Abstracts

CD105, VE-cadherin and VEGFR2 were inhibited whereas the expression of CD34 and CD45 remained unchanged, demonstrated that HDACs are involved in endothelial differentiation of progenitor cells. The proliferation of EPCs assessed by mesurements of telomerase activity and western blot analysis of PCNA showed a decreased proliferative potential, as well migration capacity assessed by wound-healing assay. Adherence capacity was also influenced by VPA, starting 12 hours after stimulation VPA has led to a decreased in adherence of EPCs. EPCs chemotaxis to anigogenic factors such as angiopoietin, VEGF, and SDF1a mesured in real time with xCELLigence system was stimulated by VPA. In vitro angiogenesis assay using Matrigel showed that in the presence of acetylated histones the number of capillary-like networks was reduced by up to 75%. Scanning electon microscopy photomicrographs showed that in the presence of VPA, EPCs lose the ability to form capilary networks on colagen scafolds, suggesting that histone acetylation inhibits the neovascularization process in vitro. Discovering the involvment of epigenetic mechanisms that regulate human vasculature development and differentiation will provide us greater insights into a variety of disorder, where the need of vasculatization is essential.

Keywords: acetylation, Epigenetics, stem cells.

MON-160

Identification of *Arabidopsis* KUMONOSU gene involved in DNA methylation and heterochromatin-associated silencing

Y. Ikeda^{1,2}, C. Becker³, L. López González¹, M.-N. Pouch-Pélissier¹, R. Pogorelcnik¹, D. Weigel³,

O. Mathieu¹ ¹CNRS UMR6293 – INSERM U1103 – GReD, Aubiere, France, ²Institute of Plant Science and Resources, Okayama University, Kurashiki, Japan, ³Max Planck Institute for Developmental Biology, Tübingen, Germany

In plants, multiple layers of epigenetic regulators are required for heterochromatin-associated silencing, including many repetitive sequences and transposable elements (Regal and Mathieu, Biochim Biophys Acta., 2011). However, the control mechanism of tissue specific heterochromatic silencing is still unknown.

Here, we report the new Arabidopsis gene affecting heterochromatin-associated silencing, KUMONOSU (KUN). We isolated kun mutant from the screening affecting transgene silencing. In kun mutant, not only transgene, many endogenous repetitive sequence and transposable elements were activated. Moreover, the expression of specific genic regions was also affected and DNA methylation level of many target genes was slightly decreased in the mutant. Interestingly, release of silencing of the reporter transgene was observed specifically in vascular tissues of kun mutant, which may indicate possible tissue-specificity in the regulation of heterochromatin silencing.

Keywords: Epigenetics, heterochromatin, Silencing.

MON-161

Impact of glucose and O-GlcNAc transferase on expression of Polycomb genes in breast cancer cells

E. Forma, P. Jozwiak, M. Brys, A. Krzeslak Department of Cytobiochemistry, University of Lodz, Lodz, Poland

Cancer is not only genetic disease but it can arise also from epigenetic abnormalities. The results of epidemiological studies indicate that obesity or hyperglycemia may increase the risk of some cancers, including breast cancers. Moreover, high blood glucose seems to be an important factor of cancer progression.

CSIII-01 - Chromatin & Epigenetics

Recent studies suggest that O-GlcNAcylation which consists in addition of N-acetylglucosamine on serine or threonine residues of proteins may play a key role in the regulation of the epigenom in response to cell metabolic status. Two enzymes are responsible for cyclic O-GlcNAcylation: O-GlcNAc transferase (OGT), which catalyzes the addition of the GlcNAc moiety to target proteins and O-GlcNAcase (OGA) which, removes the sugar moiety from proteins. The results of recent studies suggest that OGT may link cell metabolic status with transcriptional repression caused by Polycomb proteins. Abnormal OGT expression and O-GlcNAcylation are features of breast cancer cells but their role in Polycomb-dependent gene regulation remains to be elucidated. In this study we investigated the effect of hipo- normo- and hiperglycemia conditions on expression of O-GlcNAc cycling enzymes, expression of Polycomb genes, i.e. EZH2, SUZ12, RING1B and BMI-1 as well as histone modification levels in breast cancer cells (MCF7, MDA-MB-231 and Hs578t) and non-tumorigenic epithelial mammary cell line (MCF10A). Moreover, the impact of glucose and OGT down-regulation by RNAi on expression of several Polycomb target genes involved in cell differentiation and epithelial mesenchymal transition was analyzed. The results showed significant glucose-dependent changes in mRNA and protein level of OGT, OGA, EZH2 and BMI-1 in breast cell lines. Significant glucose-dependent increase in EZH2 protein expression was especially evident in poorly differentiated breast cancer cells MDA-MB-231 and Hs578t, which show high invasive phenotype: Cells grown in high glucose showed decreased BMI-1 protein and H2A ubiquitinylation levels compared to cells grown in low glucose. OGT down-regulation caused decreased O-Glc-NAcylation in cells and was correlated with reduced EZH2 protein level but not BMI-1 level. OGT interference influence expression of some Polycomb target genes. There was increased expression level of FOXC1 and CDH1 and decreased level of HOX10A in cells with OGT depletion. The results of our preliminary studies suggest direct link between aberrant O-GlcNAcylation which is a nutrient-responsive modification and EZH2dependent repression in breast cancer cells.

Keywords: O-GlcNAc transferase, Polycomb, breast cancer.

MON-162

Impact of surfactants on soft wheat meiosis A. Zhussupova, N. Omirbekova, Z. Zhunusbayeva Molecular Biology and Genetics, Al-Farabi Kazakh National

Molecular Biology and Genetics, Al-Parabi Kazakh Wallona University, Almaty, Kazakhstan

Surfactants are broadly used in the national economy as active components of detergents, and in release of the products, based on synthetic and natural fibers. Oil and chemical industries, manufacture of construction materials also serve as important consumers.

A number of works is available on various biological effects and violations of structure and functions of organisms under synthetic surfactants, most of which depict biochemical changes. However, there is absolutely no data available on the possible genetic consequences.

The objective of our study is the comparative analysis of genetic toxicity of surfactants varying in their chemical nature at the level of chromosome violations in soft wheat microsporogenesis.

Kazakhstanskaya 3 and Shagala soft spring wheat varieties were used as the study material. The following non ionogenic surfactants: Triton X-100, Triton X-305, Twin 85, Twin 65 and Twin 20, frequently used in biological research, were chosen in a 1% concentration (with five hours treatment prior to sowing in the field). Seeds, processed by the distilled water, served as control. Pollen mother cells were temporally fixed in Carnoy solution, washed three times with 70% ethanol solution. Acetocarmine staining was used for estimation of chromosome aberra-

> FEBS Journal **281** (Suppl. 1) (2014) 65–783 © 2014 The Authors. FEBS Journal © 2014 FEBS

CSIII-01 – Chromatin & Epigenetics

tions in wheat microsporocytes. Chromosome violations were considered in 13,280.00 cells under the light microscope with appropriate statistical processing.

The maximum number of chromosome violations in Kazakhstanskaya 3 is caused by Twin 85, Triton X-305, Twin 65, and Triton X-100 (29.2 \pm 0.9%, 25.7 \pm 0.9%, 25.6 \pm 0.8% and 32.5 \pm 0.7% respectively), rather than Twin 20 (19.54 \pm 0.7%), with the difference reliable at 99% probability level and similar for Shagala; with up to 16 times higher general frequency in compare with the spontaneous level.

Mutations at metaphase I are represented by multi-, univalents, clumping of chromosomes (pycnosis), shift of the metaphase plate division spindle and overflowing of nuclear matter, anaphase I – lagging chromosomes, bridges and fragments, anaphase II – bridges with fragments, asynchronous fission, and nucleus free cells. At tetrad stage violations of both nuclear (dyads, triads, pentads and hexades) and cytoplasmic (changes of tetrad walls and absence of cytokinesis) nature are found.

It is possible that the increasing frequency of the aberrant cells in wheat gametes is associated with the surfactants collision of physiological processes occurring in a cell, resulting in disturbed enzymatic system, which in turn leads to the accumulation of chromosomal defects. Spindle violation might be caused by uneven growth of its fibers, due to which fast-growing threads form bends, resulting with the chromosomes delay in the equatorial part of the cell or impaired formation of daughter cells. **Keywords:** meiosis, surfactants, wheat.

MON-163

In silico study of drought and salt tolerance in wheat

A. Fujah-Yusuf^{1,2}, M. Labhilili¹, S. Cherkaoui² ¹Molecular Biology, National Institute of Agricultural Research, ²Biology, Mohammed V University-Agdal, Rabat, Morocco

The in silico study of genes is an important step because it helps guide the interpretation of experimental results and suggest new experiments. Salt-tolerant candidate genes in wheat and other cereals include HKT, NHX, SOS, and HAK genes. Drought-tolerant candidate genes are DRG, DHN, DRF, DREB, etc. These genes were searched, identified and downloaded from the NCBI database. Comparative studies and analyses such as search for candidate genes' functions, search for protein domains, multiple sequence alignments, and phylogenetic tree construction as well as primers design were carried outusing bioinformatics tools. The RT-PCR primers associated with the candidate genes for the studied traits were designed as molecular markers to assist theprogram for improvement in cereals precisely in wheatvia marker-assisted selection.

Keywords: Bioinformatics tools, Drought & Salt-tolerant Candidate Genes, Marker-assisted selection.

MON-164

Inhibition of DNA methylation alters chromatin organization, nuclear positioning and activity of 45S rDNA loci in cycling cells of *Q. robur*

V. Zoldos¹, V. Vičić Bočkor¹, T. Horvat¹, D. Barišić², Ž. Maglica³

¹Faculty of Science, Univeristy of Zagreb, Zagreb, Croatia, ²Friedrich Miescher Institute for Biomedical Research, Basel, ³Ecole Polytechnique Fédéral de Lausanne, Lausanne, Switzerland

In *Quercus robur* there are two rDNA loci, the major (NOR-1) and the minor (NOR-2) locus. We aimed to deduce the transcriptional activity of the two rDNA loci in root tip cycling cells by

FEBS Journal 281 (Suppl. 1) (2014) 65–783 © 2014 The Authors. FEBS Journal © 2014 FEBS





exhamining the position of rDNA loci in respect to the nucleolus, organizational patterns (topology) of rDNA chromatin in metaphases and interphases using light and electron microscopy, silver staining of NORs, epigenetic signatures of rDNA chromatin and the expression levels of 18S rRNA genes before and after treatment of root tips with the DNA methylation inhibitor 5-aza-2'deoxycytidine (5-aza-2'-dC). Our data indicates that in normal physiological conditions of Q. robur cells, a situation resembling nucleolar dominance is present where only rRNA genes within the NOR-1 locus are transcriptionally active and participate in the formation of the nucleolus. To investigate the role of the other, transcriptionally inactive NOR-2 locus, we induced its reactivation by treatment with 5-aza-2'-dC. We observed that a decrease in levels of DNA methylation at NOR-2 locus and an increase in total level of rRNA transcripts do not affect the contribution of this locus in nucleolar formation, suggesting its loss of function. In addition, we propose a correlation between rDNA chromatin topology, location of different rDNA fractions related to the nucleolus, epigenetic signature and the activity of rRNA genes within the active NOR-1 locus.

Keywords: DNA methylation, rDNA locus, transcriptional activity.

MON-165

Integrative genome-wide analysis reveals novel pathways and transcription factors involved in non-small cell lung cancer drug resistance D. Li

School of Life Science and Technology, Tongji University, Shanghai, China

Background: The mechanism of acquired drug resistance in non-small cell lung cancers (NSCLC) is poorly understood. The transcriptome and epigenetic signature associated with drug resistance, in particular, are not well-defined. We performed an integrative genome-wide analysis with the aim of identifying unique gene expression patterns, pathways, transcription factors (TFs), DNase I hypersensitive sites (DHSs), and enhancers involved in drug resistance.

Methods: We conducted RNA-sequencing (RNA-Seq) to examine differentially expressed genes and pathways, DNase I sequencing (DNase-Seq) to identify DHSs and infer TFs binding sites, and chromatin immunoprecipitation sequencing (ChIP-Seq) to locate H3K4me2 histone modification across enhancers in the gefitinib-resistant NSCLC cell line PC9R and its gefitinib-sensitive parental cell line, PC9.

Results: We discovered several genes and pathways, many of which are related to the epithelial-mesenchymal transition (EMT). We also identified differential distributions of both DHSs and enhancers and inferred TFs, which are closely associated

Abstracts