening during transcriptional initiation involves intermediates with DNA breaks that subsequently require DNA repair mechanisms to ensure genome integrity.

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P07

Eradication of hematopoietic stem cells from patients with chronic myelomonocytic leukemia by combining DNA demethylating agents and histones methyltransferase inhibitors

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Introduction

Chronic myelomonocytic leukemia (CMML) is a HSC clonal disorder affecting elderly people. Heterochromatin, characterized by DNA methylation and H3K9me3, H3K9me2 and H3K27me3 marks, is involved in gene and retroelement (RE) repression. REs, including LINE-1, SINE and endogenous retroviruses (ERV) play a role in tumor development by inducing genomic instability and modulating gene expression but they can also be their Achille's heel. Indeed, massive production of REs triggers a « viral mimicry » state leading to induction of interferon (IFN) and anti-tumoral immune responses. DNA demethylating agents (DNMTis) are effective in about 40% of CMML patients. However, the response is always transient, temporally masking the phenotype of malignant HSCs without eradicating them.

Our hypothesis is that CMML HSCs could display heterochromatin alterations that restrain RE expression allowing them evading the immune system, leading to resistance to DNMTis.

Results

We showed that CD34+ HSPCs from CMML patients display a significant increase in H3K9me2 as compared to cells from age-matched controls. This is associated with an increase in G9A and GLP protein and mRNA expression, the enzymes regulating the deposition of this mark. Furthermore, H3K9me2 ChIP-seq analysis confirmed the increase of this mark in CD34+ cells from patients and more particularly at the level of REs.

The treatment of CMML CD34+ cells with DNMTis (decitabine, DAC, or 5-azacytidine), in combination with either UNC0638 or UNC0642, two potent selective G9A/GLP inhibitors, decreased their clonogenicity and serial replating in methylcellulose in a synergistic fashion, for 31/33 patients tested, while having no effect on aged healthy cells (n=15). The combination is more effective on more primitive CD34+CD38-CD90+ cells than on CD34+CD38-CD90- or total CD34+ cells while DAC affects only progenitors. Both survival and proliferation are affected at the single cell level. Assessment of variant allele frequency by targeted sequencing of patient mutations shows that only non-mutated clones survive after combination therapy, indicating that malignant HSCs are eradicated in vitro.

After four days of culture with DAC+UNC0638, but not with each treatment alone, we observed the production of double strand RNA (dsRNA), overexpression of IFN-β, IFN-stimulated genes and IFN signaling, specifically in CMML CD34+ cells. Moreover RNA-seq analysis in these conditions showed upregulation of many ERVs and antiviral signatures. The addition of an antibody neutralizing IFNAR2, the receptor for IFN-I, restored the clonogenic capacity of HSPCs in the presence of the combination, demonstrating the importance of the antiviral response in treatment efficacy.

Bone marrow from CMML patients was collected at diagnosis and used to perform xenografts in NSGS mice. The results showed that the combination significantly reduces the infiltration with tumoral hCD45+ cells in bone marrow and peripheral blood in transplanted mice.

Conclusion

These results demonstrate that targeting heterochromatin improves DNMTi efficacy for this difficult-to-treat disease.

P08

PRMT2: an anti-inflammatory epigenetic factor involved in acute myeloid leukemia aggressiveness

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INTRODUCTION

Protein Arginine Methyltransferases (PRMTs) are epigenetic factors involved in several cellular processes including regulation of gene expression through the methylation of histone tails. Overexpression and/or overactivity of the two major PRMTs (1 and 5) have been previously identified in Acute Myeloid Leukemia (AML) patients and their biological or pharmacological inhibition leads to a decreased leukemia progression in mice (PRMT1, -4, -5). Among the nine members of this protein family, PRMT2 has been found overexpressed in glioblastoma and is responsible for the H3R8me2a epigenetic mark leading to a transcription activation of target genes. We investigate the role of PRMT2 in the initiation and development of AML using a PRMT2 knock-out mouse model (PRMT2KO) and AML patient cohorts. Excessive or chronic inflammation can lead to severe pathologies including cancer. Indeed, long-term or chronic inflammation and aberrant myeloproliferation are interconnected. Therefore, we also investigated the anti-inflammatory effect of PRMT2 in an AML and a non-leukemic context.

RESULTS

PRMT2KO mice do not exhibit difference in bone marrow progenitor or mature cell populations compared to wild type mice, suggesting that PRMT2 has little or no influence in the hematopoietic stem cell maintenance in normal conditions. In contrast, we observed that PRMT2KO bone marrow-derived macrophages (BMDM) are more sensitive to lipopolysaccharide (LPS) stimulation and express higher levels of pro-inflammatory cytokine mRNA, suggesting a role of PRMT2 in the negative regulation of inflammatory processes. Analysis of datasets from a cohort of 371 AML patients (Guy Sauvageau, Leucegene project, IRIC, Montréal, QC, Canada) reveals that patients with lower PRMT2 expression display a worse survival rate compared to patients with higher PRMT2 expression. Gene Set Enrichment Analysis of this cohort show that PRMT2 should be involved in signaling pathways that are associated with the initiation and progression of inflammation, thus confirming our hypothesis. Our work focus on key players in inflammation and cancer, i.e. STAT3 and NF-kB. Moreover, ongoing epigenetic, transcriptomic, and proteomic analyses could provide new clues for better understanding the association between inflammation and leukemogenesis.

CONCLUSION

Taken together, our data suggest that PRMT2 acts as a negative regulator of inflammation. AML patients harboring a low PRMT2 expression should display a higher inflammatory phenotype, thus leading to a bad prognostic and a worse survival.

P09

Modulation of the activity of the stem cell epigenetic regulator PLZF through pollutant exposition

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The promyelocytic leukemia zinc finger protein (PLZF) is a regulator of heterochromatin and is involved in stem cell and progenitor maintenance. PLZF epigenetic activity is directly modulated by acetylation of specific lysine residues and controls its cellular localization by increasing its DNA affinity. Here, we report that environmental pollutants, such as benzene, affected directly the levels of PLZF acetylation and, thus, disturbed the heterochromatin patterning in hematopoietic cells.

P010

FULLY AUTOMATED ChIP ON TWO HISTONES USING DIAGENODE'S iDeal ChIP-SEQ KIT FOR HISTONES IN INOREVIA'S MAGELIA® PLATFORM

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ChIP-seq is the method of choice for identification of protein-DNA interactions at a whole genome scale. Despite its widespread use, this challenging application has significant limitations to hurdle, including the requirement for relatively large amounts of starting material, higher reproducibility, more efficient recovery, shorter hands-on time and increased signal-to-noise ratio. Miniaturization paired with automation represent a tangible solution to address these challenges. Magelia®, a novel multi-omics platform compatible with lower quantities of starting material, offers unique opportunities for automation of complex molecular biology applications. Its precise handling of a variety of magnetic beads paired with starting material reduction are a match for such protocols. Thanks to the platform's core technology, reduced reaction volumes with no evaporation improve kinetics for an efficient IP and background reduction while avoiding cross contamination. In this study, we showcase the compatibility of Magelia® with Diagenode's H3K9me3 and H3K4me3 antibodies and iDeal ChIP-seq kit for Histones. Total material equivalent to 25 000 cells was processed for ChIP in parallel manually and in the Magelia® respectively. Considerable hands-on time reduction (8x) was achieved with the platform. Magelia® treated samples showed equivalent or improved performance for several metrics when compared to manually treated samples, including duplication rates, relative strand cross-correlation coefficient and assigned peak correspondence. A relation between gene and peak density was identified for marker H3K4me3, which matched the density found in published studies. Peaks assigned around genes previously known to interact with H3K9me3 were confirmed. This emphasized the biological congruence of the obtained data. The iDeal ChIP-seq kit for Histones paired with the Magelia® platform, embodies a robust, effective, and automated method to tackle a complex protocol. This study validates compatibility of the platform with low initial cell quantities for ChIP applications. This solution is now part of Magelia®'s ever-growing list of applications.

THANKS to all participants

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