GAME OF EPIGENOMICS

Dubrovnik, Croatia, 24-28 April, 2016

INNOMOL
Innovation Pipeline

RB
Ruđer Bošković Institute

Game of Epigenomics

conference

Dubrovnik, Croatia, 24-28 April, 2016

Book of Abstracts

Editors

Oliver Vugrek, Ivanka Jerić, Andreja Ambriović Ristov and Ana Vidoš

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PROGRAMME

SUNDAY, 24 APRIL 2016

16:00 REGISTRATION

OPENING OF THE CONFERENCE | chair Oliver Vugrek

18:00
GOE KEYNOTE
NATURAL AND ARTIFICIAL REGULATION OF THE DNA METHYLOME
Ryan Lister | Harry Perkins Institute of Medical Research and University of Western Australia, Crawley, Australia

19:00 WELCOME PARTY

MONDAY, 25 APRIL 2016

MINISYMPOSIUM
EPIGENOMICS IN CONTEXT OF HUMAN METHYLATION DISORDERS | chair Ivo Barić

09:00
GOE PLENARY
S-ADENOSYLMETHIONINE AS AN INTEGRATOR OF HEPATIC METABOLISM
Jose M Mato | CIC bioGUNE (Center for Cooperative Research in Biosciences), Bilbao, Spain

09:45
GOE INVITED
DO DEFECTS IN METHYLATION MODIFY EPIGENETICS?
Luciana Hannibal | Albert Ludwigs University of Freiburg, Germany

10:15
GOE INVITED
EFFECT OF HYPOMETHYLATION ON ENDOTHELIAL HOMEOSTASIS
Rita Castro | University of Lisbon, Portugal

10:45
GOE INVITED
CLINICAL ASPECTS OF HUMAN METHYLATION DISORDERS
Ivo Barić | University Hospital Centre Zagreb (KBC Zagreb), Croatia

11:15
GOE INVITED
GENOME-WIDE METHYLOME SEQUENCING IDENTIFIES CTCF REGULATED GENES IMPLICATED IN DIABETIC COMPLICATIONS
Assam El-Osta | Baker IDI Heart and Diabetes Institute, Melbourne, Australia

11:45 COFFEE BREAK

SESSION I | chair Ryan Lister

12:00
GOE PLENARY
ABSENCE OF CANONICAL ACTIVE CHROMATIN MARKS IN DEVELOPMENTALLY REGULATED GENES
Roderic Guigo | Centre for Genomic Regulation (CRG), Barcelona, Spain

12:45
GOE ORAL
GRAPH-BASED DATA INTEGRATION PREDICTS LONG-RANGE REGULATORY INTERACTIONS ACROSS THE HUMAN GENOME
Tom Michoel | Roslin institute, University of Edinburgh, Scotland

13:05
GOE ORAL
CPG ISLAND EROSION, POLYCOMB OCCUPANCY AND SEQUENCE MOTIF ENRICHMENT AT BIVALENT PROMOTERS IN MAMMALIAN EMBRYONIC STEM CELLS
Anagha Joshi | Roslin institute, University of Edinburgh, Scotland

13:25

GOE ORAL

VARIABLE SELECTION IN BINOMIAL REGRESSION WITH LATENT GAUSSIAN FIELD MODELS FOR ANALYSIS OF EPIGENETIC DATA

Aliaksandr Hubin | University of Oslo, Norway

13:45 LUNCH BREAK

SESSION II | chair Ulrich Zechner

15:15

GOE INVITED

ENDOGENOUS RETROVIRUSES AND THE CONTROL OF GENE REGULATORY NETWORKS IN THE BRAIN

Johan Jakobsson | Lund University, Sweden

15:45

GOE INVITED

EXOME AND DEEP SEQUENCING OF CLINICALLY AGGRESSIVE NEUROBLASTOMA REVEAL MUTATED GENES INVOLVED IN CANCER PROGRESSION

Mario Capasso | Università degli Studi di Napoli Federico II - COINOR, Napoli, Italy

16:15

GOE INVITED

EPIGENETIC REGULATION OF NEURONAL DEVELOPMENT AND FUNCTION

Vijay Tiwari | Institute of Molecular Biology GmbH, Mainz, Germany

16:45

GOE ORAL

DELAY IN THE DEVELOPMENTAL TRAJECTORY OF INTRAGENIC DNA METHYLATION UNDERLIES ALTERED PRE-MRNA SPLICING IN AUTISM SPECTRUM DISORDERS

Michael J. Corley | Department of Native Hawaiian Health, John A. Burns School of Medicine, University of Hawaii

17:05 COFFEE BREAK AND POSTER SESSION I

Tuesday, 26 April 2016

SESSION III | chair Assam El Osta

09:00

GOE PLENARY

EPIGENOMICS FROM MAPPING TO INTERPRETATION

Jörn Walter | Saarland University, Saarbrücken, Germany

09:45

GOE INVITED

MAPPING REGULATORY AND EPIGENETIC CHANGES UNDERLYING EARLY EVENTS OF DRUG-INDUCED LIVER TUMOR PROMOTION

Rémi Terranova | Preclinical Safety, Translational Medicine, Novartis Institutes for Biomedical Research (NIBR), Basel, Switzerland

10:15

GOE INVITED

EPIGENETICS ORIGINS OF CANCER

Zdenko Herceg | Epigenetics Group International Agency for Research on Cancer (IARC), Lyon, France

10:45 COFFEE BREAK

SESSION IV | chair Boris Maček

11:00

GOE INVITED

MS-PROTEOMICS TO STUDY HISTONE POST-TRANSLATIONAL MODIFICATIONS AND CHROMATIN PLASTICITY IN CANCER, FOR CLINICAL AND BASIC RESEARCH

Tiziana Bonaldi | Department of Experimental Oncology, European Insitute of Oncology, Milano Italy

11:30

GOE INVITED

QUANTITATIVE INTERACTOMICS IN EPIGENETICS AND GENOME STABILITY

Falk Butter | Institute of Molecular Biology, Mainz, Germany

11:50

GOE INVITED

QUANTITATIVE INTERACTION PROTEOMICS FOR EPIGENETICS

Michiel Vermeulen | Radboud Institute for Molecular Life Sciences, Department of Molecular Biology, Nijmegen, The Netherlands

12:10

GOE ORAL

2D TAU GEL COUPLED WITH MS/MS TO IDENTIFY A DIFFERENT HISTONE PTMS PROFILE IN BREAST CANCER CELL LINES

A. M.Perri | Laboratory of Proteomics and Mass spectrometry, Dpt. of Experimental and Clinical Medicine, Magna Graecia University of Catanzaro, Italy

13:00 LUNCH BREAK

SESSION V | chair Zdenko Herceg

14:00

GOE PLENARY

ROLE OF NEIL DNA GLYCOLASE IN DNA DEMETHYLATION

Christof Niehrs | Institute of Molecular Biology (IMB), University of Mainz, Germany

14:45

GOE ORAL

PRECISION MEDICINE BEYOND INDIVIDUAL GENES: GLYCANS AS INTEGRATORS OF GENES AND ENVIRONMENT

Gordan Lauc | University of Zagreb Faculty of Pharmacy and Biochemistry & Genos Glycoscience Research Laboratory, Zagreb, Croatia

15:15

GOE ORAL

INHIBITION OF DNA METHYLATION RESENSITIZE TUMOR CELLS TO DEPENDENCE RECEPTORS APOPTOTIC PATHWAY TARGETING THERAPIES

Robert Dante | Cancer and Development Laboratory Centre de Recherche en Cancérologie de Lyon, France

15:35

GOE ORAL

EPIGENETIC DEREGULATION IS AN IMPORTANT MECHANISM LEADING TO ABERRANT PROTEIN GLYCOSYLATION IN HUMAN COMPLEX DISEASES

Vlatka Zoldoš | Faculty of Science, University of Zagreb, Croatia

16:05

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EPIGENOME EDITING WITH ZINC FINGER PROTEINS AND CRISPR

Rainer Ebel | Sigma-Aldrich (Merck Group), Taufkirchen, Germany

16:25

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ADVANCES IN EPIGENOMICS ON ILLUMINA NGS PLATFORMS

Szabolcs Kokeny | Illumina, USA

16:35 COFFEE BREAK AND POSTER SESSION II

19:00 CONGRESS DINNER

Restaurant ARSENAL (Gradska kavana, Street: Pred Dvorom 1, Dubrovnik)

Wednesday, 27 April 2016

SESSION VI, chair Robert Belužić

09:00

GOE PLENARY

TRANSCRIPTION AND CHROMATIN MECHANISMS DETERMINING THE OOCYTE DNA METHYLOME

Gavin Kelsey | The Babraham Institute, Cambridge, UK

09:45

GOE INVITED

H3K4OX-MEDIATED CHROMATIN CONDENSATION PROTECTS BREAST CANCER CELLS FROM THE DNA DAMAGE REPAIR MACHINERY

Sandra Peiró | Programa de Recerca en Càncer, Institut Hospital del Mar d'Investigacions Mèdiques, Barcelona, Spain

10:15

GOE ORAL

MICRORNA PROFILING OF OVARIAN CANCER

Sonja Levanat | Ruđer Bošković Institute, Zagreb, Croatia

10:45 COFFEE BREAK

SESSION VII, chair Neda Slade

11:00

GOE PLENARY

NON-CANONIC FUNCTIONS OF POLYCOMB RING1 PROTEINS

Miguel Vidal | Department of Cell and Molecular Biology, Centro de Investigaciones Biológicas (CSIC), Madrid, Spain

11:45

GOE ORAL

HIGH RESOLUTION DNA EPIGENOME PROFILING USING CPG-SPECIFIC TETHERED-OLIGONUCLEOTIDE-PRIMED SEQUENCING (CPG-TOP-SEQ)

Saulius Klimašauskas | Institute of Biotechnology, Vilnius University, Lithuania

12:05

GOE ORAL

NON-CODING TRANSCRIPTION BRINGS RNAI TO REGULATE EXPRESSION OF NUTRIENT UPTAKE GENE IN FISSION YEAST

Sneha Shah | TATA institute of fundamental research (TIFR), Mumbai, India

12:25 LUNCH BREAK

15:00 KARAKA GAME OF THRONES TOUR

Panoramic cruise on Karaka ship replica of a traditional ship from the 16th century and walking tour of the Old Town

Thursday, 28 April 2016

SESSION VIII, chair Oliver Vugrek

09:00

GOE ORAL

A SPLICING SWITCH OF THE HISTONE VARIANT MACROH2A1 COUPLES THE CHROMATIN STATE TO ENERGY METABOLISM

M. Posavec Marjanović | IMPPC, Badalona Spain, Ruđer Bošković Institute, Zagreb, Croatia

09:20

GOE ORAL

PLACENTAL DNA METHYLATION OF THE SEROTONIN TRANSPORTER GENE IS ASSOCIATED WITH MATERNAL GESTATIONAL DIABETES

Jasminka Štefulj | Ruđer Bošković Institute, Zagreb, Catholic University of Croatia

09:40

GOE INVITED

EPIGENOMICS OF S-ADENOSYLHOMOCYSTEINE HYDROLASE DEFICIENCY

Oliver Vugrek | Ruđer Bošković Institute, Department of Molecular Medicine, Laboratory for Advanced Genomics, Zagreb

10:10 CLOSING SESSION

ABSTRACTS

Natural and artificial regulation of the DNA methylome

Ryan Lister (1)

1) The Harry Perkins Institute of Medical Research and The University of Western Australia, Crawley, Australia

The vertebrate body plan and organs are shaped during a highly conserved embryonic phase called the phylotypic stage, however the mechanisms that guide the epigenome through this transition and their evolutionary conservation remain elusive. Here we report widespread DNA demethylation of thousands of enhancers during the phylotypic period in zebrafish, Xenopus and mouse. These dynamic enhancers are linked to essential developmental genes that display coordinated transcriptional and epigenomic changes in the diverse vertebrates during embryogenesis. Phylotypic stage-specific binding of Tet proteins to (hydroxy)methylated DNA, and enrichment of hydroxymethylcytosine on these enhancers, implicated active DNA demethylation in this process. Furthermore, loss of function of TET1/2/3 in zebrafish caused reduced chromatin accessibility and increased methylation levels specifically on these enhancers, indicative of DNA methylation being an upstream regulator of phylotypic enhancer function. Overall, this reveals a novel regulatory module associated with the most conserved phase of vertebrate embryogenesis and uncovers an ancient developmental role for the Tet dioxygenases. Finally, we are developing new molecular tools for precise targeted manipulation of the epigenome in order to establish the causal relationships between epigenomic modifications and genome regulation.

S-Adenosylmethionine as an integrator of hepatic metabolism

JOSÉ M MATO (1)

1) CIC bioGUNE- CIBERehd, Derio, Bizkaia, Spain

Keywords: S-Adenosylmethionine, methylation, liver metabolism, fatty liver.

S-adenosylmethionine (SAMe) methylates a myriad of substrates, including DNA, proteins and lipids, provides the sulfur atom for glutathione and taurine synthesis, and the amino group to make polyamines. Here, we provide a look at how SAMe regulates global hepatic metabolism. We show that hepatic SAMe functions as an integrator of the cellular metabolic status and that its hepatic depletion alters an unparalleled diversity of cellular processes ranging from the synthesis of lipids and amino acids to mitochondrial polarization and redox status. We also identify a serum-specific metabolic signature characteristic of mice lacking Mat1a, the main enzyme involved in hepatic SAMe synthesis, and found that around one-third of patients with nonalcoholic fatty liver disease (NAFLD) showed this signature, which may guide patient selection for SAMe treatment.

Do defects in methylation modify epigenetics?

LUCIANA HANNIBAL (1), UTE SPIEKERKÖTTER (2), HENK J BLOM (1)

1) Laboratory of Clinical Biochemistry and Metabolism, Department of General Pediatrics, Adolescent Medicine and Neonatology, University Medical Centre Freiburg. Mathildenstrasse 1, 79106 Freiburg, Germany

2) Department of General Pediatrics, Adolescent Medicine and Neonatology, University Medical Centre Freiburg. Mathildenstrasse 1, 79106 Freiburg, Germany

Keywords: methylation, epigenetics, S-adenosylmethionine, homocysteine, vitamin B12

Epidemiological findings as well as experimental results from in vitro and animal model studies suggest that elevated homocysteine (Hcy) causes DNA hypomethylation. Because DNA methylation is a key modulator of gene expression, its imbalance may trigger pathological processes by altering the expression of a myriad of genes. The long-hypothesized regulation of gene expression by the endogenous concentration of S-adenosylmethionine (AdoMet) and S-adenosylhomocysteine (AdoHcy), or by the AdoMet/AdoHcy ratio, constitutes an important link between DNA methylation and diseases in Hcy, folate and vitamin B_{12} metabolism.

Ex-vivo examination of DNA and protein methylation in human umbilical vein endothelial cells grown in the presence of a specific inhibitor of AdoCys hydrolase (leading to elevated AdoHcy) showed inhibition of both protein and DNA methylation. Protein methylation was more sensitive to the inhibitory effects of elevated AdoHcy than DNA methylation. In consecutive studies, we demonstrated that in mice, rats and humans, hyperHcy does not alter DNA methylation. The only exception was observed in rats under a combination of a diet high in methionine and low in B-vitamins. Under this dietary condition, global DNA methylation decreased more than 25%. These findings are in contrast with several publications showing reduced global and gene specific DNA methylation in hyperHcy models and the proposed positive effects of folic acid on DNA hypomethylation. Our studies were first to investigate global protein methylation in rodents and humans. The finding that protein methylation was substantially more sensitive to inhibition by elevated AdoHcy compared to changes in DNA methylation in the mouse and rats models is intriguing, and a matter of active ongoing investigation.

Current research in our team focuses on the identification of specific proteins undergoing disturbed methylation due to AdoHcy accumulation, and the elucidation of the DNA and protein methylomes in inborn errors of metabolism severely affecting the remethylation pathway. Our findings will fuel future research not only relevant to inborn errors of homocysteine and methylation metabolism, but also in common disorders like cancer, vascular diseases and congenital defects where epigenetics is thought to play a preponderant role.

Clinical aspects of human methylation disorders

Ivo Barić (1), Danijela Petković Ramadža (2)

1) University Hospital Center Zagreb & University of Zagreb, School of Medicine

2) University Hospital Center Zagreb

Keywords: Hypermethinonemia, inherited methylation defects,

Inherited methylation disorders are a group of four metabolic disorders which are likely largely underdiagnosed. These are methionine adenosyltransferase I/III deficiency (recently named Mudd's disease) and glycine N-methyltransferase, S-adenosylhomocysteine hydrolase and adenosine kinase deficiencies, respectively. They have in common the affection of the transmethylation processes in the metabolic pathway between methionine and homocysteine. Clinical presentation of methylation disorders depends on the disorder and can vary significantly. Mudd's disease is clinically benign in the majority of patients, but those with plasma methionine higher than app. 800µmol/L are at higher risk for developing central nervous system signs and symptoms. Typical abnormality on brain images is demyelination with oedema of white matter, resulting in separation of myelin layers - the so-called vacuolating myelopathy. Glycine N-methyltransferase deficiency has been reported in only five individuals who haven't had clinical problems which could be accurately attributed to this disorder. However, their plasma methionine concentrations are in the range which can be associated with clinical signs and symptoms . S-adenosylhomocysteine hydrolase in its severe form is associated with foetal hydrops, brain anomalies, liver failure and severe muscular hypotonia leading to respiratory failure and death in early infancy. Patients with a milder phenotype had muscle disease with increased creatine kinase activity and various combinations of developmental delay, behavioral disorders, myelination delay, microcephaly, strabism, coagulopathy and liver disease. Adenosine kinase deficiency is a multisystemic disease with neonatal onset. The patients had in various combinations muscular hypotonia, dysmorphy (particularly frontal bossing), psychomotor retardation, epilepsy, liver disease and recurrent hypoglycaemia. Biochemical hallmark of this group of disorders is isolated hypermethioninemia. However, it is not always present in early infancy making the newborn screening inaccurate. Mild hyperhomocysteinemia has been reported in several patients. Plasma S-adenosylmethionine and S-adenosylhomocysteine are crucial metabolites for biochemical clarification of isolated hypermethioninemia. Treatment options are of limited benefit. Dietary methionine restriction can be useful in patients with Mudd's disease if they are symptomatic and/or have plasma methionine above 800 µmol/L and in some aspects in patients with S-adenosylhomocysteine hydrolase deficiency and adenosine kinase deficiency. S-adenosylmethionine supplementation may be useful in some patients with Mudd's disease.

References: This work has been done as a part of the "European network and registry for homocystinurias and methylation defects (E-HOD)" project co-funded by the European Union in the framework of the Health Program (No.2012_12_02).

Effect of hypomethylation on endothelial homeostasis

Madalena Barroso (1), Isabel T. Almeida (2), Rita Castro (3), Diane E. Handy (4)

1) 1Metabolism and Genetics Group, Research Institute for Medicines (iMed.UL), University of Lisbon, Lisbon, Portugal and Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, USA.

2) Metabolism and Genetics Group, Research Institute for Medicines (iMed.UL), University of Lisbon, Lisbon, Portugal.

3) Metabolism and Genetics Group, Research Institute for Medicines (iMed.UL) and Department of Biochemistry and Human Biology, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal.

4) Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, USA.

Keywords: hypomethylation; homocysteine; DNA and histone hypomethylation

Hyperhomocysteinemia is an independent risk factor for cardiovascular diseases (CVD) by mechanisms incompletely defined. S-Adenosylhomocysteine (SAH) is the metabolic precursor of homocysteine that accumulates in the setting of hyperhomocysteinemia and is a negative regulator of most cell methyltransferases. Thus, we suggest that methylation imbalance, caused by excess SAH, may contribute to homocysteine's vascular toxicity by disrupting endothelium homeostasis and favoring the establishment of a pro-atherogenic phenotype.

Decreased nitric oxide (NO) bioavailability is a principal manifestation of underlying endothelial dysfunction, an early marker of atherosclerosis and CVD. Excess SAH was found to alter NO bioavailability, by decreasing endothelial nitric oxide synthase (eNOS) protein and activity, to decrease cellular production of NO.

Another important feature of endothelial dysfunction is oxidative stress. Glutathione peroxidase-1 (GPx-1) is a selenoprotein and a major cellular antioxidant. The expression of a subset of selenoproteins (including GPx-1) is dependent on a specific methylation of the selenocysteine-tRNA (Sec-tRNA). SAH accumulation was found to induce $tRNA^{Sec}$ hypomethylation, leading to a suppression of GPx-1 expression and promoting oxidative stress and pro-inflammatory activation of endothelial cells.

SAH-induced upregulation of adhesion molecules promoted leukocyte adhesion to endothelial cells and their trans-endothelial migration, illustrating the physiological relevance of SAH-induced endothelial cell activation. Excess SAH also decreased global DNA methylation. In addition, ICAM-1 (intercellular adhesion molecule 1) was found to be upregulated by a DNA methyltransferase inhibitor, suggesting that its expression may be regulated by DNA hypomethylation. Analysis of its promoter methylation; however, showed that it was demethylated in untreated cells, implying that it may be regulated by factors other than DNA methylation in response to excess SAH.

Lastly, SAH-induced suppression of the epigenetic regulator EZH2 (enhancer of zeste homolog 2) resulted in histone hypomethylation and activation of the inflammatory mediator NFkB (nuclear factor kappa B). These results identify a new, critical link between SAH and inflammatory responses, demonstrating that EZH2 suppression and the resulting NFkB activation may contribute to the adverse

effects of excess SAH in the vasculature.

Overall, these studies implicate SAH as a modulator of epigenetic mechanisms by compromising RNA, DNA, and histone methylation, favoring the establishment of a pro-atherogenic phenotype and contributing to homocysteine-associated CVD.

Genome-wide methylome sequencing identifies CTCF regulated genes implicated in diabetic complications

Ishant Khurana (1), Anna Syreeni (2), Mark Ziemann (1), Antony Kaspi (1), Merlin Thomas (1), Carol Forsblom (2), Mark Cooper (1), Per-Henrik Groop (2), Assam El-Osta (1)

1) Baker IDI Heart & Diabetes Institute, Australia

2) Folkhälsan Institute of Genetics, Folkhälsan Research Center, Biomedicum Helsinki, Helsinki, Finland

Keywords: Methylation, Chromatin, Transcription, Diabetes

Building on the first genome-wide hyperglycemic atlas of the vascular epigenome, we now expand this map using the The Finnish Diabetic Nephropathy Study (FinnDiane) and show differential methylated regions (DMRs) of key transcription factor binding sites. In this cohort we show epigenetic changes derived from white blood cells for genes implicated in diabetes such as mTOR, EPHA1, EFNB2, FGFR4 and BGN. These epigenetic changes are also observed in HMEC (human microvascular endothelial cells) as well as primary HAECs (human aortic endothelial cells). Methylation sequencing of the FinnDiane cohort identified that mTOR (mechanistic target of rapamycin) gene regulation was subject to differential CpG methylation at CTCF binding sites. These clinical findings were tested *ex vivo* in primary diabetic HAECs that confirm low glucose transition to high glucose conditions increased mTOR gene expression. The significance of DNA methylation on mTOR was confirmed using the DNA methylation inhibitor, 5-aza-2'-deoxycytidine (5adC). We show exon-specific mTOR expression is DNA methylation dependent and hypothesize alternative splicing of mTOR is mediated by Pol II pausing conferred by DNA methylation. Chromatin immunoprecipitation implicates DNA methylation of mTOR regulates CTCF binding in primary HAECs stimulated by hyperglycemia and 5adC. These results highlight glucose-derived epigenetic changes of genes implicated in the progression of diabetic complications.

Absence of canonical active chromatin marks in developmentally regulated genes

Roderic Guigo (1)

1) Center for Genomic Regulation (CRG) and Universitat Pompeu Fabra (UPF), Barcelona, Spain

Post-translational modifications of histones define an evolutionarily conserved “code” that governs differential gene expression. Trimethylation of histone H3 at lysine 4 (H3K4me3) and at lysine 36 (H3K36me3), for instance, correlate with active transcription, whereas H3K9me3 and H3K27me3 are usually linked to transcriptional repression. The combinatorial behavior of histone modifications along regulatory regions—reflecting and/or influencing the specific arrangement of transcription factors—modulates the expression levels of genes, conferring them with a unique temporal and spatial transcriptional program. Computational models have been developed that can predict gene expression from histone modifications with great accuracy.

A number of recent reports, however, indicate that expression of certain genes may occur in absence of histone modifications canonically associated to active genes. The modENCODE project reported that some expressed genes lacked H3K4me3. Hödl and Basler found that cells that lack H3K4 methylation, respond to developmental signaling pathways by activating target gene expression in Drosophila wing imaginal discs. Chen et al. observed that pre-midblastula transition (pre-MBT) genes have particularly low levels of H3K4me38. More recently, Zhang et al. reported that genes within yeast heterochromatic regions can be transcribed in absence of active histone marks. We have recently shown that active transcription in the absence of chromatin marking is actually a general feature of genes that are strongly regulated during development. We analyzed data produced by modENCODE in whole animals and tissues in fly and worm, characterized the fly transcriptome by RNASeq and the epigenome by ChIPSeq in two spatially well-defined and relatively homogeneous developmental fly tissues, and carried out targeted experimental validations in isolated cells. All these analyses strongly suggest that expression of genes regulated during fly development can occur in the absence of marks typically associated with active genes, and, indeed, this expression does not seem to be affected by perturbations of the histone methyltransferase system. Conversely, we found that chromatin marking is associated not only to transcriptional levels, but also to transcriptional and post-transcriptional stability—an association that appears to be conserved through metazoan evolution.

Graph-based data integration predicts long-range regulatory interactions across the human genome

Dr. Tom Michoel (1)

1) The Roslin institute, University of Edinburgh

Keywords: DNAse-seq, Hi-C, CAGE

Motivation: Transcriptional regulation of gene expression is one of the main processes that affect cell diversification from a single set of genes. Regulatory proteins often interact with DNA regions located distally from the transcription start sites (TSS) of the genes. We developed a computational method that combines open chromatin and gene expression information for a large number of cell types to identify these distal regulatory elements.

Results: Our method builds correlation graphs for publicly available DNase-seq and exon array datasets with matching samples and uses graph-based methods to filter findings supported by multiple datasets and remove indirect interactions. The resulting set of interactions was validated with both anecdotal information of known long-range interactions and unbiased experimental data deduced from Hi-C and CAGE experiments. Our results provide a novel set of high-confidence candidate open chromatin regions involved in gene regulation, often located several Mb away from the TSS of their target gene.

Availability: To query the predicted interactions, a webservice was developed:

http://dhsgen.roslin.ed.ac.uk.

CpG island erosion, polycomb occupancy and sequence motif enrichment at bivalent promoters in mammalian embryonic stem cells

Anna Mantsoki (1), Dr. Anagha Joshi (1), Dr. Guillaume Devailly (1)

1) The Roslin institute, University of Edinburgh

Keywords: chromatin modifications, ES cells, bivalency

In embryonic stem (ES) cells, developmental regulators have a characteristic bivalent chromatin signature marked by simultaneous presence of both activation (H3K4me3) and repression (H3K27me3) signals and are thought to be in a 'poised' state for subsequent activation or silencing during differentiation. We collected eleven pairs (H3K4me3 and H3K27me3) of ChIP sequencing datasets in human ES cells and eight pairs in murine ES cells, and predicted high-confidence (HC) bivalent promoters. Over 85% of H3K27me3 marked promoters were bivalent in human and mouse ES cells. We found that (i) HC bivalent promoters were enriched for developmental factors and were highly likely to be differentially expressed upon transcription factor perturbation; (ii) murine HC bivalent promoters were occupied by both polycomb repressive component classes (PRC1 and PRC2) and grouped into four distinct clusters with different biological functions; (iii) HC bivalent and active promoters were CpG rich while H3K27me3-only promoters lacked CpG islands. Binding enrichment of distinct sets of regulators distinguished bivalent from active promoters. Moreover, a 'TCCCC' sequence motif was specifically enriched in bivalent promoters. Finally, this analysis will serve as a resource for future studies to further understand transcriptional regulation during embryonic development.

Variable selection in binomial regression with latent Gaussian field models for analysis of epigenetic data

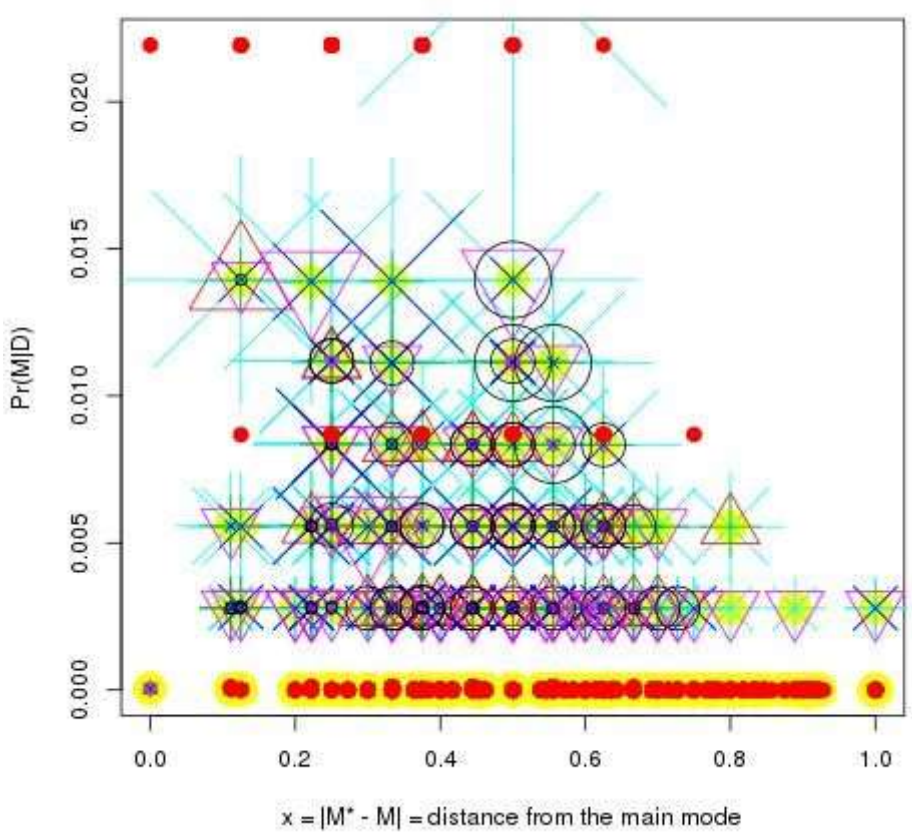
Aliaksandr Hubin (1), Geir Storvik (1), Paul Grini (1)

1) University of Oslo

Keywords: Epigenomics of Arabadopsis thaliana, Local meta-heuristics, Combinatorial optimization, High performance computations, Statistical modeling

Epigenetic observations are represented by the total amount of reads from a particular cell and the amount of methylated reads, which are reasonable to model via a Binomial distribution. There are numerous factors that might influence the probability of success from a particular region. We might also expect spatial dependence of these probabilities. We incorporate dependence on the covariates and spatial dependence of probability of being methylated for observation from a particular cell by means of a binomial regression model with a latent Gaussian field. We finally divide genome into a number of regions to reduce computational effort and avoid heteroscedasticity and carry out efficient mode jumping MCMC with simultaneous model selection with respect to different model selection criteria across different choices of covariates of the regression model for each region in order to draw from posterior distributions of parameters of the models and models jointly and finding the best choice of covariates that influence methylation structure in all of the regions within the genome.

References: E. I. George and R. E. Mcculloch. Approaches for bayesian variable selection. Statistica Sinica, pages 339–374, 1997. H. Rue, S. Martino, and N. Chopin. Approximate bayesian inference for latent Gaussian models by using integrated nested laplace approximations. Journal of the Royal Statistical Sosciety, 71(2):319–392, 2009 G. Storvik. On the flexibility of metropolis-hastings acceptance probabilities in auxiliary variable proposal generation. Scandinavian Journal of Statistics, 38:342–358, 2011.



Caption: The figure contains the space of visited models mapped on a 2d space by multidimensional scaling approach based on the distance matrix between the addressed models represented by binary vectors. The radiuses of the circles are proportional to the posterior mass of the corresponding models.

Endogenous retroviruses and the control of gene regulatory networks in the brain

Johan Jakobsson (1)

1) Lund University

Keywords: transposons, epigenetics, brain, histone modifications, long non-coding RNAs

The mammalian brain is an extremely complex organ with thousands of different types of neurons serving a wide variety of functions. How this complexity is achieved is largely unknown. More than half of the human genome is composed of TEs and it is becoming increasingly clear that TEs can act as gene regulatory elements since they are very well suited to influence gene expression and may play an important role in controlling and fine-tuning gene network. For example, a series of recent studies indicate that endogenous retroviruses (ERVs) participate in the control of gene expression during early mouse and human development and there is also a remarkable cell-type specific expression of these elements during different stages of early human development. Still, if and how ERVs act as regulatory elements in somatic cells including the brain is poorly explored, both in terms of normal development and disease states.

In this study, we characterize the expression profile of human endogenous retroviruses (HERVs) in the human nervous system during embryonic development. The data shows that HERVs are dynamically expressed in the developing human nervous system, based on both regional and age related differences. The expression patterns of HERVs was also monitored in hNPCs and upon their differentiation towards mature neurons, revealing a dynamic control also in this in vitro model of human neural development. In addition, we show that in hNPCs, a select number of HERVs are repressed by H3K9me3 recruited by the epigenetic co-repressor TRIM28. These data demonstrate that HERVs have the capacity to act as regulatory RNAs in the brain. In addition, HERVs may act as epigenetic hubs that attract repressive histone marks, which may mediate transcriptional silencing of nearby genes.

Taken together, these results establish a dynamic transcriptional control of HERVs during brain development and indicate that these elements play a critical functional role in regulating transcriptional networks human cells which in turn influence the progeny they produce. Considering that the genomic composition of HERVs varies considerably between species, this mechanism has the potential to mediate human-specific gene regulatory network. These experiments will open up for further studies on the role of ERVs in driving human brain evolution, their role in contribution to individual variation and their implication in human brain.

Exome and deep sequencing of clinically aggressive neuroblastoma reveal mutated genes involved in cancer progression

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Neuroblastoma is a malignancy of the developing sympathetic nervous system that often presents as metastatic at the time of diagnosis that is under the age of 5 for 90% of children. Aggressive tumors show survival rates of less than 50%. The complete spectrum of somatic mutations of the most aggressive forms of neuroblastoma is still to be defined. Here we sought to identify additional potential cancer drivers in high-risk and ultra-high-risk (high-risk patients with any adverse event within 36 months from diagnosis) neuroblastoma.

Whole exome sequencing was performed for 17 ultra-high-risk germline and tumor pairs to identify somatic mutations and deep targeted sequencing of 134 genes selected from the initial screening in an additional set of 48 germline and tumor pairs (62.5% were ultra-high-risk and high-risk), 17 ultra-high-risk tumors and 17 human-derived neuroblastoma cell lines.

Combining both cohorts we found 22 significantly mutated genes, many of which implicated in cancer progression processes. Of these, fifteen (68.2%) were highly expressed in neuroblastoma supporting the biological rationale for their involvement in this malignancy. CHD9, annotated as cancer driver in public databases, was the most significantly altered gene (4.0% of cases) after ALK. Other genes (PTK2, NAV3, NAV1, LRRC17, PXDN, FZD1, ARHGEF10L and ATRX) expressed in neuroblastoma and involved in cell invasiveness and migration were mutated at frequencies between 4% and 2%. Pathways implicated in cell survival, proliferation and motility (focal adhesion and regulation of actin cytoskeleton) were the most frequently disrupted affecting 14.1% of cases, suggesting potential novel therapeutic strategies to prevent disease progression. Rare potentially pathogenic germline variants were significantly enriched in BARD1, CHEK2 and AXIN2.

To conclude, the combination of whole exome and deep targeted sequencing in a discovery and validation cohort experimental design, identified novel cancer genes in clinically aggressive neuroblastomas. Our analyses demonstrate that infrequently mutated genes may have pathway-level implications in leading tumor progression and suggests possible novel strategies for therapeutic interventions in aggressive forms of neuroblastoma.

Epigenetic Regulation of Neuronal Development and Function

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1) Institute of Molecular Biology (IMB)

Cell-fate specification during mammalian development involves stable resetting of transcriptional programs and the role of chromatin-mediated regulation in this process has been increasingly appreciated. The nervous system is the most complex organ in all mammalian organisms. The last decade has observed extensive research in understanding how this complexity is generated during neuronal development. Despite exciting progress, very little is known about the function of epigenetic mechanisms in neurogenesis and neuronal activity. Using epigenetics, genomics and molecular biology tools in combination with extensive computational biology approaches we are investigating i) the crosstalk of transcription factors with chromatin during specification of neuronal fate, ii) dynamics of chromatin accessibility during neurogenesis and iii) the function of novel epigenetic regulators during neuronal development. In addition, we are also investigating whether neuronal- activity-dependent gene regulation involves epigenetic reprogramming.

Delay in the developmental trajectory of intragenic DNA methylation underlies altered pre-mRNA splicing in autism spectrum disorders

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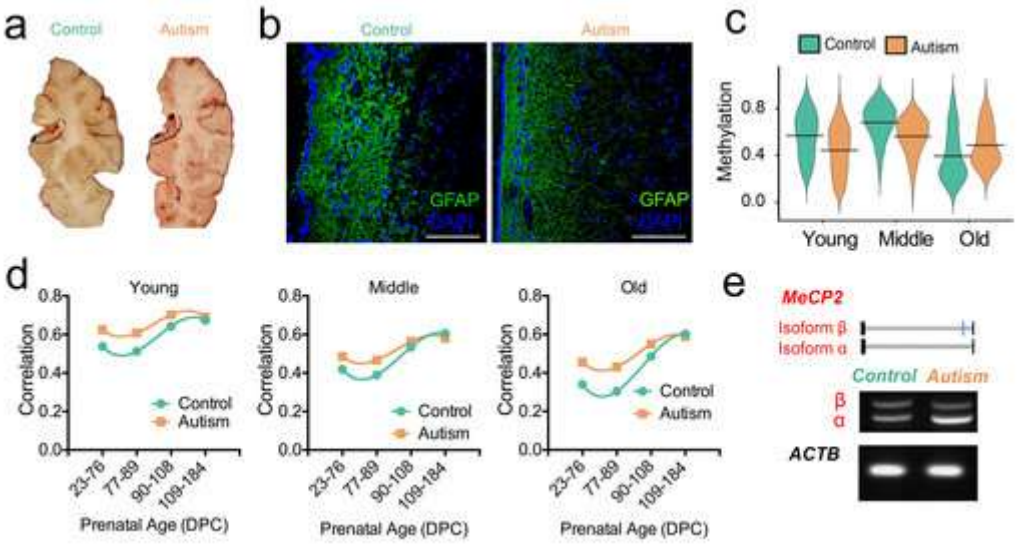
Keywords: autism, DNA methylation, postmortem brain, neurodevelopment, splicing

Autism spectrum disorders (ASD) are clinically heterogeneous neurobehavioral diseases suspected to originate *in utero* from perturbations in neural stem cell niche regions of the developing brain, when DNA methylation undergoes extensive changes. However, whether the normal dynamics of DNA methylation in neural stem cell compartments is compromised in ASD is unknown (Fig. a). Here, we report significant DNA methylation defects in the subventricular zone (SVZ) of the lateral ventricles from postmortem brain of autism-diagnosed compared to typically developing individuals (Fig. b,c). By integrating these data with DNA methylation profiles at prenatal stages of brain development, we observed that the normal trajectory of DNA methylation states, predominately over gene bodies, is disrupted in ASD (Fig. d). Such altered intragenic DNA methylation states are associated with aberrant pre-mRNA splicing events of ASD-relevant neurodevelopmental genes, including *MeCP2* (Fig. e). These findings suggest an "epigenetic delay" in conditioning the chromatin landscape during neurodevelopment, leading to deleterious transcriptomic events in ASD. Our comparative and integrative analyses of the DNA methylome and RNA transcriptome in 34 postmortem SVZ tissue specimens from ASD cases and age-matched, typically developing individuals reveal new insights into the developmental timing and mechanisms underlying the etiology of ASD.



Caption: a) Postmortem brain tissue dissections of neurogenic niche b) subventricular zone of lateral ventricles (SVZ). c) Altered DNA methylation, d) delayed developmental trajectory and e) aberrant splicing in autism SVZ compared to age-matched control.

Epigenomics - from mapping to functional interpretation

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Epigenetics is important to understand mechanisms controlling cellular development (epigenesis), the (in)stability of genetic regulation (functional genomics) and effects of (non-mendelian) inheritance. Epigenetic control involves multiple molecular levels including various forms of DNA-methylation, multiple histone modifications and numerous forms of non-coding RNAs. By combining these multiple layers the single genome of an organism is epigenetically "transformed" into hundreds of different cell type specific epigenomes during development and differentiation. The International Human Epigenome Consortium IHEC aims to provide a comprehensive catalogue of cell type specific epigenomes of primary human cells in healthy and diseased state. In my talk I will present data generated by the german epigenome consortium DEEP, a major partner in IHEC. I will discus novel insights in cell type specific epigenome topologies and their functional relevance for development and disease. In the second half I will present a novel modelling approach to understand the mechanisms controlling the maintenance of epigenetic patterns in replicating cells.

Mapping regulatory and epigenetic changes underlying early events of drug-induced liver tumor promotion

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Keywords: Drug induced liver carcinogenesis, Epigenetic biomarkers, Regulatory landscape, DNAseI sequencing

Predicting drug-induced non-genotoxic carcinogenesis during preclinical development of novel therapeutics intended for chronic administration in humans is a major challenge for drug safety scientists. Gene regulatory interactions underlying the early stages of non-genotoxic carcinogenesis are poorly understood but could potentially be leveraged as mechanism-based early biomarkers [1, 2]. Here we have used a well characterized in vivo model of Phenobarbital (PB)-mediated liver tumor promotion to better understand the regulatory and epigenetic changes that underlie early events of drug-induced liver tumor promotion. DNaseI hypersensitive sites (DHS) are markers of regulatory DNA and have supported the discovery of all classes of cis-regulatory elements including enhancers, promoters, insulators, silencers and locus control regions [3]. Using DNase I sequencing adapted to frozen liver tissue we have created comprehensive and accurate genome-wide open chromatin maps, and highlighted PB-mediated chromatin remodeling at proximal and distal regions within a broad range of transcriptionally responsive genes. We identify 2,600 differential DHS regions, largely situated in distal putative regulatory regions (6.3% at proximity to the Transcriptional Start Sites, -2kb/+1kb) and have combined these with gene expression, transcription factor motif, and chromatin modification data. Applying this integatred cistrome profiling approach to rodent strains and species that exhibit differential sensitivity to PB-mediated liver tumor promotion will help identify regulatory elements and their cognate transcription factors responsible for PB-mediated liver tumor promotion and provide a resource for the identification of translatable mechanism-based early biomarkers for drug induced carcinogenesis.

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Epigenetics origins of cancer

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It is now widely accepted that cancer is also epigenetic disorder and that epigenetic changes play key roles in cancer development and progression. The fact that epigenetic alterations are, in contrast to genetic changes, reversible has an important implication for cancer treatment and prevention. Epigenetic inheritance include DNA methylation, histone modifications and RNA-mediate silencing all of which are essential mechanisms that allow the stable propagation of gene activity states from one generation of cells to the next. Epigenetic states are profoundly altered in human cancer and epigenetic deregulation have been observed in virtually all types of human cancers, although the precise underlying mechanisms remain poorly understood. Recent years have witnessed a remarkable pace of discoveries in epigenetics and epigenomics which will revolutionize our understanding of cancer and other complex diseases. This should help to elucidate the mechanism underlying tumourigenesis, identify specific epigenetic targets and the critical windows of vulnerability. The intrinsic reversibility of epigenetic changes represents a tremendous opportunity for the development of novel strategies for cancer treatment and prevention. Recent conceptual and technological advances in epigenetics and ongoing efforts aiming to identify epigenetic targets that could be exploited in cancer prevention and therapy as well as molecular epidemiology will be discussed.

MS-proteomics to study histone post-translational modifications and chromatin plasticity in cancer, for clinical and basic research

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Keywords: histone post-translational modification, epigenetics, mass spectrometry, chromatin proteomics,cancer, enhancers

Chromatin is a highly dynamic, well-organized and yet ill-defined protein-DNA-RNA structure that controls various DNA-dependent processes. A large number of site-specific post-translational modifications of histones (hPTMs) contribute to the maintenance and modulation of chromatin plasticity, gene activation, DNA replication and repair, and a variety of other biological processes and disease states. The observation of the diversity, frequency and co-occurrence of histone modifications at distinct genomic loci led to the notion that these marks create a molecular barcode, read by effector proteins that translate it into a specific transcriptional state, or process, on the underlying DNA. However, the molecular details of its working mechanisms are only partially characterized. More recently, various technological progresses have enabled the detection of these PTMs on an increasing number of non-histone proteins, involved in a variety of biological processes.

Recent achievements made Mass Spectrometry (MS) and quantitative proteomics excellent tools to help understanding how histone and non-histonic PTMs mediate the structural-functional state of chromatin. My team contributed to the field by setting-up distinct MS-proteomics strategies, combined with various biochemical methods of enrichment of chromatin and extra-chromatin proteins, to investigate chromatin plasticity and nuclear dynamics governed by post-translational modifications.

The talk will offer an overview of the MS-proteomics strategies developed to gain insights into chromatin biology, with emphasis on: the proteomic dissection of chromatin regulatory regions; the hPTMs-analysis of clinical specimens and histone-methyl-proteome profiling.

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Quantitative interactomics in epigenetics and genome stability

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Keywords: mass spectrometry/histone/protein-nucleic acid interactions

Mass spectrometry is a powerful tool in the life sciences and can be used to identify proteins in complex mixture. The raise of quantitative methods allows comparing samples directly for the abundance of each protein. This is used in expression proteomics, but is also useful to study protein interactions with diverse baits. We apply quantitative mass spectrometry to explore protein interactions with nucleic acids and histone marks. In the past, we have focused on the unbiased identification of interacting proteins using stable isotope labeling of amino acids in cell culture (SILAC)-based assays. For example, in the DNA pull-down approach, a bait DNA fragment carrying the sequence or modification of interest or a corresponding control are exposed in parallel to the same nuclear proteome. Binders enriched at the DNA bait show a SILAC ratio different from one-to-one and can thus be distinguished from the vast majority of background binders. The technique can be used accordingly for RNA-protein interactions to identify RNA-binding proteins. We have investigated telomeric repeat containing RNA (TERRA) as a special example of a long non-coding RNA. We have found epigenetic regulators to bind to TERRA and demonstrate that interactors affect localization and expression of this RNA. Recent improvements in algorithms for label-free quantitation have extended the applicability of quantitative mass spectrometry to more diverse species. Just recently, we expanded the studies of histone modification specific binders previously done in human and mouse to two bird species using label-free quantitation.

The dynamic interactome and genomic localization of Polycomb complexes during stem cell differentiation

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While the core subunits of Polycomb group (PcG) complexes are well characterized, little is known about the dynamics of these protein complexes during cellular differentiation. We used quantitative interaction proteomics and genome-wide profiling to study PcG proteins in mouse embryonic stem cells (mESCs) and neural progenitor cells (NPCs). We found the stoichiometry and genome-wide binding of PRC1 and PRC2 to be highly dynamic during neural differentiation. Intriguingly, we observed a downregulation and loss of PRC2 from H3K27me3-marked chromatin during differentiation, whereas PRC1 was retained at these sites. Additionally, we found PRC1 at enhancer and promoter regions independent of PRC2 binding. Overexpression of NPC-specific PRC1 interactors in mESCs revealed that the subunit switching observed during differentiation can affect PRC1 recruitment. In summary, our integrative analyses have uncovered dynamic PcG subcomplexes and widespread co-localization with active chromatin marks during differentiation.

2D TAU gel coupled with MS/MS to identify a different histone PTMs profile in breast cancer cell lines

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Keywords: 2D electrophoresis, PTMs, histones, breast cancer, mass spectrometry

Eukaryotic DNA is packaged around the nucleosomes at distinct levels of compaction associated with the transcriptional state of chromatin. The nucleosome octamer is composed by two copies each of H3, H4, H2B and H2A core histones which wrap about 147 base pairs of DNA. The linker histone H1 is important for maintenance of higher-order chromatin state. All the histones and their variants are subjected to various post-translational modifications (PTMs) that often occur in the tail domains that protrude away from the nucleosome. Chemical modifications to histones include acetylation, methylation, citrullination, ubiquitination, sumoylation, ADP-ribosylation, proline isomerization and phosphorylation. Histone post-translational modifications play an important role in the regulation of gene expression and are critical for the development and progression of many types of cancer, including breast cancer. Numerous hPTMs are simultaneously present in various histones to form a "histone code" that is read by specialized proteins to facilitate downstream functions in chromatin.
The aim of this study was to identify the histone PTMs pattern in breast cancer cell lines.
Histones were extracted from MCF7 breast cancer cells and from MCF10 mammary epithelial cell line. Two-dimensional electrophoresis was performed. In the first dimension histone proteins were separated in a triton-acid-urea (TAU) gel on basis to differences in their hydrophobicity and charge. Lanes, containing charge-separated histones on TAU gel, were cut and transferred to the top of a 15% SDS polyacrylamide gels for the second dimension electrophoresis. Protein spots were in-gel digested and purified. Peptides obtained were analyzed on a quadrupole-orbitrap mass spectrometer Q-Exactive, and resulting data were processed with Proteome Discoverer software Proteomic approach allowed us to identify numerous post-translational modifications in many sites of all the core histones and in their variants. Many PTMs were also found on the linker histone H1. We identified many modifications known in the literature as well as new modifications never described.
This study revealed different qualitative hPTMs profiles in the two breast cell lines which can provide new insight on their possible implication in breast cancer and can offer new tools for future clinical applications.

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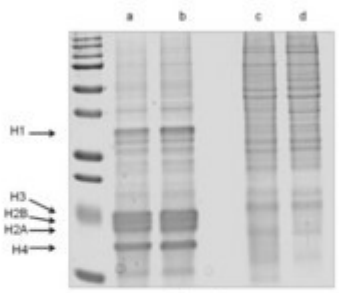


Fig. 1

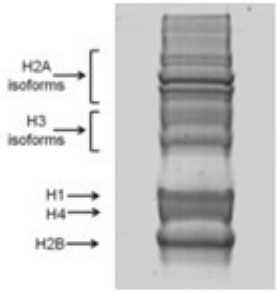
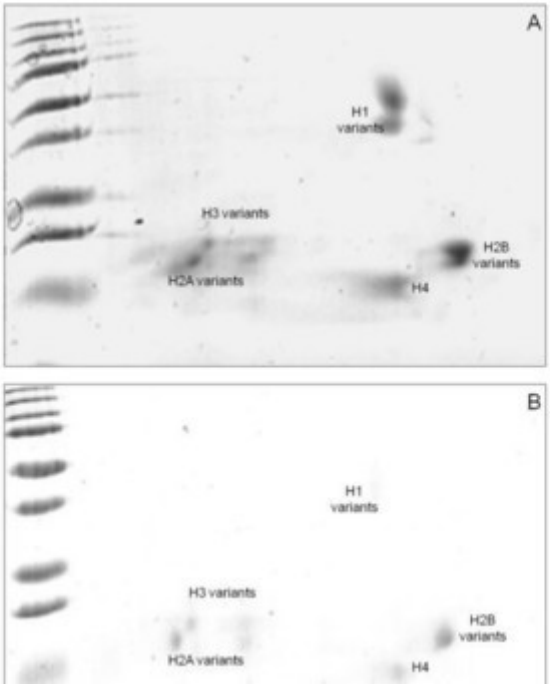


Fig. 2



Caption: Fig. 1: SDS-PAGE of histones extracted from MCF7(a) and MCF10 cells (b). Total protein content is shown in c and d. Fig. 2: Histones from MCF7 were separated on a short TAU gel. Fig. 3: 2D TAU/SDS PAGE of histones from MCF7 (A) and MCF10 cells (B).

Precision medicine beyond individual genes: Glycans as integrators of genes and environment

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Keywords: precision medicine, patient stratification, glycome, protein glycosylation

Glycosylation is an essential posttranslational modification generated by a complex biosynthetic pathway comprising hundreds of glycosyltransferases, glycosidases, transcriptional factors, ion channels and other proteins. This process results in the creation of branched oligosaccharide chains, called glycans, which become integral part of proteins and significantly contribute to their structure and function. Since glycans are created without the genetic template, alternative glycosylation creates an additional layer of protein complexity by combining genetic variability with past and present environmental factors. Individual variability in glycome composition is very large, but glycosylation of an individual protein seems to be under strong genetic influence, with the heritability of the IgG glycome being up to 80%[8]. Structural details of the attached glycans are of great physiological significance and many pathological conditions are associated with various types of glycan changes. Since the onset of genome wide association studies (GWAS), thousands of genetic loci have been associated with different diseases and traits. However, in the last few years it is becoming increasingly clear that GWAS studies are only a beginning of the understanding of complex human diseases. Hypotheses generated in these studies have to be put in the context of complex biology of life and a more elaborate approach that combines different 'omics phenotypes is needed to understand disease mechanisms and perform patient stratification that transcends genomics. Glycomics, as by far the most complex epiproteomic modification, has an immense potential in this respect, which is only beginning to be investigated.

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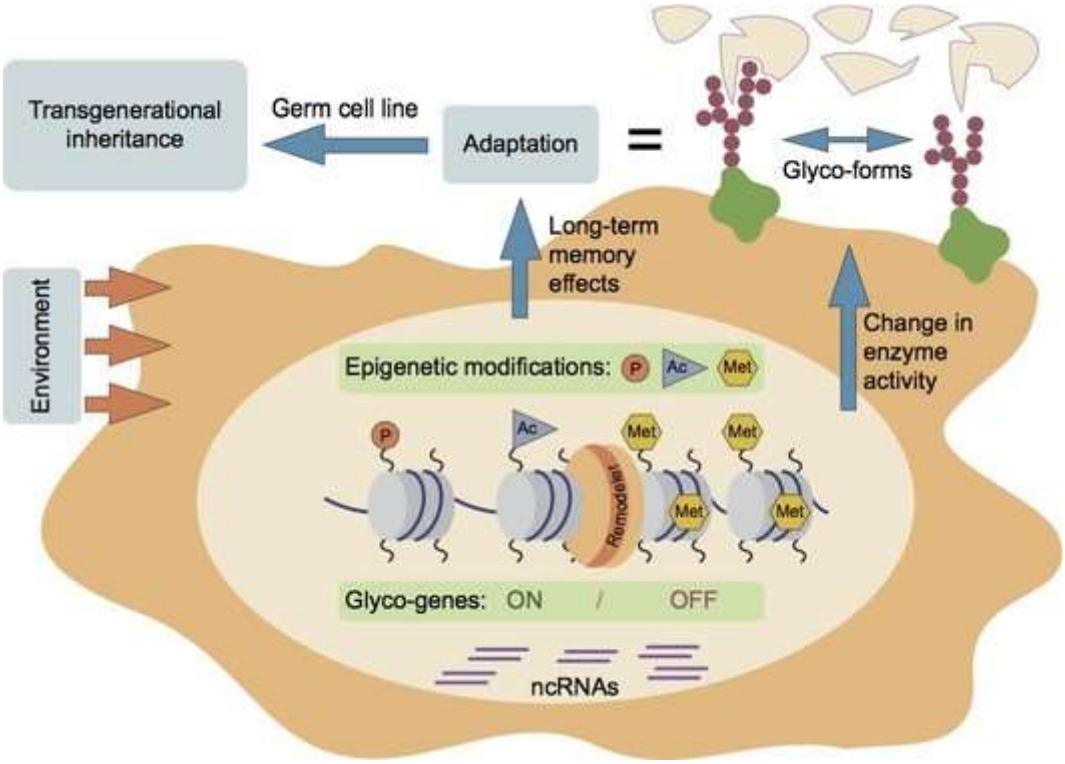

Caption: Glycan structures result from interaction between genes and environment. Glycans do not have a direct genetic template, but sequence variants in hundreds of genes that participate in protein glycosylation affect the final outcome of this complex pathway. Furthermore, epigenetic mechanisms transpond both current and past environmental signals to either activate, or deactivate specific branches of the pathway, produce altered glycan structures and consequently affect performance of a protein, cell or the entire organism. (Lauc G, Vojta A, Zoldoš V Epigenetic regulation of glycosylation is the quantum mechanics of biology. BBA Gen Subjects, 1840:65-70, 2014.)

Inhibition of DNA methylation resensitize tumor cells to Dependence Receptors apoptotic pathway targeting therapies

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Keywords: DNA methylation, inhbitor, cancer, apoptosis, dependence receptors

The alterations of DNA methylation level and patterns are a common feature of human cancer cells. A global DNA hypomethylation has been observed in many cancers, despite this reduced level of genomic methylation, localized hypermethylation are also observed

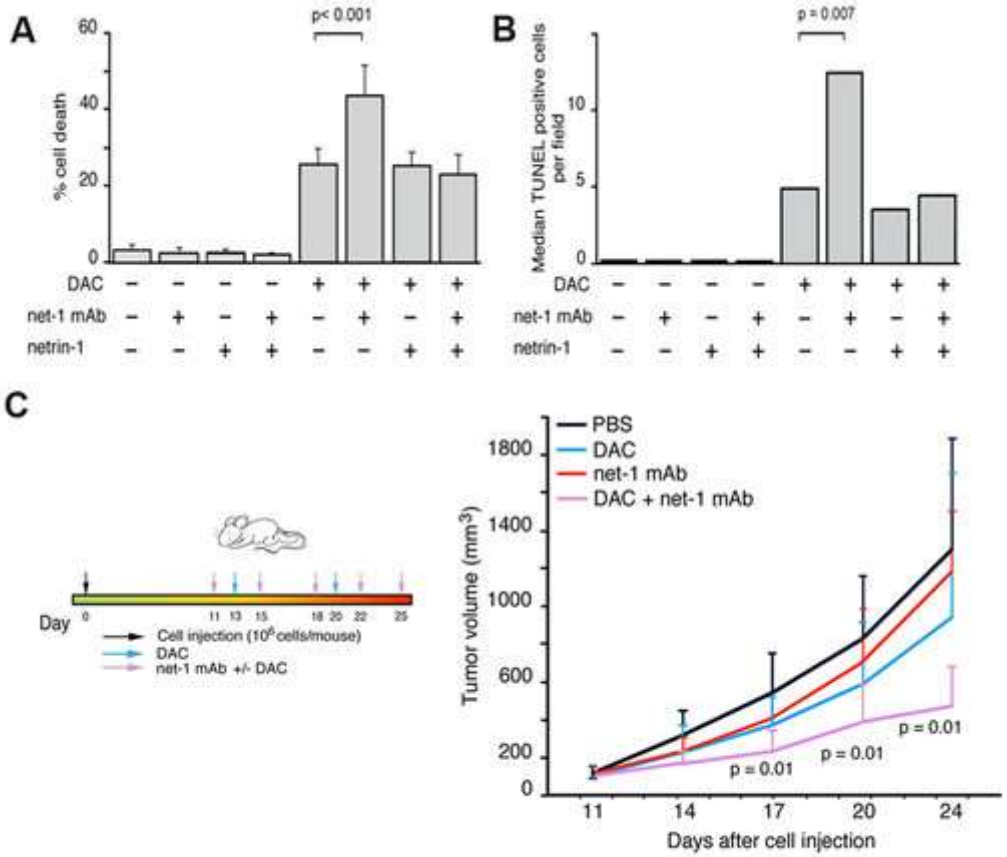
Hypermethylation of CpG islands lead to the loss of expression of the genes possessing this structure at their 5' end. Among them, genes belonging to the pro-apoptotic dependence receptors (DR) pathway undergo epigenetic disruption in human tumors, including DNA hypermethylation. In contrast to most cellular receptors, a dual role characterizes these transmembrane receptors: in presence of their respective ligand, they provide a classic positive signal, while the absence of ligand triggers a cascade of signaling events leading to apoptotic cell death.

We hypothesized that epigenetic events could play a crucial role in the fine-tuning of the DR pathway, and impact tumor development and aggressiveness. Therefore, we described the consequences of experimental manipulations of epigenetic marks on gene expression and their effects on apoptosis both *in vitro* and *in vivo*.

Structural decoding of the Netrin-1-UNC5 interaction and its therapeutical implications in cancers

Mélodie Grandin[1], Markus Meier[1], Jean Guy Delcros[1], Denise Nikodemus[1], Raphael Reuten[1], …, Robert Dante[2], Manuel Koch[2], Patrick Mehlen[2] and Jörg Stetefeld[2]

1 Co-first authors, 2 Co-senior authors. Cancer Cell, in press



Caption: Decitabine treatment is sufficient to resensitize tumor cell to netrin-1 interference in vitro and in vivo. In the human lung cancer cells H460, decitabine treatments induced apotosis in vitro (A, B), and reduced tumor growth in xenograft models (C).

Epigenetic deregulation is an important mechanism leading to aberrant protein glycosylation in human complex diseases

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Keywords: epigenetics, DNA methylation, N-glycosylation, glyco-gene expression,complex diseases

Most eukaryotic proteins are modified by covalent addition of glycan molecules that considerably influence their structure and function. Glycans are complex molecules synthesised through biochemical pathways involving many genes. The final glycan structure is largely influenced by external and intrinsic factors where epigenetic mechanisms play mediator role between environment and the expression of genes coding for gylcosyltransferases (i.e. glyco-genes). Aberrant glycosylation is profoundly involved in virtually every complex disease including inflammatory, autoimmune diseases and cancer. Using epigenetic inhibitor of DNA methyltransferases, 5-aza-2'-deoxycytidine, on human cells in culture we have shown that many glyco-genes are regulated by DNA methylation and that this has a consequences on glyco-phenotype. By analysing expression and methylation in parallel on several unrelated datasets representing different types of cancer we found the intersection of the glyco-genes with altered expression/methylation suggesting that epigenetic deregulation of glyco-genes is probably one of the most common way leading to aberrant glyco-phenotypes described in cancer. Our results also points to glyco-genes as a new important group of genes with changed expression in cancer through aberrant DNA methylation. We have shown as well that epigenetic deregulation of other glycosylation-related genes, such as transcription factors, has an effect on N-glycome composition and the disease outcome such as the case of MODY (Maturity Onset Diabetes of the Young) subtype of diabetes. Using epi-CRISPR tool, consisting of deactivated Cas9 nuclease and catalytic domain of the DNA methyltransferase DNMT3A, we aim to target methylation at certain CpG sites shown to be relevant for transcriptional regulation of the *HNF1A* gene (Zoldoš et al. 2012). This transcription factor is regulating a glyco-gene *MGAT4A* involved in proper glycosylation of GLUT-2 transporter and GSIS (Glucose Stimulated Insulin Secretion) mechanism. In addition to glycomic studies (i.e. study of composition of plasma and cell secretom *N*-glycome) we are also focusing on glycoproteomic studies. The glycoprotein of our interest is immunoglobulin G (IgG). Recently, the first genome wide association studies (GWAS) identified genetic loci associated with IgG glycosylation (Lauc et al. 2013). Some of these GWAS loci show pleiotropy with inflammatory bowel disease (IBD) and several other inflammatory diseases where IgG glycosylation changes have also been reported. By analysis of promoter methylation of five GWAS loci we were able to show that two of them were differentially methylated in IBD patients compared with healthy people, suggesting that epigenetic deregulation could be on the basis of aberrant IgG glycosylation observed in IBD (Dias et al. 2014) and that aberrant IgG glycosylation might be an important element in IBD development and progression.

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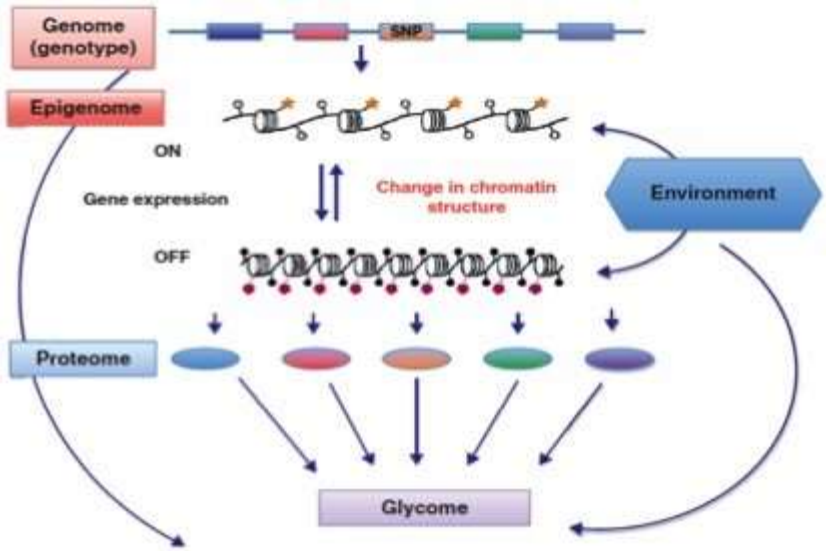

Caption: Glycome (a composition of glycans on proteins of a certain cell or secretome) is influenced by genetics (i.e. single nucleotide polymorphisms, SNPs) as well as by intrinsic and external factors mediated by epigenetic mechanisms (i.e. DNA methylation and histone modifications).

Epigenome Editing with Zinc Finger Proteins and CRISPR

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Keywords: Cas9-p300 histone acetylation

Histone acetylation, carried out by histone acetyltransferases (HATs), plays a fundamental role in regulating chromatin dynamics and transcriptional regulation. The importance of histone acetylation in cancer has been clinically validated with several inhibitors of HDACs shown to be effective as anti-tumor agents. A method for site-restricted histone acetylation would create a platform for establishing causality associated with this epigenetic mark at defined genomic loci, as well as provide a new tool to upregulate a wide variety of both coding and non-coding genes. Both zinc finger proteins (ZFP) and newly developed CRISPR systems offer powerful new options for targeted gene regulation. Here we describe an approach for efficient targeted histone acetylation using ZFP and CRISPR. First, we show that fusions of the p300 HAT catalytic domain and engineered ZFPs can lead to targeted histone acetylation and substantial increases in expression of targeted endogenous genes. Next, we demonstrate that gRNA can successfully direct nuclease-deficient Cas9-p300 fusions to increase levels of histone acetylation and endogenous genes expression. This proof-of-principle work elucidated an Epigemone Editing approach with potential utility for research and therapeutic applications.

References: Together with our partner Biovit Sigma-Aldrich is Gold sponsor of the meeting. Within this package is presentation.

Transcription and Chromatin Mechanisms Determining the Oocyte DNA Methylome

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Keywords: Genomic imprinting, oocytes, DNA methylation, CpG islands, single-cell profiling

Genomic imprinting represents a paradigm of epigenetic gene regulation in mammals. Imprinting depends, in the first place, on the establishment of distinct epigenetic states in the oocyte and sperm at imprinted control regions (ICRs), a sub-set of the CpG islands that acquire DNA methylation differentially in the gametes. Focusing on the oocyte, we show that transcription is the major determinant of the DNA methylation landscape, including at ICRs. To understand the mechanistic connections between transcription and de novo methylation, we have developed ChIP-seq methods to profile histone modifications in growing oocytes implicated in promoting or antagonising DNA methylation. We identify chromatin states permissive for and resistant to DNA methylation characterised by reciprocal enrichment of H3K4me2/me3 and H3K36me3, a finding reinforced by the consequences of ablating H3K4 demethylases and methyltransferases on the DNA methylation landscape. We detect a step-wise modulation of chromatin state at CpG islands destined for DNA methylation in the oocyte, highlighting a developmental switch in H3K36me3 as a key determinant of their de novo DNA methylation. Finally, we identify that oocytes attain a unique chromatin landscape as they complete their development. Key to such studies is the development of methods for genome-wide profiling of epigenetic marks in low numbers of cells, including at the single-cell level, which could have application widely in epigenetic studies, particularly for rare cell types and for investigating cell-to-cell heterogeneity in epigenetic marks at critical developmental transitions.

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H3K4ox-mediated chromatin condensation protects breast cancer cells from the DNA damage repair machinery

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Keywords: Chromatin, histones, epithelial to mesenchymal transition, DNA damage response, breast cancer

Histone tail modifications are key in regulating many cellular processes. Oxidation of H3 on lysine 4 (H3K4ox) is carried out by lysyl oxidase–like 2 protein (LOXL2) and is associated with transcriptional repression. The LOXL2 enzyme is overexpressed in many tumour types, in which its expression correlates with poor prognoses. Using an H3K4ox-specific antibody, we determined that the H3K4ox modification is enriched in triple-negative (TN) breast cancer cells, correlating with high LOXL2 levels in these cells. Additionally, it is found primarily in heterochromatin in these cells, as shown by ChIP-seq. We now show that this modification controls heterochromatin compaction and inhibits the DNA damage response (DDR), which is interesting in light of recent work showing that the mutational rate in cancer cells is higher in heterochromatin than in euchromatin. A LOXL2 knockdown resulted in a reduction in H3K4ox levels and a change in chromatin conformation towards a more "open" state. Under these conditions, DNA lesions are exposed, DDR is activated, and TN cells die in few days. Treating TN breast cancer cells with a LOXL2 inhibitor also sensitizes the cells. Together, these results reveal a role for oxidized H3 in DDR, providing a new mechanism by which this modified H3 affects chromatin compaction and opening a therapeutic window for treating TN breast tumours.

MicroRNA profiling of ovarian cancer

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Keywords: microRNA, ovarian cancer

Ovarian carcinoma is the leading cause of death from gynecological malignancies in the western world. Its high death rate is a result of the fact that most patients (>60%) are diagnosed at an advanced stage of the disease. Ovarian cancer is a heterogeneous disease with respect to histopathology, molecular biology, and clinical outcome.

The purpose of this study was to select most promising targets through miRNA screenings of ovarian cancer, that would give an predicting tool in ovarian cancer development.

MicroRNAs can act as either oncogenes or tumor suppressors, and they are highly specific for tissues and developmental stages. It is known that their profile is more accurately correlated with cell differentiation and development status compared to mRNA expression profile. Many miRNAs have been implicated in the development of several human cancers; some are associated with loss of tumor suppressor miRNAs and some with the overexpression of oncogenic miRNAs. The gene networks orchestrated by these miRNAs are still mostly unknown.

Our experience with ovarian cancer so far came out with the role of Hh-Gli signaling pathway in ovarian cancer development (1,2). Also, from literature, and our previous data shown that BRCA1and 2 genes are contributing to hereditary breast and ovarian cancer development (3,4).

The most important first step is selection of samples (good quality and particular type of ovarian tumor) and controls of healthy ovaries (fallopian tube tissue) with adequate pathohistological expertise. We are concentrated on serous ovarian cancer as one of most malignant and most difficult to detect in an earlier stage.

We isolated RNA using Absolutely RNA miRNA kit for miRNA microarray screening.Quality of isolated RNA was tested on Agilent 2100 Bioanalyzer using Agilent RNA 6000 Nano Kit and Agilent Small RNA Kit.

We provided miRNA microarray screening using Agilent miRNA microarray8x15K version 3(Agilent Microarray platform at the Rudjer Boskovic Institute) and miRNA labeling and Hyb kit of 8 cancer tissue samples and 8 control samples.

The Biostatistical analysis of expressed microRNAs using moderated t-test (limma) considered only microRNAs with at least 1.5 fold change. Resulting p-values were adjusted for multiple hypothesis testing based on the false discovery rate by the Benjamini-Hochberg method.

Our first results highlihted candidate miRNAs which we intend to connect to Hh-Gli signaling pathway which have to be verified and investigated further in future projects.

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High resolution DNA epigenome profiling using CpG-specific Tethered-Oligonucleotide-Primed sequencing (CpG-TOP-seq)

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Keywords: epigenomics, DNA methylation,

Methylation of cytosine to 5-methylcytosine (5mC) in CpG dinucleotides acts as a key epigenetic modification affecting gene regulation and cellular differentiation in high eukaryotes. Dysregulation of 5mC patterns is frequently associated with various complex human diseases including cancer. Current approaches for the determination of the modification status of CpG sites can be divided into a) bisulfite conversion-based methods, b) restriction endonuclease-based methods and c) affinity capture-based techniques. The gold standard bisulfite sequencing (BS-seq) can infer modification information of each cytosine at a single-base resolution. Besides its unique advantages, whole genome BS-seq analysis unavoidably generates large amounts of data, making it prohibitively expensive for large scale populational studies; moreover, the majority of the reads (50–80%) provide little or no information about the CpG methylation (1). The other groups of methods provide more affordable inroads into the DNA methylome structure although at significant sacrifice in resolution and/or informativity. Typically, enrichment-based profiling strategies include steps of random fragment generation, affinity enrichment and end-sequencing yielding rather long stretches of genomic DNA corresponding to a detectable signal (resolution defined by the average fragment length). Here we propose a new concept in analysis of DNA modification patterns that bridges the existing economy-*versus*-resolution gap. This strategy is based on selective covalent tagging of target sites (the unmodified fraction of CpG of dinucleotides or 'unmethylome') using an engineered version of the M.SssI methyltransferase and a synthetic cofactor analog carrying a reactive functional group. Each tagged CpG site is then used to chemically tether a DNA oligonucleotide (instead of a biotin moiety previously used in the mTAG-seq technique (2)), which serves to prime the template-dependent DNA polymerase activity at the tagged nucleotide itself, thereby producing daughter strands that sequentially include the CpG site and an adjoining genomic region. Amplification and sequencing of the produced CpG-nested fragment pool permits a unequivocal genomic mapping of each tagged CG site with single nucleotide precision. The validity of this new approach (named CpG-specific Tethered-Oligonucleotide-Primed sequencing, CpG-TOP-seq) combined with Ion Torrent Proton sequencing is demonstrated by pilot genome-wide studies of model bacterial and human DNA samples. Our studies show a greater than 95% read utility, high resolution and sensitive mapping of the target sites, and demonstrate the capacity of CpG-TOP-seq to uncover unique epigenetic features currently approachable only by the gold standard BS-seq. Unlike BS-seq, it avoids sequencing the entire genome, thereby providing a cost-effective alternative for high-resolution genome-wide profiling of DNA modification.

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A splicing switch of the histone variant macroH2A1 couples the chromatin state to energy metabolism

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Keywords: histone variant, NAD+, metabolism, splicing switch,mitochondria

The macro domain of the histone variant macroH2A1.1 is an evolutionarily conserved binding module for NAD+ derived metabolites (ADP-ribose, poly-ADP-ribose, O-acetyl-ADP-ribose) of up to date unknown physiological function. By analyzing various mouse tissues, we found the histone variant and splicing isoform macroH2A1.1 to be uniquely and dominantly expressed only in skeletal muscle. We could show that through a switch in alternative splicing the macroH2A1.1 isoform is rapidly induced during myogenic differentiation. Myotubes deprived of the macroH2A1.1 isoform displayed a mitochondrial intrinsic defect with reduced respiratory capacity. We further observed that the NAD+-related metabolite-interacting domain of macroH2A1.1 was essential to sustain optimal mitochondrial activity but dispensable for a second function related to cell fusion and regulation of adhesion and migration genes. By binding and inhibiting ADP-ribosylated PARP-1, macroH2A1.1 lowered nuclear NAD+ consumption and allowed the nucleocytosolic accumulation of the NAD+ precursor NMN and thus the NMN-dependent maintenance of mitochondrial NAD+ and NAD+-dependent function. Altogether, our results suggest that macroH2A1.1-containing chromatin regulates mitochondrial respiration in a manner independent of gene regulation by establishing a novel nucleocytosolic buffer of NAD+ precursors.

References: We thank Pura Muñoz Canoves, Andreas Ladurner, Carles Cantó, Eduard Gallardo for tools, Sonia-Vanina Forcales, Ivan Ahel lab for helfull discussions and HUGTiP and IMPPC facilities. This work was supported by grants from MINECO, AFM-Téléthon. MPM was supported by a FI fellowship (AGAUR) and MB is a Ramón y Cajal fellow. Work in the Buschbeck lab is further supported by the Deutsche José Carreras Leukaemie Stiftung, the Asociación Española Contra el Cáncer and the Fundació Internacional Josep Carreras.

Placental DNA methylation of the serotonin transporter gene is associated with maternal gestational diabetes

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Keywords: foetal programming, gestational diabetes,placenta, DNA methylation, serotonin transporter

Epigenetic alterations occurring in response to various prenatal exposures are considered to play a role in foetal programming of lifelong health outcomes. Intrauterine exposure to maternal hyperglycemia has been linked to increased risk for later-life obesity, metabolic disorders and cardiovascular diseases, however, underlying mechanisms are poorly understood (1). Serotonin signalling is increasingly being recognised as an important contributor to obesity and metabolic disorders such as diabetes (2). In particular, the serotonin transporter gene (*SLC6A4*), encoding a transmembrane protein responsible for extracellular serotonin availability, has been implicated in regulating food intake, body weight and energy balance. Due to its capability to adapt to various environmental conditions, the placenta is considered to be a central actor in foetal programming (3). In the present study we used human term placental tissue as an easily obtainable *ex vivo* model to investigate the potential association between the infant's *SLC6A4* methylation status and maternal gestational diabetes mellitus (GDM). We hypothesised that maternal hyperglycemia might induce changes in placental *SLC6A4* DNA methylation. The study included 50 mother-infant dyads enrolled at the Department of Obstetrics and Gynaecology, Clinical Hospital Center Zagreb, School of Medicine, University of Zagreb. Eighteen mothers were diagnosed with GDM and 32 were normoglycemic (control group). All infants were healthy, of normal birthweight and born at term by planned Cesarean section. Tissue samples were collected from the foetal side of the placenta immediately after delivery and DNA methylation was quantified at seven CpG sites within the *SLC6A4* promoter region using bisulfite sequencing. In addition, all placental samples were genotyped for *SLC6A4* promoter polymorphism (5-HTTLPR/rs25531) and analysed for *SLC6A4* mRNA levels. Methylation levels at the investigated loci were highly correlated with each other (*P<0.0001* for all combinations). Average methylation across all seven CpG sites showed no association with infant sex, genotype, gestational age, birthweight, maternal age at delivery, pregestational body mass index, gestational weight gain, parity, and nicotine, alcohol and vitamins use (*P>0.05*). On the other hand, average methylation as well as methylation at two individual CpG sites was significantly decreased in the GDM as compared to the control group (Figure 1). Between group differences were further

supported by negative correlation of methylation levels with maternal gestational glucose levels (*P=0.0291* for average methylation). In addition, a significant negative correlation was observed between *SLC6A4* methylation and mRNA levels (*P=0.0104* for average methylation). The obtained results provide the first evidence for a potential role of *SLC6A4* epigenetic alterations in mechanisms underlying foetal programming in infants exposed to intrauterine hyperglycemia.

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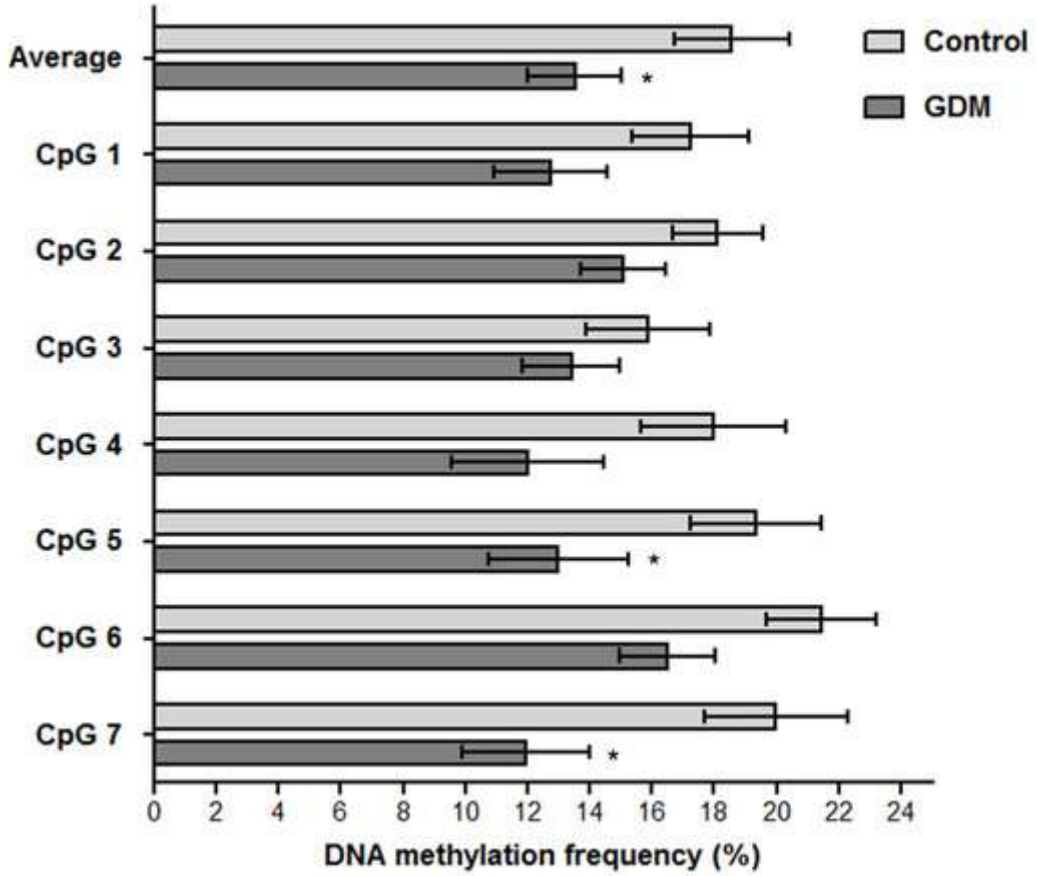

Caption: Placental SLC6A4 methylation (mean±sem) in the control and gestational diabetes mellitus (GDM) group. Average methylation is the mean of seven analysed CpG sites. CpG sites 1 to 7 correspond to positions 4728, 4769, 4780, 4811, 4846, 4848 and 4853, respectively, in NG_011747.2 (GeneBank). * P<0.05

Non-coding transcription brings RNAi to regulate expression of nutrient uptake gene in fission yeast

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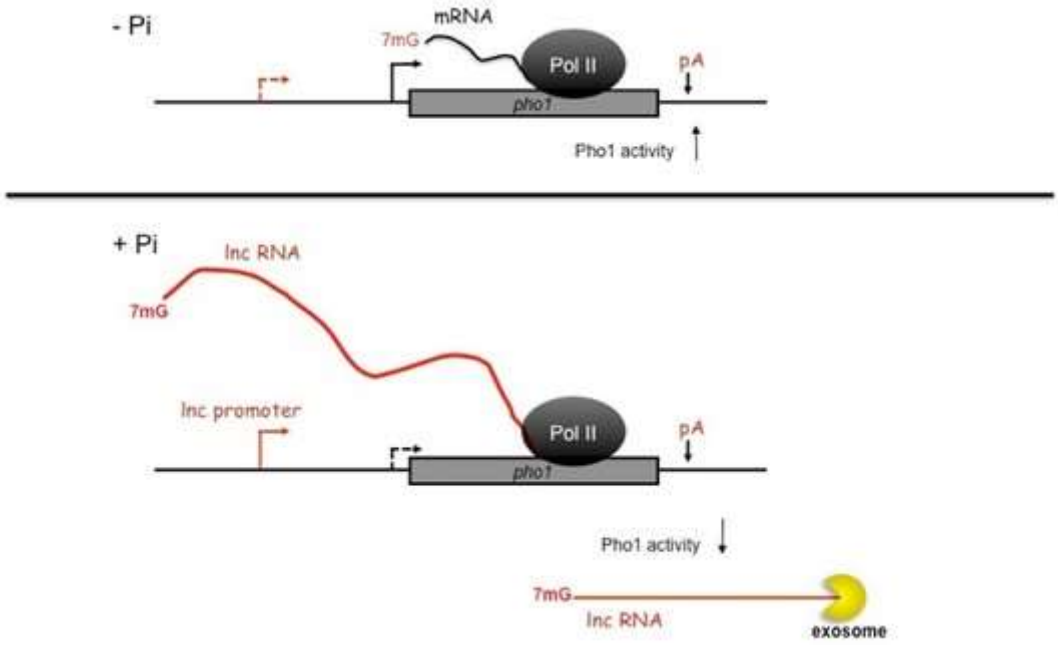
Keywords: lncRNA, heterochromatin, Mmi, exosome, transcription

Elucidating the role of RNA polymerase II non-coding (nc) transcription has been the focus of a number of recent studies. Scenarios have been described in various model systems where either the act of nc transcription, or ncRNA itself, are able to regulate expression of a protein-coding gene. The mechanistic details, however, are not very well understood.

We show that in the fission yeast Schizosaccharomyces pombe, upstream nc transcription represses expression of the protein (Pho1) involved in uptake of extracellular phosphate. Interestingly, the nuclear exosome is co-transcriptionally recruited via RNA binding protein Mmi1 and appears to regulate transcription termination and degradation of ncRNA. Our data suggests that non-coding transcription functions to facilitate the dynamic regulation of gene expression by creating a transient heterochromatic state in response to environmental cues. We believe that our study elucidates a molecular mechanism involved in repression of gene expression via non-coding transcription.

References: Shah S, Wittmann S, Kilchert C, Vasiljeva L. lncRNA recruits RNAi and the exosome to dynamically regulate pho1 expression in response to phosphate levels in fission yeast.Genes Dev. 2014 Feb 1 28(3):231-44.



Caption: Figure 1. Model describing ncRNA mediated regulation of a protein-coding gene (pho1) in S. pombe. In the absence of phosphate in the media pho1 mRNA expression is induced resulting in high acid phosphatase activity. However, in high phosphate conditions, long ncRNA (lncRNA) is transcribed from upstream of pho1 mRNA promoter. Transcription of lncRNA results in transcriptional downregulation of pho1 mRNA and protein levels. Transcription termination and post-transcriptional degradation of ncRNA is dependent on the exosome complex. Thus, via transcription of lncRNA, pho1 mRNA expression is regulated in response to extracellular phosphate in the environment.

Non-canonic functions of Polycomb RING1 proteins

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Keywords: Polycomb, RING1A/RING1B, H2A ubiquitination, replication, replicative stress

Epigenetic regulation uses a diversity of molecular machines fundamentally acting through chromatin modifications. The Polycomb system, identified during the genetic analysis of development of the fly Drosophila, plays important roles not only during ontogeny, but also in adult life, in homeostatic processes that ensure cell renewal from pluripotent progenitor cells. Deregulated Polycomb activity concurs often in oncogenic cell transformation. The Polycomb system includes two major, unrelated biochemical entities endowed with distinct histone modifying activities: protein ligase that monoubiquitinates histone H2A and lysine methyltransferase that modifies histone H3, corresponding to Polycomb Repressive Complexes (PRC) of type 1 and 2, respectively. PRC1 and PRC2 activities are intertwined and through these histone modifications and chromatin compaction-related mechanisms perform an accepted canonical function as transcriptional repressors (1).

Type 1 PRC are biochemically heterogenous, except for the heterodimeric E3 ubiquitin ligase, common to all variants. In such protein ligase, RING1A (or its paralog RING1B) are essential components that, together with a PCGF subunit, bring a specific E2-ubiquitin module to nucleosomes for modification of histone H2A lysine 119. I will report on findings showing that, in addition, to transcriptional repression, RING1 proteins play a role in replication of specific genomic regions, and also in the responses to replicative stress (2). Such housekeeping function(s) rely on both E3-dependent and independent activities of RING1 proteins in proliferating cells.

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EPIGENOMICS OF S-ADENOSYLHOMOCYSTEINE HYDROLASE DEFICIENCY

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S-adenosylhomocysteine (AHCY) hydrolase deficiency is a multisystem methylation disorder caused by point mutations in exons of *ahcy* gene. Although many advances have been made since the discovery of the disease in 2002, molecular mechanisms of disease pathology are still elusive. Using AHCY deficiency as model system may allow new insights in understanding related methylation disorders thereby deciphering players of the complex epigenomic network. To do so, we have engaged in omics approaches, from SILAC and mass spectromety (MS) to pyrosequencing and next generation sequencing (NGS) such as RNAseq. As shown recently, AHCY deficiency causes abnormal hypermethylation at imprinting control regions, showing for the first time adverse effects on the methylation status of patient DNA. Preliminary results of MS and NGS will be discussed to show our progress in understanding disease pathology and impact on the cellular methylome.

POSTER PRESENTATIONS

P1

DNA methylation level is regulated by stearoyl-CoA desaturase 1 in pancreatic beta-cells but not in alpha-cells

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Keywords: SCD1, DNA methylation, pancreatic cells, diabetes

Type 2 diabetes (T2D) is multifactorial disorder characterized by chronic hyperglycemia due to impaired insulin secretion from pancreatic β-cells, elevated glucagon secretion from pancreatic α-cells and insulin resistance in target tissues. It is widely accepted that elevated circulating free fatty acids (FFAs) and stored lipids are associated with T2D pathogenesis and cause pancreatic cells failure. Current data support the view that continuing exposure to environmental factors, such as high concentration of FFAs, may provide pancreatic cells with inheritable epigenetic modifications [1]. It was recently shown that stearoyl-CoA desaturase 1 (SCD1), which plays significant role in FA metabolism, regulates inflammatory gene expression by changing DNA methylation level in 3T3 adipocytes [2]. In the present study we tested the hypothesis that global DNA methylation in pancreatic α- and β-cells is related to SCD1 expression or activity. The experiments were carried out on clonal β (INS-1E) and α (αTC1-6) pancreatic cell lines. To induce lipotoxicity, the cells were treated with 0.4 mM palmitic acid (16:0) per 16 hours. We showed that inhibition of SCD1 by specific inhibitor (A939572) as well as silencing of SCD1 gene expression by siRNA, leads to decrease in global DNA methylation level in β pancreatic cells after lipotoxicity induction. DNA hypomethylation caused by SCD1 was also accompanied by lower methyltransferase 1 (Dnmt1) gene expression and drop in Dnmt1 protein level. In contrast, we observed DNA hypermethylation in β-cells after SCD1 overexpression. Interestingly, in pancreatic α-cells SCD1 inhibition affects neither global DNA methylation level nor Dnmt1 gene expression. Obtained results suggest that SCD1 regulates level of DNA methylation in pancreatic β- but not in α-cells.

References: References: 1. Vanessa Derenji Ferreira de Mello et al., Ann. Med. 46 (2014) 103-113 2. Malgorzata Malodobra-Mazur et al., Int. J. Biochem. Cell Biol. 55 (2014) 40-50 Acknowledgement: This research was supported by National Science Centre, Poland, grant no UMO-2013/10/E/NZ3/00670.

P2

Implication of epigenetic events in the crosstalk between tumor cells and stromal cells

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Keywords: Cancer-associated-fibroblasts, breast cancer, DNA methylation, Methylated DNA Binding-Proteins

There is strong evidence that cancer associated fibroblasts (CAFs) are able to drive tumor progression. However, it remains unclear how CAFs are able to reprogram tumor cells, indeed little is know about how these CAFs instruct tumor cells epigenomes to engage specific differentiation. In this study we hypothesize that CAFs can induce epigenetics modifications on tumor cells leading to tumor cell reprograming. CAFs were isolated from breast cancer tissues and conditioned medium of cultured CAFs (CAFs-CM) was collected to culture breast cancer cell lines. In this model, we analyze thanks to whole genome approach the possible epigenetics modifications induce by the CAFs-CM on tumor cell. Differentially expressed genes upon CAFs-CM treatment were identified by parallel sequencing of total RNA. A strong correlation was found between genes up-regulated by CAFs-CM and up-regulated by Decitabine a DNA hypomethylating drug. These preliminary results suggest an involvement of DNA methylation/epigenetic modifications in tumor cell reprogramming by CAFs. To further explore this hypothesis, DNA methylation patterns, Methylated DNA Binding Proteins deposition, and chromatin modifications will be investigated.

P3

Reduced promoter methyilation of MyD88 and ASC/TMS1 genes in tumor tissue of patients with lung and larynx carcinoma

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Keywords: methylation, inflammation, cancer, MyD88, ASC/TMS1

Aberrant DNA methylation of promoter region CpG islands is associated with gene silencing and serves as an alternative to mutation-induced inactivation of tumor suppressor genes in human cancers. Chronic inflammation and infection have been recognized among major risk factors for the most common types of cancer. Several lines of evidences are linking cancer, inflammation and infection. For example, transcription factor nuclear factor-κB (NF-κB), major inducer of inflammation is activated by many cancer risk factors (cigarettes smoke) and is constitutively active in most cancer. Many epidemiological studies have shown that inflammatory/infection conditions precede most cancers. Activation and controlling mechanisms of inflammation and infection are regulated by NF-κB and almost exclusively relied on receptors of innate immunity, known as Pattern Recognition Receptors (PRR). Thus, suppression of these proinflammatory pathways may provide opportunities for both prevention and treatment of cancer. The aim of presented study was to evaluate methylation status of ASC/TMS1 and MyD88 genes, key adaptor molecules in innate immunity signaling. We were using pyrosequencing approach, method that is still golden standard in methylation analysis, to define methylation status of promoter regions of genes of interest. Here we found that both MyD88 and ASC/TMS1 exhibit reduced methylation status of promoter regions in tested tumor tissues, comparing to healthy tissue. Also, we found different methylation pattern of specific tested CpG islands characteristic for lung and larynx cancer.

P4

The Effect of P73 Isoforms on DNA Methylation in Cancer Cells

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Keywords: TP73, p73 isoforms, TAp73α, ΔNp73α, global DNA methylation

p73 exists in multiple isoforms which could be divided into two groups: one containing transactivation domain (TA) and another amino-terminally truncated (ΔN) isoforms. While TAp73 isoforms show tumor-suppressive functions similar to those of wild-type p53, ΔNp73 isoforms inhibit wild-type p53 as well as TAp63 and TAp73, and are considered as potential oncogenes. ΔNp73 isoforms are overexpressed in many tumors correlating with enhanced chemoresistance and poorer disease outcome. DNA methylation is a form of epigenetic regulation, which adds methyl groups to cytosine residues, regulating gene activity. Hypermethylation of CpG islands in promoter regions inhibits transcription of genes silencing its expression. Using inducible *tet-on* system, we examined the impact of increased expression of TAp73α and ΔNp73α on global DNA methylation using Illumina Human Methylation 450 BeadChip. Statistically significant change was not found upon induced expression of any p73 isoform.

References: Moll UM., Slade N.(2004): p63 and p73: Roles in development and tumor formation. Mol Cancer Res 2: 371-386. Daskalos A., Logotheti S., Markopoulou S., Xinarianos G., Gosney JR., Kastania AN., Zoumpourlis V., Field JK., Liloglou T. (2011): Global DNA hypomethylation-induced ΔNp73 transcriptional activation in non-small cell lung cancer. Cancer Letters 300: 79-86.

P5

5-Azacytidine and 5-aza-2'-deoxycytidine sensitize colorectal cancer cells to topoisomerase inhibitors.

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Keywords: apoptosis, clonogenicity, combinatorial therapy, DNA methylation, irinotecan

Colorectal cancer (CRC) was the third most common cancer worldwide with 1.36 million new cases and nearly 0.7 million deaths in 2012. Currently, 5-fluorouracil, irinotecan, and oxaliplatin constitute the cornerstone of chemotherapy for CRC. Nevertheless, efficient drug combinations remain to be found for patients with CRC. We demonstrate that DNA demethylating agents, 5-azacytidine (5-aza-C) and 5-aza-2'-deoxycytidine (5-aza-dC, decitabine), sensitize human CRC cells (HCT116, DLD-1) to inhibitors of topoisomerase I (irinotecan) and topoisomerase II (etoposide, doxorubicin, mitoxantrone). The sequential treatments with DNA demethylating agents and topoisomerase inhibitors reduced cell viability more effectively than those of individual compounds at equal or even higher concentrations and it was associated with increased apoptosis of CRC cells. The cells had been pretreated either with 5-aza-dC (1 μM) or 5-aza-C (4 μM) for 48 hours followed by culture medium change and treatment with 5-azanucleosides along either with irinotecan (5-75 μM), etoposide (5-50 μM), doxorubicin (0.05-0.9 μM), or mitoxantrone (0.05-1 μM). Two or three days later, the cells were collected for an appropriate assay (MTS assay, DNA fragmentation analysis). The CRC cells were pretreated with DNA demethylating agents one or two days before topoisomerase inhibitors treatment in order to allow the cells to divide a few times, because passive loss of cytosine methylation needs at least two DNA replication cycles to become permanent. Moreover, we decided to investigate whether combinatorial DNA demethylating agents and topoisomerase inhibitors treatments could impair CRC cell colony-forming ability more effectively than those of individual compounds. For this purpose, we performed long-term colony formation assay. In DLD-1 cells, etoposide (0.1-1 μM) alone reduced the surviving fraction (SF) in a concentration-dependent manner, whereas low-dose 5-aza-dC (0.1 μM) caused a 21.5% reduction in clonogenicity. The sequential treatment with 5-aza-dC and etoposide decreased clonogenic growth as compared to each compound alone. The same enhancing effect was observed in HCT116 cells. The results of our studies may contribute to improving the effectiveness of current treatments for CRC and possibly other cancers.

References: 1. This project was supported by a grant from the National Science Centre, Poland (2015/17/N/NZ3/03737), by Wroclaw Centre of Biotechnology, programme The Leading National Research Centre (KNOW) for years 2014-2018, and by IITD Statute 3.

P6

Histone Methyltransferase SetD1B regulates NF-kB target gene expression under inflammatory conditions

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Keywords: Epigenetics, Periodontics, Histone methylation

Pro-inflammatory conditions in diabetes and periodontal disease result in the activation of NF-κB, which in turn regulates the transcription of pro-inflammatory cytokines, causing changes in the histone methylation state at gene promoters. In general, changes in histone methylation state are accomplished by histone methyltransferases and histone demethylases. Here we have focused on the effect of inflammatory conditions on SetD1B, a lysine-specific transferase that trimethylates the histone mark on lysine 4 as a major epigenetic mechanism involved in active gene transcription. Objective: To determine the epigenetic mechanism by which inflammatory conditions affect gene expression. Methods: Mice were given IP injections of streptozocin for the diabetic model and were administered intragingival injections of LPS for the periodontitis model. Human periodontal ligament (PDL) cells were stimulated with LPS and/or high glucose (HG). Gene expression analysis of IL-1b was conducted using RT real-time PCR. Binding of NF-kB, SetD1 and histone methylation state was examined on specific IL-1b gene promoter regions using chromatin immunoprecipitation analysis with P65, SetD1B and H3K4me3- specific antibodies to determine relative enrichment. Immunofluorescence was used to assess nuclear translocation of NF-kB. Results: Micro-CT images revealed an increase in alveolar bone loss in the periodontitis, diabetes and diabetic periodontitis animal models. The average distance from the CEJ to the ABC was significantly increased in the periodontitis (1.6-fold) and diabetic periodontitis mouse models (2.0-fold). Histochemical analysis demonstrated increased infiltration of inflammatory cells and disorganization of periodontal ligament fibroblasts in the periodontitis, diabetes and diabetic periodontitis models. Inflammatory conditions increased occupancy of NF-kB on the IL-1b gene promoter upon treatment with LPS (1.3-fold), HG (1.8-fold) and HG+LPS (2-fold). NF-kB enrichment was accompanied by increased gene expression of SetD1B when stimulated with HG+LPS (3.1-fold). Upregulation of SetD1B resulted in increased occupancy of H3K4me3 on IL-1b gene promoter upon stimulation with LPS (1.4-fold), HG (1.6-fold) and HG+LPS (1.3-fold), which correlated with increased IL-1b gene expression when treated with LPS (1.9-fold), HG (2.1-fold) and HG+LPS (4-fold). The NF-kB inhibitor BOT64 and SetD1B siRNA decreased IL-1b gene expression each by 1.5-fold in LPS-stimulated cells. Conclusion: LPS and HG challenged PDL cells were associated with NF-kB enrichment on inflammatory gene promoters and increased gene expression of SetD1B. Inhibition of NF-kB and SetD1B affected downstream gene expression in LPS-challenged cells. Studies using NF-kB inhibitors and histone methylation modifiers may provide the basis for future Epi-therapeutics to improve the periodontal health. This research was supported by an NIH individual fellowship 1 F30 DE024352-01.

P7

3D conformation analysis of Jmjd3 locus during mouse corticogenesis reveals an intronic enhancer

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Keywords: Jmjd3, Chromatin Conformation, Enhancer

JMJD3, also known as KDM6B, is an H3K27me3-demethylase that is required for the proper formation of the neural lineages [1,2]. Despite its central role, little is known about the regulation of *Jmjd3* itself during this process. To decipher the underlying mechanisms we took advantage of an *in vitro* model that recapitulates cortical development through the sequential generation from mouse ES cells to neural stem cell, neural precursors and neurons, respectively. Our study supports a model in which the induction of *Jmjd3* expression during corticogenesis relies on an intronic enhancer that we showed, by a 3C approach (Chromosome Conformation Capture), to physically interact with the *Jmjd3* promoter at the time of induction. Strikingly, we also observed that this process correlates with the production of a short non-coding RNA from the enhancer region (e-RNA), further conserved in human. Functional analyses aiming to delineate the respective roles of the enhancer region and its e-RNA in this process are currently in progress.

References: 1 : Jepsen et al, Nature 450(7168) (2007) 415-9 2 : Burgold et al, PLoS ONE 3(8) (2008) e3034

P8

STAT1 mediated high-order chromatin structure enhances therapeutic efficacy through viral mimicry

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Keywords: cancer epigenetics, High order chromatin structure, DNA looping, transcription

The cancer immune-editing hypothesis emphasize that cancer cells undergo robust transformation to r educe their immunogenicity and escape the immune recognition that otherwise would lead to their eli mination. However, some of the anti-tumor effects of chemotherapeutic agents are known to induce t ype I interferon (IFN) signaling pathways that enhance the immunogenicity of dying cancer cells, and th ereby stimulate anti-tumor immune response. Although type I IFN signaling is best known for their anti -viral interference, they are now acknowledged as one of the key evoke cancer cell autonomous immu ne response that is crucial for the therapeutic success. Upon receiving type I IFN signals, STAT1 is activ ated by IFN receptor bound Janus kinase to regulate the induction of defined set of genes. Although a l arge number of genes are regulated in a complex manner, how transcription factor STAT1 determine t he finely tuned combination of gene transcription is very unclear. So, given the growing appreciation o f chromatin structure as an important element regulating gene expression, we hypothesize that STAT1 mediates high order chromatin structure to facilitate transcriptional regulation by physical proximity. H ere we have demonstrated that treatment of chemotherapeutic agents trigger transcription of endoge nous retroviral genes, which in turn activates cancer cell autonomous viral mimicry. The finely tuned re gulation of multiple gene transcription is stimulated by signal transducer and activator of transcription 1 (STAT1) mediated high-order chromatin structure. Transcriptional regulation is abrogated when phys ical proximity is destroyed by disruption of STAT1 binding. In addition, chemotherapeutic efficacy is abr ogated when STAT1 mediated high order chromatin structure is destroyed. Overall, our results suggest that transcriptional regulation of type I IFN related gene signatures by STAT1 mediated high order chro matin structure is crucial for the efficient anti-tumor treatment.

P9

Proteome analysis of the mammary epithelial hierarchy

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Keywords: Proteomics, mammary gland, progenitor cells, epigenomics, breast cancer

Mounting evidence suggests that progenitor cells become deregulated in cancer making them promising cellular targets for chemoprevention. In the breast, basal and luminal progenitors are remarkably active, expand in response to hormones and are associated with aggressive breast cancer subtypes. Accurately defining the molecular composition of mammary stem/progenitor cells is key to our understanding the mechanisms that govern their proliferation and self-renewal and become deregulated in breast cancer. Known cell surface markers now enable us to purify cells enriched for distinct steps of the mammary epithelial hierarchy, yet so far comprehensive profiling of these cells has largely been restricted to mRNA expression analysis. Here, we use proteomics to identify several epigenomic regulators that are differentially expressed across the luminal and basal, mammary epithelial subsets. Screening of matched epigenetic inhibitors further demonstrates these epigenetic proteins to be essential for the colony-forming capacity of murine mammary progenitors *in vitro*. These enzymes have the potential to serve as novel therapeutic targets in breast cancer.

References: This work is supported by the Canadian Breast Cancer Foundation (CBCF); Canadian Cancer Society Research Institute; Prairie Woman On Snowmobiles and Hold'Em For Life (Toronto). A.C. holds CBCF postdoctoral fellowship; A.S holds Canadian Institutes of Health Research doctoral research award and Frank Fletcher Memorial Fund scholarship.

P10

CCNA1 promoter methylation distinguishes oral lesions

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Keywords: DNA methylation, OLP, OLL, CCNA1, oral mucosa

Altered DNA methylation is one of the possible factors responsible for different human diseases. The model on which we explore changes in DNA methylation in disease are potentially malignant lesions of the oral mucosa, oral lichen planus (OLP) and oral lichenoid lesions (OLL). These two lesions are difficult to distinguish clinically and histopathologically, while the therapy for them is quite different (Mravak-Stipetić et al, ScientificWorldJournal 2014). Therefore, there is a strong need to find new approach that would easily distinguish OLP from OLL. Epigenetic biomarkers, such as methylated genes could point to changes even before they can be clinically detected. Herein, we will examine the methylation status of important cell cycle regulator, CCNA1 gene (cyclin A1) in normal oral mucosa and oral precancerous lesions. We will identify CCNA1 promoter methylation in healthy subjects and in those with changes in the oral mucosa. Namely, our previous study have shown that different methylation of CCNA1 gene could be a good biomarker of mucosal diseases of the cervix (Nina Milutin Gašperov et al, PLoS ONE 2015). So, we assumed that this gene could also be changed substantially in the oral mucosa, particularly in the OLP and OLL diagnosis. Moreover, the study of Hanahan et al. (Cell 2011) showed that methylation of CCNA1 promoter was significantly associated with HPV positivity in head and neck squamous cell carcinoma (HNSCC). Hence, we will also investigate the HPV status in precancerous lesions as well as in healthy specimens. The OLP and OLL diagnoses are based on clinical examination and confirmed by histopathology. The method of methylation specific polymerase chain reaction (MSP) will be used to determine the CCNA1 gene promoter methylation status. The methylation profiles will be correlated with different diagnosis of the oral mucosa as well as the controls. Our preliminary results point out the CCNA1 promoter methylation differences between normal and probably malignant lesions of the oral mucosa.

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P11

Epigenetic modification of „euchromatic“ satellite DNAs in the red flour beetle Tribolium castaneum

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Keywords: satellite DNAs, Tribolium castaneum, H3 histone modification, DNA methylation

Eukaryotic genomes contain a considerable fraction of satellite DNAs (long arrays of tandemly repeated sequences) highly prevalent in pericentromeric heterochromatin. Nevertheless, information about satellite DNA distribution and their corresponding chromatin organization outside of these regions is rather limited. The availability of a whole-genome assembly mapped to *Tribolium castaneum* chromosomes makes possible examination of satellite DNAs on the genome-wide scale. Recently, using a combination of bioinformatics and experimental approaches, we identified and studied content, distribution, and structural features of satellite DNAs throughout the entire genome. We detected that besides one highly abundant satellite DNA in pericentromeric regions, several different satellite DNAs are distributed exclusively in euchromatic chromosomal arms of all chromosomes (Pavlek et al. 2015). We hypothesize that satellite DNA islands in the euchromatic regions could be important factors in the modulation of gene expression through the formation of micro-heterochromatic environment. We therefore decided to test epigenetic status of selected satellite DNAs. First we used IF/FISH to recover correlation between distribution of heterochromatic or euchromatic H3 histone modifications (H3K9me3 and H3K4me3, respectivelly) and satellite DNAs. In addition, we explored DNA methylation status of these satellite DNAs by Southern blot analyses of genomic DNA digested with methylation sensitive restriction endonucleases.

References: Martina Pavlek et al., DNA Res. 22 (2015) 387-401.

P12

The efect of dBigH1, embrionic variant of Drosophila melanogaster histone H1, on nuclear reprogramming

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Keywords: histone variant, dBigH1, embrionic extract, chromatin, nuclear reprogramming

It is known that differentiated cells can be reprogrammed when exposed to an oocyte or an embryonic extract. In this work, we followed early reprogramming events in S2 cells treated with DREX (*Drosophila* early-embryo extract), and complemented this data with results obtained from S2 cells overexpressing dBigH1, together with *in vitro* chromatin reconstitutions using the same extract. We have observed that a simultaneous loss of somatic H1 linker histone is not necessary for the chromatin incorporation of the embrionic linker histone dBigH1. Observed decreasement in H3K4me3, H3 acetylation and changes in transcription are in correlation with previosly known information of dBigH1 being transcriptional repressor in early embryogenesis. Decrease in the number of HP1a foci in extract-incubated nuclei reflects reorganization of heterochromatin during the reprogramming process. We have also shown that dBigH1 incorporation into chromatin is ATP-facilitated. The affects of depletion dNAP-1 and dNLP histone chaperons and Acf1 subunit of the ACF chromatin remodeler have shown that dNAP-1 has a significant role in chaperoning not only dBigH1 but also H1 in this reprogramming system. The combined approach of the three systems has shown to be useful tool to follow the changes in the epigenetic landscape and in nuclear processes connected to the presence of the embrionic linker histone.

P13

Protein and methyl donors in maternal diet influence imprinted gene expression in offspring without altering DNA methylation

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Keywords: methyl donors, imprinted genes, protein restriction, nutritional programming

It is now admitted that the status of maternal nutrition can influence the epigenetic state of the fetal genome and may constitute a programming effect predisposing to specific diseases later in life. Methyl donors constitute a class of nutrients which influence on pregnancy outcomes and fetal health is widely acknowledged. Methyl donor compounds mediate one-carbon metabolism and influence the availability of S-adenosylmethionine (SAM) which is the primary methyl donor for a wide range of methylation reactions, including DNA and histone methylation. However, very few studies addressed the consequences of an excess of methyl donors during pregnancy and of the possible interactions with the macronutrient content in maternal diet. Using a rat model of nutritional programming, we showed that an excess of methyl donors interacts with protein content in maternal diet and that this interaction influences fetal and postnatal growth. The expression of several imprinted genes from the IGF system was influenced by protein and methyl donor content in maternal diet. Protein restriction during gestation decreased the expression of growth promoting genes (Igf2, Plagl1) and increased the expression of the growth restricting gene Igf2r. Methyl donor supplementation tended to attenuate this effect. During the postnatal period, methyl donor supplementation was associated with an overexpression of Igf2 but a reduced expression of Igf1 in offspring liver, and a reduced growth, mostly when associated with a maternal protein restricted diet. Despite large changes in gene expression levels, DNA methylation at several Imprinted Control Regions (ICRs) of imprinted genes was not affected by maternal diet. The Plagl1 gene promoter was undermethylated in the liver of offspring born from protein restricted dams and methyl donor supplementation attenuated this effect during the postnatal period. To the contrary, a regulatory region in the Igf1 gene was overmethylated in the protein restricted group. Our results suggest that protein restriction rather than methyl donor supplementation influenced imprinted genes expression in rat liver at birth, whereas methyl donors had a major effect in the postnatal period. However, the impact on DNA methylation of imprinted control regions was rather weak, suggesting that transcriptional deregulation occurred through other mechanisms.

References: 1. Giudicelli F et al., Plos One

P14

Identifying major sources of variability in DNA methylation analyses

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Keywords: DNA methylation analysis, PMR value, sources of variability, methods study

Aberrant DNA methylation is associated with various diseases, and a vast number of disease-specific methylated loci have been identified. However, diverging methylation frequencies are commonly reported for the same locus in the same type of disease, underscoring the need for standardization of experimental pipelines. The aims of the present work were to investigate various sources of variability in PCR-based DNA methylation analyses, identify the major pitfalls, and suggest guidelines in order to minimize methodological bias and potentially reduce the divergence among reported methylation frequencies. The potential sources of variability in PCR-based DNA methylation analyses that have been investigated include different investigators, time point of analysis, bisulfite kits, sample storage conditions, template input amounts, and choice of reference for normalization. For all analyses, six gene promoters were analyzed across 20 colon cancer cell lines by quantitative methylation-specific PCR, which was used as a model. The resulting percent of methylated reference (PMR) values were compared. In total, over 200 PCRs have been performed. Most crucial is the choice of reference for normalization, as well as the template input in the PCR. In general, use of different parameters such as variable input amount, different bisulfite kits, and different storing conditions for samples in the same experiment caused important PMR variations. Additionally, even among basically identical PCRs with use of a highly standardized protocol, a minimum level of variation was observed. Altogether, standardization of the experimental pipeline, as well as testing of in-lab reproducibility, is highly recommended for all labs performing DNA methylation analyses.

References: The study was supported by grants from the South-Eastern Norway Regional Health Authority and partly by the Research Council of Norway.

P15

DNA methylation: Smoking and quitting, a longitudinal analysis

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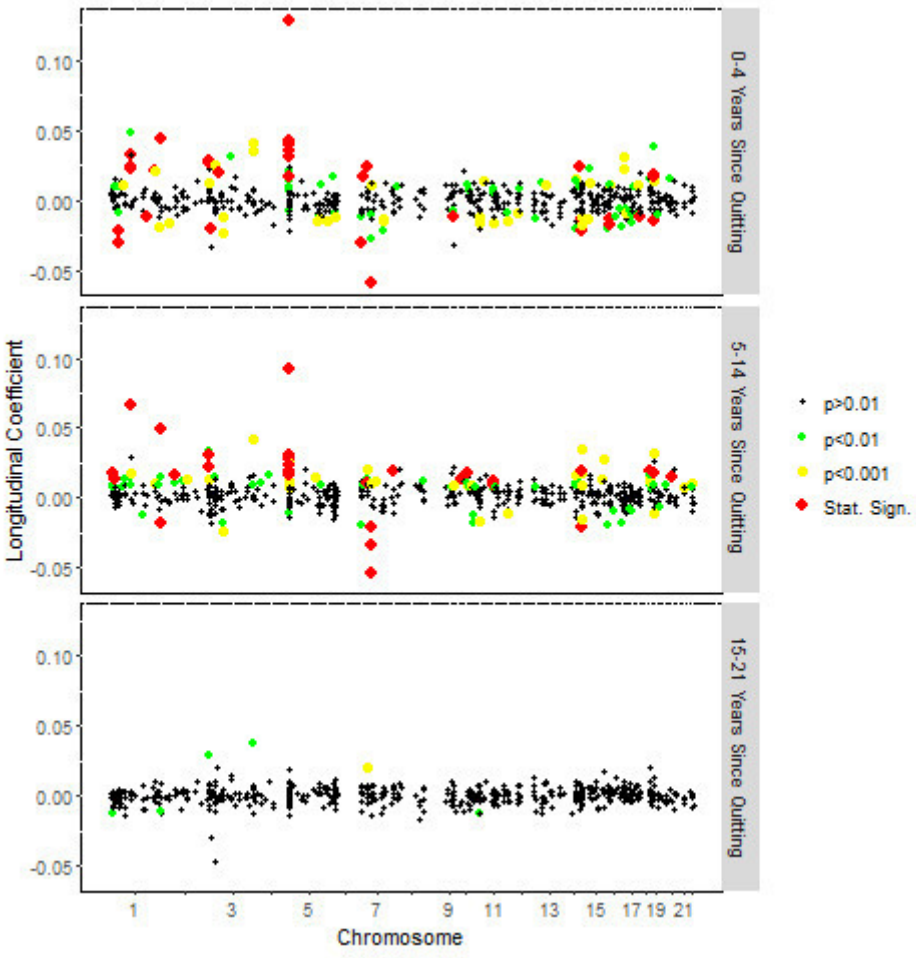
Keywords: smoking, methylation, longitudinal study

The association between smoking tobacco and DNA methylation has received a great deal of attention, and the evidence for epigenome-wide, persistent effects continues to grow through revealing cross-sectional studies. However, few large-scale investigations have examined the smoking-methylation association longitudinally, i.e. with multiple time-points for each individual. Our study seeks to determine the association between smoking habits and methylation changes by examining whole blood methylation in current smokers, ex-smokers and individuals who have never smoked, both cross-sectionally and at two time-points separated by 5 to 9 years. Our longitudinal analysis of smokers vs non-smokers shows that continued smoking has a heterogeneous effect on whole blood methylation, with some CpG sites becoming further differentiated between smokers and non-smokers (N=9, smallest Bonferroni-corrected p-value $4.3*10^{-4}$), while others stabilize or re-approach one another (N = 5, smallest Bonferroni-corrected p-value $5.1*10^{-16}$). Past evidence suggests that cumulative pack-years (a measure of duration and intensity) has a significant effect on methylation levels. Our results indicate that intensity of smoking may be a more important factor than duration, with 35 sites showing significance for the former, and only one for the latter (smallest Bonferroni-corrected p-value $2.2*10^{-15}$). Longitudinal results confirm that after quitting smoking most sites associated with smoking revert very quickly to the levels of never smokers. The most rapid reversion occurs in the first 14 years after quitting, with reversion rates correlated to the initial difference in methylation levels. Examination of the disease pathways associated with CpG sites significant in the longitudinal analysis may reveal mechanisms linking smoking and short- and long-term clinical outcomes. Understanding these mechanisms will help to construct the molecular map from exposure to disease, and can contribute to developing therapies to mitigate the adverse clinical effects of cigarette smoke exposure.

References: 1. S. Zeilinger S et al. PLoS One 8 (2013) e63812. 2. R.C. Richmond et al. Hum. Mol. Genet. 24 (2015) 2201-2217.



Caption: Rate of change of methylation M-value per year in comparison to never smokers for each CpG site under investigation longitudinally (N=590). The panel display results for individuals for various times since quitting smoking.

P16

Changes of cell cycle regulation in HepG2 cells through contribution of four independent pathways after targeted silencing of AHCY hydrolase

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Keywords: methylation, AHCY hydrolase, cell cycle, proliferation, cancer

Most methylating enzymes use AdoMet as methyl group donor to methylate their substrate. AdoHyc is additional product of transmethylation reactions and is also proven to be one of the strongest competitive inhibitors of methyltransferases. Proper activity of AHCY hydrolase is therefore essential for fast removal of AdoHyc in order to avoid global alterations of cell methylation. Recently, it has been reported that level of AHCY hydrolase expression might have impact on changes in cell characteristics usually associated with so called cancer phenotype: cell cycle regulation, cell proliferation and migration. To investigate possible changes, targeted silencing of AHCY hydrolase in hepatocellular carcinoma cell line was performed using shRNA. Western blott was used to determine the amount of cell cycle regulating proteins involved in various independent signaling pathways. Metabolic cell activity was measured by MTT viability test and Neutral red uptake assay to analyze differences in cell proliferation, while transwell chamber assay was used to study changes in cell migration. Western blott analysis suggests G2/M cell cycle arrest induced through p53 independent GADD45a pathway. GADD45a could both act through strong Tyr-15 phosphorylation of Cdc2 or interaction with p21. High levels of p27 indicate G1/S arrest through inactivation of CyclinE/CDK2 complex. Further changes include Ras independent activation of MEK/ERK pathway possibly through regulation by p21/PAK/P-cRaf(Ser338) cascade. Other than MEK/ERK activation, G1 passage promotion is also caused by degradation of p53 through Chk2 signaling. We found that both metabolic activity tests show decrease and indicate reduced cell proliferation. Also, slower cell migration is seen using transwell assay. Progression to cancer phenotype usually starts with alterations of cell cycle since its control is necessary for preventing unrestrained cellular growth. Our research indicates complex changes in regulation of cell cycle after AHCY hydrolase silencing with competing contribution of various pathways. Inhibitors of AHCY are currently being investigated as potential cancer treatments, thus understanding their effect on cell cycle is critical to predict possible treatment outcomes as well as for effective mixed drug therapies.

P17

AHCY interaction with galectin-3 identified by new Gateway vectors for bi-molecular fluorescence complementation and pooled ORFs screening strategy

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Keywords: AHCY, BiFC, galectin-3, Gateway, high-throughput

AHCY is the single mammalian enzyme that removes S-adenosyl homocysteine (SAH) – a byproduct in cellular methylation reactions. AHCY controls the methylation potential of the cell, thereby ensuring the efficient methylation of DNA, mRNA, tRNA, lipid and protein (1). Methylation is the key epigenetic regulator of expression of many vital proteins, thus it is no surprise that AHCY deficiency causes developmental defects and is potentially lethal. Our aim was to study AHCY interaction network since it shapes intracellular dynamics and function of a protein. Interaction studies often use bi-molecular fluorescence complementation (BiFC) to reveal the formation and cellular localization of protein complexes, however large-scale approach is lacking. We developed the tool for fast human protein interactions screening that combines Gateway-ready BiFC vectors and ORF pooling. First we have validated the vectors, and in a high-throughput screen we identified galectin-3 as the new AHCY interaction partner. Using Cell Cognition, machine-learning free software, we analyzed the intracellular localization of this complex based on BiFC fluorescence pattern. Complex localized in vesicles, identified as compartments of the endosomal pathway, a known galectin-3 trafficking route. We report this Interaction here for the first time in a live cell system, after it was first captured by the pull-down screen in 2014 (2). Galectin-3 is a ubiquitous protein involved in intracellular traffic and transport of nuclear proteins, a possible route for AHCY nuclear translocation. This approach offers a platform to rapidly identify and localize new protein interactions inside living human cells, a basis for understanding the function of an interaction.

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P18

Heat*seq: A Web-application To Contextualize A High-Throughput Sequencing Experiment In Light Of Public Data

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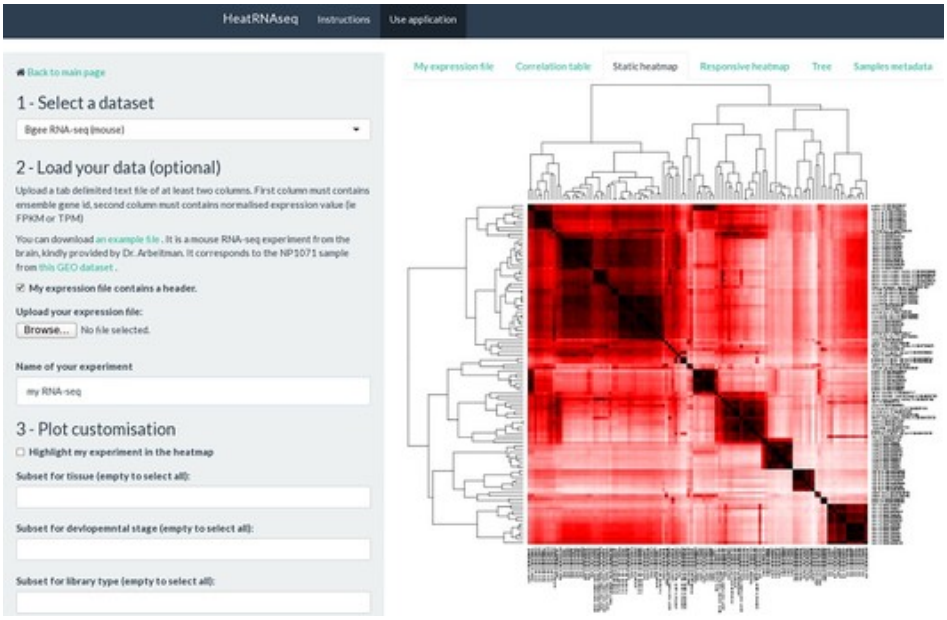
Keywords: ChIP-seq, RNA-seq, CAGE, webtool, NGS

With the establishment of better protocols and decreasing costs, high-throughput sequencing experiments such as RNA-seq or ChIP-seq are now accessible even to small experimental laboratories. However, comparing one or few experiments generated by an individual lab to the vast amount of relevant data available in public domain might be hindered due to lack of bioinformatics expertise. Though several user friendly tools allow such comparison gene or promoter level, a genome-wide picture is missing. We developed Heat*seq, a free, open-source web-tool that allows comparison at genome-wide scale of any experiments provided by the user to public datasets (RNA-seq, ChIP-seq and CAGE experiments from Bgee, Blueprint epigenome, CODEX, ENCODE, FANTOM5, modEncode and Roadmap epigenomics) in human, mouse and drosophila. Correlation coefficients amongst experiments is displayed as an interactive correlation heatmaps. Users can thus identify clusters of experiments in public domain similar to their experiment in minutes through a user-friendly interface. This fast interactive web-application uses the R/shiny framework allowing the generation of high-quality figures and tables that can be easily downloaded in multiple formats suitable for publication. Heat*seq is freely available at http://www.heatstarseq.roslin.ed.ac.uk/.



Caption: Screenshot of Heat*seq

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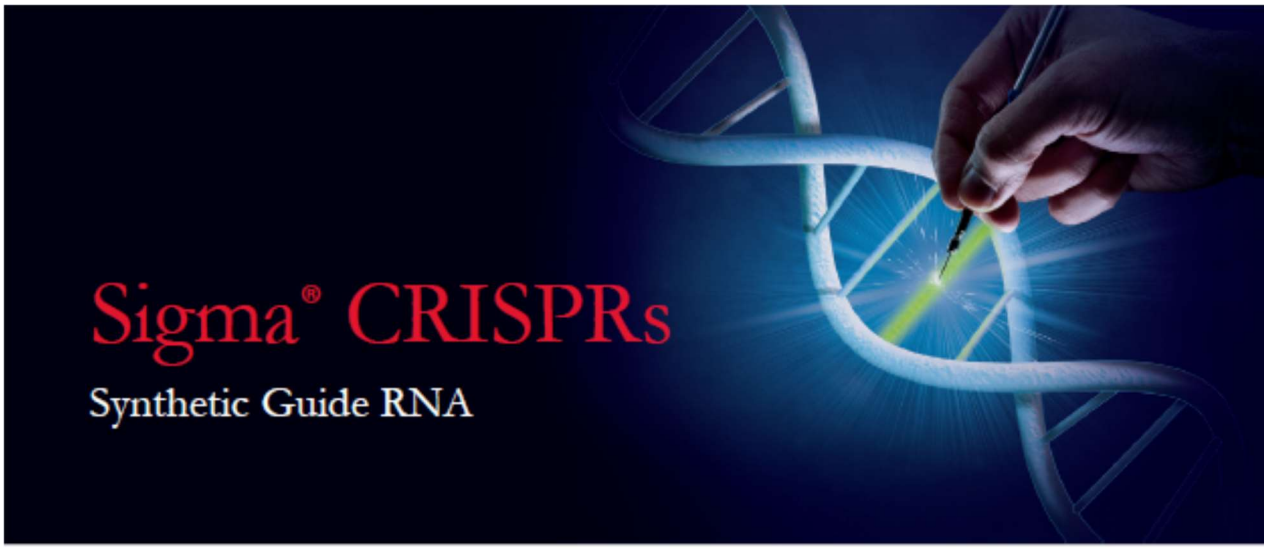

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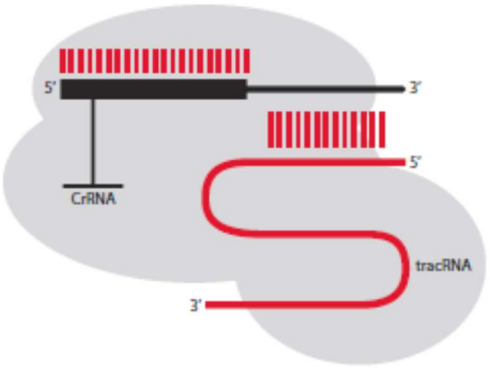

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