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Introduction:

The sense of collaboration was prominent at the second biennial '*European Epigenomics and Stem Cells-2011 Meeting*' held by GeneExpression Systems in Paris in November which recognized the promise of epigenomics and stem cells research. The two-day intensive single track meeting was arranged in six scientific sessions. The meeting brought together renowned international scientists from high caliber academic institutions and industry leaders, contributing seminars and posters displaying the latest topics in epigenomics and stem cells research. There were about 60 participants around the world attended this focus theme conference. This report covers few representative talks from academia and biotech industry those were presented in the meeting.

Chromatin biology

In general, covalent chemical modifications to the DNA and to histones, histone variants, nucleosome positions, small noncoding RNAs and the level of chromatin compaction all contribute to chromosomal structure and to the activity or silencing of genes. These chromatin-level alterations are defined as epigenetic when they are heritable from mother to daughter cell. The great diversity of epigenomes that can arise from a single genome permits a single, totipotent cell to generate the 250 cell types of an adult individual. The epigenetic changes are a prerequisite for reprogramming from one cell type to another during cell differentiation. In addition, maintenance of one particular epigenome during chromatin replication is crucial for clonal expansion of cell lineages and tissues. Both the reprogramming and the epigenome maintenance function require SNF2 enzymes, which catalyse nucleosome dynamics. However SNF2 remodeling processes are complex and not easily dissected in humans since there are as many as 53 different SNF2 remodeling factors. The conference was inaugurated by **Karl Ekwall** (Karolinska Institute, Huddinge, Sweden), who presented an overview of chromatin remodeling and histone modification changes during cell differentiation. Ekwall's group is using well-defined eukaryotic yeast (*S. pombe*) model system and genome wide methodology for mapping *S. pombe* nucleosomes positions *in vivo* and *in vitro*. Using MNase mapping of chromatin studies, he speculated that involvement and cooperation of Topoisomerase I and chromatin remodelers are essential in nucleosome disassembly and the control of gene expression. Possibly, this mechanism maybe relevant for epigenetic programming of stem cells since it was also shown by others while studying pluripotency in mouse embryonic stem cells. Dr. Ekwall also presented data (on the epigenetic regulation of blood cell differentiation) genome-wide DNA methylation studies which suggest reduction of CpG methylation at some specific genes during the differentiation process and drastic changes in global histone modification levels, such as H3K27me3 and H3K27ac. Additionally, changes were also observed in DNA methylation and histone modification patterns in samples from acute myeloid leukemia AML patients and exploring further to develop prognostic tools.

The mammalian genome contains numerous regions known as facultative heterochromatin, which contribute to transcriptional silencing during development and cell differentiation. **Mathieu Gerard** (CEN, Gif-sur-Yvette, France) has analyzed the pattern of histone modifications associated with facultative heterochromatin within the mouse imprinted Snurf-Snrpn cluster, which is homologous to the human Prader-Willi syndrome genomic region. He has shown that the maternally-inherited Snurf-Snrpn 3Mb region, which is silenced by a potent transcription repressive mechanism, is uniformly enriched in histone methylation marks usually found in constitutive heterochromatin, such as H4K20me3, H3K9me3 and H3K79me3. In addition, he demonstrated that H3K36me3 is markedly enriched at the level of pericentromeric heterochromatin in mouse embryonic stem cells and fibroblasts. This data suggested that H3K36me3 function is not restricted to actively transcribed regions only and may contribute to the composition of heterochromatin, in combination with other histone modifications.

Although, non-canonical Wnts enhances cardiac gene expression in adult progenitor cells but the mechanism is unclear. **Masamichi Koyanagi** (Kyushu University Hospital, Beppu, Japan) discovered that Wnt5a increased several histone lysine demethylases such as JmjC-domain containing family, JMJD2B/2C. JMJD2 family demethylate trimethylated histone H3 at lysine 9 (H3K9me3), thereby removing repressive histone modifications. Therefore, increased JMJD2 might epigenetically modify gene expression thereby opening chromatin structures at silenced promoters and modulate the differentiation of APCs. Dr. Koyanagi's results presented in the meeting suggested that non-canonical Wnt5a induces epigenetic remodelling by removing repressive chromatin marks and thereby sensitize APCs for acquiring a cardiac cell fate.

Epigenetic modifications during development & assay development

Amongst the numerous modifications occurring in cells during normal development or pathological conditions, the epigenetic modifications is a field where the knowledge is currently rapidly evolving, together with the tools allowing studying such modifications. **Vincent Dupriez** (PerkinElmer, Villebon sur Yvette, France) presented homogenous, non-radioactive assays developed at PerkinElmer to study histone methylation and acetylation, as well as the interaction of modified histones with reader domains. Some of these epigenetic assays includes: Amplified Luminescent Proximity Homogeneous Assay, Lanthanide Chelate Excite-based assay that uses Europium labelled anti-marker antibody in Time-Resolved Fluorescence Resonance Energy Transfer method. Microscope imaging provides a powerful useful method to analyze the cell-specific or sub-cellular modifications. Using High Content Assay, HCA ImagAmp™, Dupriez provided data on sensitivity of detection. In addition, he also showed many examples of complementary signal transduction analysis in stem cells, looking at the cAMP and protein phosphorylation. mRNA splicing is presently accepted as a co-transcriptional process and could thus be influenced by local DNA methylation. **Sofia Kouidou-Andreou** (Aristotle University School

of Medicine, Thessaloniki, Greece) analyzed data on CpG methylation, alternative splicing, and exonic splicing enhancers and concluded that DNA methylation is an alternative splicing-defining parameter, expected to play a catalytic role in cellular differentiation. She concluded that p53 silent sporadic mutations in human cancers well correlated with embryonic stem cells and methylated CpGs.

Epigenetics in stem cells and pluripotency

Myc is a key molecule in transformation, cell reprogramming, and its expression is critical to maintain embryonic stem cells (ESC) in the undifferentiated state. However, the mechanism by which Myc performs these tasks has not yet been elucidated. ATAD2, a gene activated in many cancer types, has recently been shown to cooperate with Myc therefore raising the question of its involvement in pluripotency and embryonic stem cell biology. Therefore, **Fayçal Boussouar** (Université Joseph Fourier, Grenoble, France) has shown that in embryonic stem cells, Atad2 is a target of and is positively regulated by the master pluripotency genes, Oct4 and Nanog. The identification of the Atad2-dependent transcriptome in ES cells was also demonstrated that Atad2 links the Oct4/Nanog activity to a specific gene functional network, involving TGF β , which suggests that it could stimulate cell proliferation. In conclusion, these results suggested that Atad2 is a critical gene functioning at the interface between ES cell pluripotency and proliferative capacity. **Francesco Neri** (Università di Siena, Siena, Italy) observed that Myc directly up-regulates all core components of the Polycomb Repressive Complex 2 (PRC2) both in ESC and in differentiated cells. PRC2 is a transcriptional repressive complex that catalyzes histone H3 methylation of Lysine 27 at promoters of developmental regulators contributing to silence their expression. He has also shown that silencing of both c-Myc and N-Myc in ESC induces the reduction of PRC2 expression leading to loss of stemness and reduction of proliferation. Analysis of epigenetic modifications and expression at PRC2 target genes revealed that c-Myc and N-Myc silencing induces the de-repression of “bivalent” developmental regulators by reduction of H3K27me3 signal on their promoters. In conclusion, Myc proteins contribute to maintain ESC pluripotency by controlling the expression of developmental regulators via the polycomb PRC2 complex.

Repressor Element-1 Silencing Transcription factor (REST) is a transcriptional repressor able to recruit a range of chromatin-modifying activities through its N and C-terminal repressor domains. Chromatin immunoprecipitation (ChIP) studies have revealed that REST binds to several thousand loci genome-wide in embryonic stem cells (ESCs) and neural stem cells (NSCs), though its exact role and mechanism of action remain unclear. In ESCs, REST is known to bind to several pluripotency gene promoters, including *Nanog*, *Sox2* and *Pou5f1* (*Oct4*) and also shares several common targets with these transcription factors, yet it is not required to maintain ESC pluripotency. However, knockout of REST function in ESCs was shown to lead to a delay in the

normal repression of pluripotency genes upon differentiation. **Angela Bithell** (Kings College London, London, United Kingdom) presented recent work on the role of REST during ESC neural induction and suggested that REST acts to co-ordinate the normal repression of the pluripotency programme with activation of the neural differentiation programme and is important for the correct differentiation of neural progenitor cells. Most importantly REST binds to core pluripotent genes and plays a role in pluripotency network. REST maintains a repressive epigenetic signature in embryonic stem cells; however it is not required in pluripotency cell maintenance. Human embryonic and induced pluripotent cells acquire progressive epigenetic changes during *in vitro* expansion. To ensure the safety of these cell types in future clinical applications the causes and consequences of culture induced modification of the epigenome need to be understood. **Ernst Wolvetang** (University of Queensland, St Lucia, Australia) discovered that ascorbate, a medium component present in all serum-free culture media for human pluripotent cells, leads to genome wide, yet specific demethylation of DNA. **David Sassoon's group** (Université Paris VI/Pierre et Marie Curie, Paris, France) identified a parentally imprinted gene, PW1/Peg3 from a screen for early regulators of stem cell progression during organogenesis. To study PW1 expression and regulation further, they have generated a bac recombineered transgenic mouse model carrying the PW1 locus in which the β -galactosidase reporter gene was placed into the last exon. This mouse model not only showed expression in the adult muscle stem cells but also revealed expression in stem cell niches in all adult tissues identified. The data presented in the meeting suggested that PW1 plays a role in the regulation of the cell cycle consistent with their previous observations demonstrating a functional link between PW1 and p53 signalling.

Epigenetic changes during differentiation

Pluripotent embryonic stem cells (ESCs) possess a relatively open chromatin structure, yet the role of chromatin compaction in embryonic stem cell pluripotency and differentiation has not been tested. Linker histone H1 is the major chromatin architectural protein in mediating higher order chromatin folding and is essential for mammalian embryogenesis. Multiple H1 subtypes exist in mammals that differ in expression during development and cellular differentiation. The compound H1 null ES cells that have an especially low level of H1 proteins due to the depletion of several major H1 subtypes display global chromatin decondensation. Yuhong Fan (Georgia Institute of Technology, Atlanta, USA) found that these compound H1 null ES cells are compromised in their capacity to differentiate. Additionally, evidence shown that H1 participates in regulation of pluripotency genes and in mediating histone mark changes necessary for silencing pluripotent genes during ESC differentiation. In conclusion, these results suggested that H1 plays a critical role in embryonic stem cell pluripotency and differentiation.

Chromodomain proteins including members of the HP1 and Polycomb families of proteins function as guardians of the transcriptome, assuring the silencing of repeated DNA sequences, but

also the timely expression of morphogenic genes during development, and the transient transcriptional repression of inducible genes in the adult organism. Therefore, tools to control chromodomain proteins open new avenues for cellular reprogramming. **Christian Muchardt's group** (Institut Pasteur, Paris, France) have identified a novel mechanism for the regulation of HP1 chromatin-interaction. Altogether, Muchardt's presentation clearly demonstrated that Peptidylarginine Deiminase 4 functions as a regulator of HP1-mediated repression and that excessive activity of this enzyme may participate in the transcriptional reprogramming observed in multiple sclerosis patients.

Stem cell propagation & *in vivo* applications

The development of methods to produce induced pluripotent stem cells (iPS) has created new opportunities to cure the hemoglobinopathies and other monogenic blood disorders. However, to realize these promises, three major problems must be overcome: Methods to reliably produce iPS free of genotoxic mutations or epimutations must be standardized; methods to correct the genetic defect must be improved, and methods to differentiate the iPS into transplantable hematopoietic cells must be developed. Significant progress has been made in the methods to produce transgene-free iPS. Methods to correct mutation in iPS can be divided in three broad categories: homologous recombination to directly correct each gene, virus mediated random integration of therapeutic transgenes followed by characterization of the integration site or creation of landing pad in which the therapeutic genes are integrated.

Eric Bouhassira (Albert Einstein College of Medicine, Yeshiva University, New York, USA) has focused on the latter method because identification of one or a few safe landing pads that could be used to insert therapeutic genes to correct all hereditary blood diseases would be much more economically efficient than having to design and test in rigorous clinical trials dozen or even hundreds of vectors and zinc fingers to correct the large number of mutations that causes the hemoglobinopathies and other red cell disorders. In order to test this strategy Bouhassira's group has developed a zinc-finger strategy to correct alpha-thalassemia hydrops fetalis in iPS and have shown that it was possible to completely restore expression of alpha-globin genes by inserting LCR-driven globin transgenes at specific landing pad. Transplantation of hematopoietic cells derived from iPS into immuno-deficient mice remains very inefficient because iPS derived cells are epigenetically programmed to produce embryonic hematopoietic cells that lacks cells renewal capacity. Long-term culture of hES cells requires the use of animal derived matrices which are variable and can result in xenogeneic contamination. In order to expand the use of hES cells for potential clinical applications it is important to have a fully defined xeno-free culture system for long term propagation of hES cells. **Martial Hervy** (Corning Life Sciences, Corning, NY, USA) has developed a synthetic, xeno-free surface, Corning® Synthemax™ Surface that supports long-term self-renewal of multiple hES cell lines in several chemically defined commercial media. hES cells

expanded on Synthemax Surface maintained stable proliferation rates, phenotypic markers, normal karyotype and pluripotency. In addition, the hES cells were able to differentiate into functional cardiomyocytes on the same surface by simply changing medium composition. In summary, Synthemax Surface in combination with defined medium provides a xeno-free culture system for expansion and differentiation of stem cells and will be useful for both research purposes and production of cells for cellular therapies.

In a context of chronic blood supply difficulties, generating cultured RBC (cRBC) *in vitro* after amplification of stem cells makes sense. cRBCs can be now cultured *in vitro* from various human stem cells: hematopoietic, embryonic or induced pluripotent stem cells (hiPSCs). The hiPSC technology represents a potentially unlimited source of RBCs and opens the door to the development of a new generation of allogeneic transfusion products. The most proliferative source of stem cells for generating cRBC is the cord blood but indeed limited in term of hematopoietic stem cells and dependent on donations. Pluripotent stem cells are thus the best candidates. Critical advances have allowed leading towards the *in vitro* production of functional RBC from iPSCs in few years. Proof of concept of generating cRBC from iPSCs has been done but needs to be optimized to lead to a clinical application in blood transfusion. **Luc Douay** (Université Pierre et Marie Curie-Saint Antoine Hospital, Paris, France) highlighted the potential applications for alloimmunized patients and those with a rare blood group. In addition, he has shown that only 3 hiPSC clones would have been sufficient to match more than 99% of the patients in need of RBC transfusions.

Stem cell therapy & drug discovery

Stem cell therapy for myocardial regeneration after infarction is a relatively new and revolutionary concept in cardiology emerging from encouraging pre-clinical results. Several stem/progenitor cell populations e.g., hematopoietic stem cells, endothelial progenitor cells (EPC) and mesenchymal stem cells (MSC), isolated from the bone marrow, are being studied for their therapeutic effects. However, functional improvements observed to date in almost all clinical trials were only moderate positive. The major problem is the low-rate survival of transplanted cells and their poor retention into the inflammatory conditions of the infarcted myocardium. In this context, clinical benefits remain transient and only attributed to transplanted cell-associated paracrine effects stimulating angiogenesis and protecting surviving cardiomyocytes (CMC).

Alexandrina Burlacu's group (Institute of Cellular Biology and Pathology, Bucharest, Romania) has evaluated the paracrine properties of MSC in hypoxic conditions. The results demonstrated that MSC were resistant to hypoxia-induced apoptosis and the synthesis and/or release of pro-angiogenic factors were preserved or even improved when MSC were incubated under hypoxic versus normoxic conditions. Furthermore, hypoxic MSC retained the paracrine protective effects on CMC and secreted chemoattractant factors for endothelial cells (EC).

Dynamic assessment of EC by using xCELLigence system revealed opposite effects of EPC- and MSC-conditioned media with regard to their ability to support EC adhesion and proliferation. In conclusion, her results demonstrated that combining different stem/progenitor cell populations might result in an additive effect that could better promote myocardial regeneration after infarction. **Sarah Crawford** (Southern Connecticut State University, New Haven, CT, USA) presented the latest updates on the epigenetics focus in solid tumour development; especially she highlighted the role of microenvironment in tumour progression giving an example of k-ras mutations, which are hallmarks of pancreas cancer.

In recent years innovative cell therapeutic strategies have been developed for diverse clinical applications. Stem cell transplantation with hematopoietic stem cells derived from bone marrow, peripheral blood and cord blood for the curative treatment of leukemia and immune disorders is still the gold standard. But beyond the reconstitution of malignant hematopoiesis cell-based therapies are increasingly intended to regenerate and replace deceased tissue and to modulate the immune system. The majority of therapies focus on cardiovascular, neurological and immunological applications. While many basic questions like cell source, cell dose and mode of action are still not resolved. **Christine Guenther** (Apceth GmbH und Co. KG, München, Germany) presented recent clinical trials on some of the applications of stem cell therapy. The alteration of the native environment of biological systems via means of external chemical influence and internal pharmaceutical impact has increased the likelihood of exposure of the developing embryo to new and powerful teratogens. Consequently, that raises an urgent need for novel methods of testing developmental toxicity. For this purpose, pluripotent stem cells of various organisms have been employed to create experimental models that simplify aspects of studying human embryonic developmental toxicity *in vitro*. In the past years, stem cell based predictive assays have been developed to diverge from testing upon a single cell type in order to increase the method's predictivity upon specific tissues. **Nicole zur Nieden** (University of California, Riverside, CA, USA) has previously shown that differentiation of mouse embryonic stem cells into osteoblasts is an accurate endpoint to classify compounds according to their bone toxicity. In the meeting she presented the newest developments on assay improvement along with toxicological data sets for pharmaceutical compounds. These results open up new vistas to develop novel stem cell-based predictive assays for the study of embryonic stem cells.

Outstanding accomplishments

On behalf of the scientific committee Krishnarao Appasani (GeneExpression Systems, Inc, USA) honoured Professor **Karl Ekwall** (Karolinska Institute, Huddinge, Sweden), the keynote speaker on the first day with "*Epigenomics Innovator Award*".

Final thoughts

Epigenomics and Stem Cells are becoming an *'inter-twined research arenas'* and regulate gene expression. The message from the meeting is there are lot of issues has to be addressed before we take stem cells to clinic to treat human diseases. Most of the attendees felt that this was a *"unique, coherent, well-organized, target meeting for learning cutting-edge technology and meeting authorities in the epigenomics and stem cells fields.*

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