1st Hungarian Epigenetics Meeting

20-21 September, 2012.

Semmelweis University, Nagyvárad Tér, Budapest

Organizers: Tamás Arányi and Bálint L. Bálint.

Scientific Organizing Comity:
Tamás Arányi, Bálint L. Bálint, Imre Boros, András Falus and László Nagy

Speakers:

Imre Boros Szeged University, Hungary
László Nagy Debrecen University, Hungary
Peter Holland University of Oxford, UK
Takuya Imamura Kyoto University, Japan
András Páldi Genethon, France

Details and registration:

http://epigenetics-hungary.eventbrite.com/

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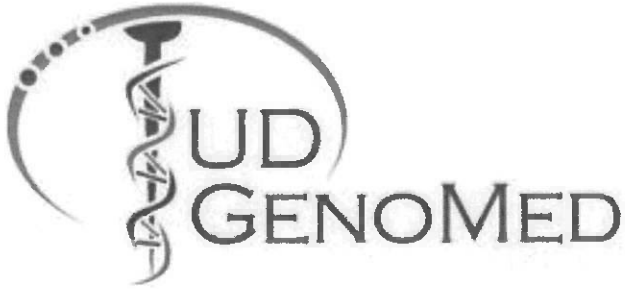

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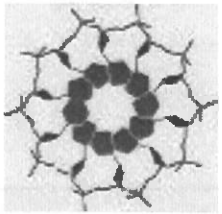

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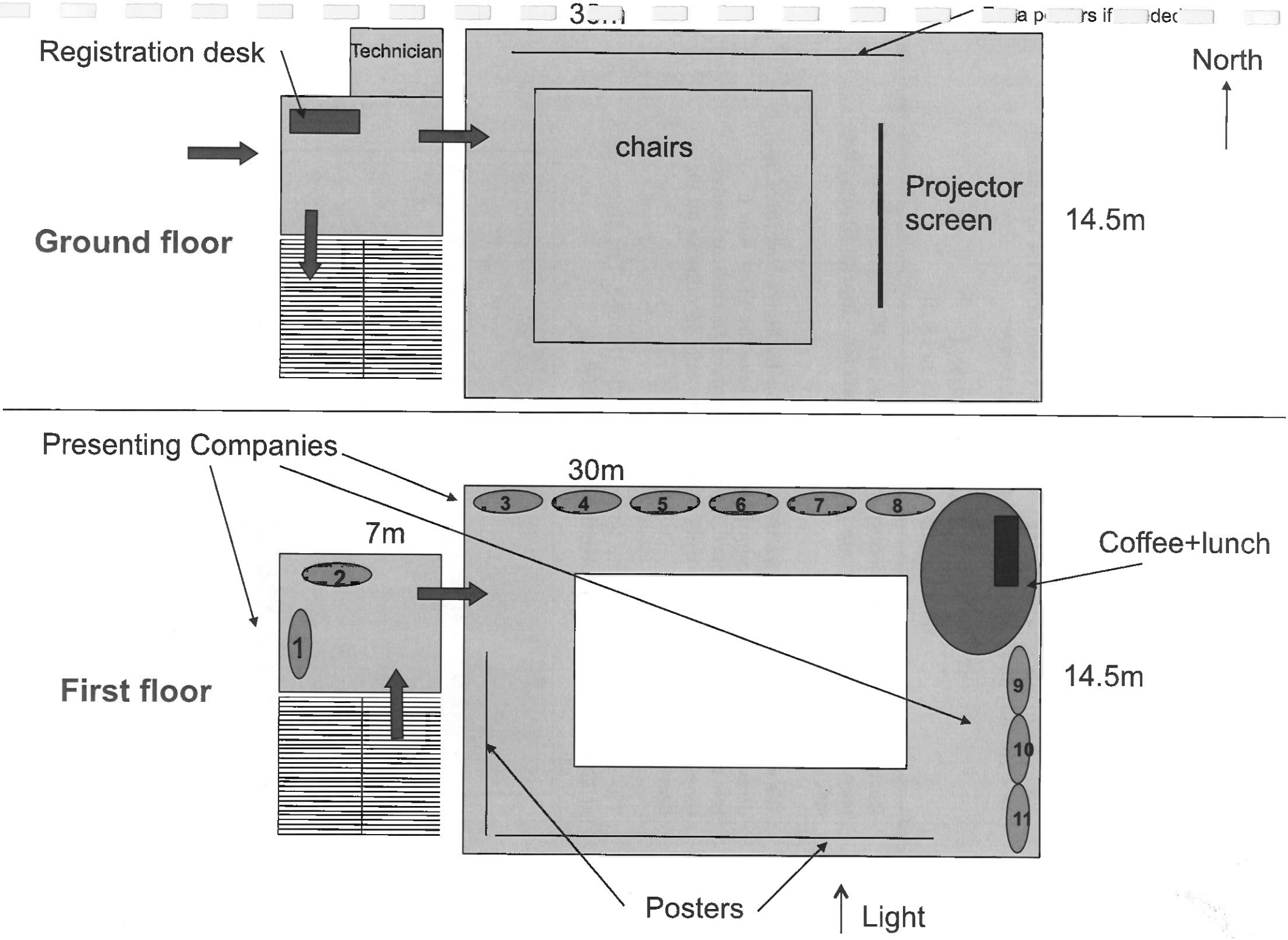
Start	End	Presenter	Title	Time
12:00	12:45	2012.09.20.	Registration desk: OPEN	0:45
12:45	13:00		Opening of the conference	0:15
13:00	13:30	Andràs Pàldi	Epigenetics: a new paradigm of what?	0:30
			TRANSCRIPTION REGULATION AND HISTONE TAILS	
13:30	14:00	Làszlò Tora	Towards the understanding of histone acetyl transferase complexes in transcription regulation and cellular differentiation	0:30
14:00	14:30	Imre M. Boros	BRIEF TOUR GUIDE TO THE DROSOPHILA CHROMATIN LANDSCAPE	0:30
14:30	15:30		COFFEE AND POSTERS, Registration desk: OPEN	1:00
15:30	16:00	Laszlo Nagy	PRMT1 produced arginine methylation and PRMT8 provide selective and coordinated retinoic acid-driven gene expression during the differentiation of mouse embryonic stem cells	0:30
16:00	16:15	Izabella Bajusz	The SET domain of Enhancer of zeste is involved in the recognition of chomatin domains to be inactivated	0:15
16:15	16:30	Ferenc Müller	The interplay between transcription initiation codes and nucleosome positioning signals on core promoters during embryo development	0:15
16:30	17:00	Gábor Szabó	Nucleosome-DNA cohesion is highly sensitive to certain H3 modifications and to superhelical twist.	0:30
18:30	22:30		GALA DINNER	4:00

Start	End	Presenter	Title	Time
9:15	10:00	2012.09.21.	Registration desk: OPEN	0:45
10:00	10:30		DNA METHYLATION AND CHROMATIN	0:30
10:00	10:30	Peter WH Holland	Genome evolution in animals: clues from the homeobox genes	0:30
10:30	10:45	Csaba István Pongor	Effect of methylation on the nanomechanical properties of DNA	0:15
10:45	11:00	Tamás Fischer	Chd1 chromatin remodelers maintain nucleosome organization and repress cryptic transcription.	0:15
11:00	12:00		COFFEE AND POSTERS, Registration desk: CLOSED	1:00
12:00	12:30	Takuya Imamura	Promoter-associated noncoding RNAs for the sequence-specific epigenetic alterations during mammalian development	0:30
12:30	12:45	Larry Jia	Sequence and Strand Specific DNA Hydroxymethylation Analysis	0:15
12:45	13:00	Zsofia Nemoda	Blocking effect of 5-(hydroxy)methylcytosine on Taq DNA polymerase	0:15
13:00	13:30	Andras Falus	Hypermethylation of histidine decarboxylase gene induces tumor surveillance	0:30
13:30	14:30		LUNCH, Registration desk: CLOSED	1:00

Start	End	Presenter	Title	Time
14:30	14:30	2012.09.21.	EPIGENETIC MECHANISMS IN DISEASES	
14:30	15:00	Iannis Talianidis	Epigenetic mechanisms regulating liver development, metabolism and disease	0:30
15:00	15:15	Richárd Bártfai	Epigenetic makeup of the centromeres in the human malaria parasite, P. falciparum	0:15
15:15	15:30	Lóránt Székvölgyi	Chromosomal R-loops and single-strand nicks mark higher-order chromatin structures	0:15
15:30	16:15		COFFEE AND POSTERS, Registration desk: CLOSED	0:45
16:15	16:30	Magdalena Koszarska	Mutations altering epigenetic regulation in acute myeloid leukemia: isocitrate dehydrogenase 1 and 2	0:15
16:30	16:45	Viktor Szegedi	Epigenetic correlation of anxiety	0:15
16:45	17:00	Alexandra Kalmár	Gene expression analysis of laser microdissected colonic cells and identification of DNA methylation-regulated genes in colorectal cancer	0:15
17:00	17:15	Lajos A. Réthy	Tales on a clinical case and epigenetics with small and few introns - an old fashioned detective story	0:15
17:15	17:45	Béla Molnár	Circulating nucleic acids are biomarkers of colorectal diseases and TLR9 dependent activators of immune and regenerative response, depending on the methylation status	0:30
17:45	18:00		Poster prizes	0:15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1st Hungarian Epigenetic Meeting

Gala Dinner

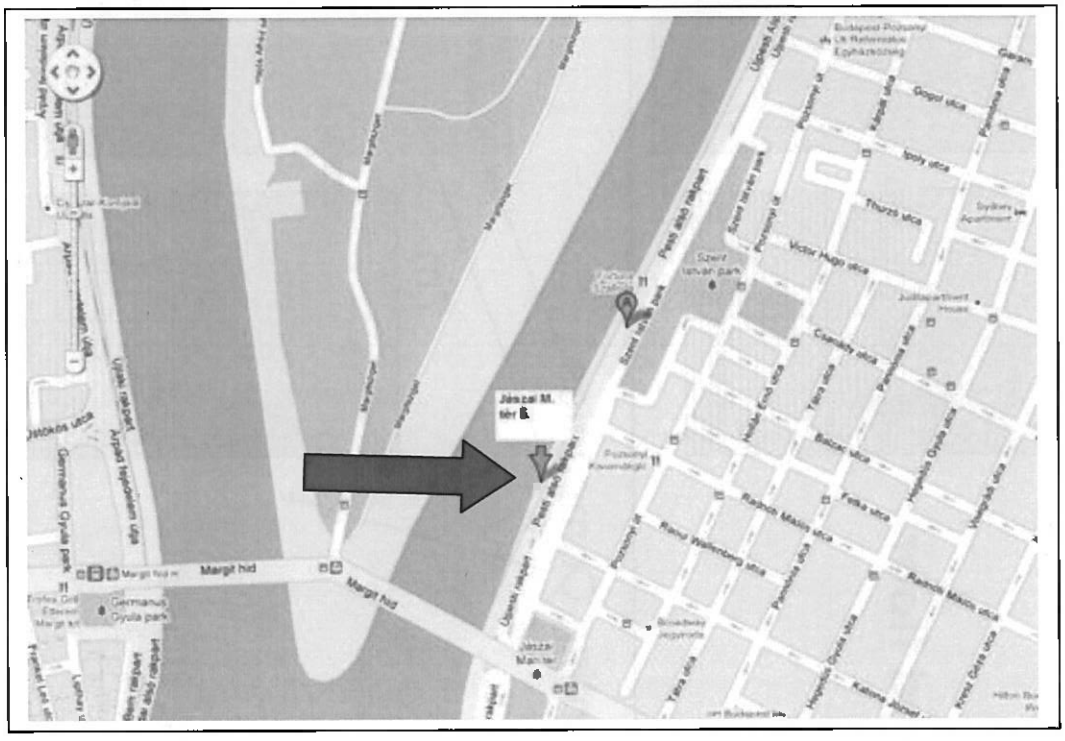
All participants who payed the Gala Dinner registration fee are invited on Thursday 20-th of September to the Gala Dinner.

Name of the ship: SIRONA
Boarding time: from 18.15 to 18.30

Boarding place: Carl Lutz rakpart, gate No6, see RED ARROW on map below (100m North from the Jászai Mari tér – Margit Bridge, Pest side).

We recommend to travel from Nagyvarad ter by Metro No2 (blue line), direction Ujpest to the Nyugati Square and take the Tram 4 or 6 towards Széll Kálmán square. Leave the tram at Jászai Mari square (one station - or walk, the walk will take you only 15 minutes on the Szent István körút).

The ship will leave for a two hours tour on the Danube. The dinner ends at 22.15 and we have to leave the boat till 22.30.



Buffet menu

1. Mixed starters
2. Hortobagyi palacsinta and grilled chicken breast with grilled vegetables and rice.
3. Dessert
4. Coffee

Drinks: welcome champaigne, mineral water and coffee - served by the waiters and included in price.

Alcoholic drinks: two glases of wine or one beer / alternativelly two soft drinks - included in price and payed with coupons.

Extra drinks can be payed at the waiters (HUF or EUR).

If you have any questions please contact the organizers:
Tamas Aranyi: +36 1 279 3143
Balint L. Balint: +36 30 961 6119

Details about the ship: Perfect Tours, Ibolya Morvai: +36 70 366 9651

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Hypermethylation of histidine decarboxylase gene induces tumor survaillence

The role of histamine in local immune response and on tumour growth is still not fully uncovered. In this lecture two approaches will be introduced.
First, the role of histamine will be demonstrated in tumor (melanoma) growth using mouse melanoma cells manipulated via stable transfection with sense mouse HDC mRNA, a mock control, and an antisense HDC RNA segment, respectively.
Gene expression profiles and in silico pathway analysis of transgenic mouse melanomas, secreting different amounts of histamine show a histamine H1 receptor dependent suppression of expression of the tumor suppressor insulin-like growth factor II receptor and the antiangiogenic matrix protein fibulin-5.
Second, HDC-knockout mice show a high rate of colon and skin carcinogenesis. HDC is expressed primarily in CD11b+Ly6G+ immature myeloid cells (IMCs) that are recruited early on in chemical carcinogenesis. Transplant of HDC-deficient bone marrow to wild-type recipients results in increased IMC cell mobilization and reproduces the cancer susceptibility phenotype of HDC-knockout mice. In addition, mouse CT26 colon cancer cells directly downregulate HDC expression at epigenetic manner through promoter hypermethylation and inhibit myeloid cell maturation. These data indicate key protective role of histamine in myeloid cell differentiation and IMCs in early cancer development.

Falus et al Trends in Immunol., 2001. 22: 648
Pos et al Cancer Res., 2005;65 :4458
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Yang et al, Nature Med., 2011, 17: 87

A. Kalmár 1, S. Spisák 1, O. Galamb 1, B. Wichmann 1,2, F. Sipos 1, K. Tóth 1, K. Leiszter 1, A.V. Patai1, A. Scholler1,3, B. Molnár1,2, Z. Tulassay1,2
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2 Molecular Medicine Research Unit, Hungarian Academy of Sciences, Budapest, Hungary
3 2nd Department of Surgery, Semmelweis University, Budapest, Hungary

Gene expression analysis of laser microdissected colonic cells and identification of DNA methylation-regulated genes in colorectal cancer development

INTRODUCTION: Changes of the DNA methylation pattern is proven to be an important process during colorectal tumorigenesis. These alterations can be analysed by whole genome microarray combined with laser captured microdissection (LCM).
AIMS & METHODS: Our aim was to identify mRNA expression patterns using LCM samples to determine the underexpressed genes in colorectal adenoma and tumor compared to the healthy stage. Furthermore, we aimed to analyse the possible mechanisms of downregulation, such as cancer-related methylation using a cell culture model. From 6 colorectal cancers and 6 adenomas, 5000 epithelial cells were collected separately from the healthy and the pathological regions by laser capture microdissection and the downregulated genes were identified. In parallel, HT29 colon adenocarcinoma cells were demethylated with 5-aza-2'deoxycytidine and the upregulated genes were determined. The selected genes were validated by RT-PCR. Bisulfite sequencing and immunohistochemistry for a selected gene (PTGDR) were also performed.
RESULTS: During the adenoma-carcinoma sequence 95 genes showed gradually decreasing expression and in the cell culture model 66 upregulated genes were identified after demethylation. Between these groups, there were 17 overlapping genes (eg.: CDKN2B, POU2F3, PTGDR), which were validated on independent samples by RT-PCR. Reduced expression of PTGDR was confirmed by immunohistochemistry along the adenoma-carcinoma sequence. In the promoter region of the gene three CpG islands were predicted, where the methylation status was determined.
CONCLUSION: The regulation of the identified genes showing decreased expression during the adenoma-carcinoma sequence progression can be associated with DNA methylation.

Andràs Pàldi

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Epigenetics: a new paradigm of what?

The term "epigenetics" is more and more popular in the scientific literature. This increasing popularity is accompanied by an ever-increasing confusion about its exact meaning and – as a corollary – about the role it may play in living organisms. Some consider epigenetics as just another mechanism of gene regulation, while others think of epigenetics as a new paradigm for heredity. Settling this debate is beyond the ambition of my talk, my aim is to give a brief overview of the main questions and conceptual issues and point out some contradictions.
The term "epigenetic" is usually used in two general contexts: epigenetic inheritance and epigenetic mechanisms.
Epigenetic inheritance is used to designate a set of heritable phenomena that cannot be explained by the transmission of the DNA. According to a more conservative interpretation, epigenetic inheritance is restricted to the mitotic and/or meiotic transmission of a gene's functional state. Maintenance of the cellular phenotype after division is an example of mitotic epigenetic inheritance. When transmitted through meiosis, the epigenetic characters do not follow Mendelian inheritance making the classical linkage analysis inefficient. In all cases however, genetically identical cells or organisms display different heritable phenotypes.
The existence of this "Lamarckian" inheritance is now widely recognized and accepted. This became only possible because of the huge progress in our understanding of the molecular bases of epigenetic phenomena. The best-characterized molecular mechanisms that contribute to epigenetic inheritance are related to chromatin biochemistry. It is now clear that these biochemical mechanisms bring about two apparently opposing features of the chromatin: stability and the capacity to change.
However, our understanding is still not satisfactory and raises a number of crucial practical and conceptual questions.

Bianca P. Hennig, Katja Bendrin, Yang Zhou and Tamás Fischer*

Biochemistry Center (BZH), Heidelberg University, Heidelberg, 69120, Germany.

Chd1 chromatin remodelers maintain nucleosome organization and repress cryptic transcription.

Proper chromatin organization is essential for defining transcription units and maintaining genomic integrity in eukaryotes. Mutations affecting the chromatin structure can lead to increased cryptic transcription and genomic instability. In an effort to understand how transcription units are correctly defined, we screened a selection of deletion mutants in Schizosaccharomyces pombe, by monitoring cryptic transcript levels at selected loci. We found that deletion of hrp1 and hrp3, the S.pombe paralogs of the Chd1 subfamily of ATP-dependent chromatin remodelers, causes strong accumulation of antisense transcripts at the tested loci. High resolution genome-wide expression profiles revealed that these mutants show significant accumulation of antisense transcripts at about 40% of the genome, but remarkably, the level of coding mRNAs is mostly unaffected. Nucleosome mapping experiments uncovered a specific role for Chd1 remodelers in the positioning of nucleosomes in gene coding regions. While the arrangement of nucleosomes in promoter regions was similar to WT, nucleosome organization within coding regions was remarkably irregular in hrp1Δhrp3Δ strain. Such irregular nucleosome structure can lead to enhanced cryptic promoter activity within transcription units. We extended our analysis to other mutations associated with enhanced cryptic transcription activity, such as set2Δ, alp13Δ, and FACT complex subunit pob3Δ. While nucleosomes were severely depleted in the pob3Δ strain, nucleosome positioning was less affected. In sharp contrast, nucleosome organization in the alp13Δ and set2Δ strains was indistinguishable from WT. These data indicate multiple mechanisms in the repression of cryptic promoter activity in eukaryotic cells.

Csaba István Pongor /presenter/
[Department of Biophysics and Radiation Biology, Semmelweis University, Budapest, Hungary.]
Pasquale Bianco
[Laboratory of Physiology, DEB, University of Florence, Florence, Italy]
Miklós Kellermayer
[Department of Biophysics an

Effect of methylation on the nanomechanical properties of DNA

In mammalian cells 60-90% of cytosine residues are methylated by different enzymes. The methylation sites are unevenly distributed across the genome and are often found in clusters called „CpG islands". Approximately 70% of promoters in the human genome contain or are preceeded by CG-rich regions, suggesting that methylation is important in the regulation of gene expression. Cyclization-kinetics and nucleosome-binding assays indicate that methylation may influence the flexibility of DNA. However, the direct effect of methylation on the mechanics of DNA is yet unknown. To investigate the impact of methylation on DNA mechanics, here we manipulated single molecules of methylated dsDNA and compared their nanomechanical properties with those of unmethylated DNA.
A 3300-base-pair sequence of lambda-phage DNA composed almost entirely of CpG islands was cloned by using PCR containing dm5CTP to produce the fully methylated product. Individual DNA molecules were mechanically manipulated in stretch and relaxation cycles by using custom-built dual-beam counter-propagating optical tweezers. Force versus extension data were fitted with the extensible wormlike-chain model to obtain the persistence length (entropic component of rigidity) and the stretch modulus (enthalpic component of rigidity) of dsDNA. Methylation reduced the persistence length of dsDNA from 39±3 nm to 30±2 nm and the stretch modulus from 1225±115 pN to 373±30 pN. The methylation-induced changes in the nanomechanical properties of dsDNA may play an important role in the regulation of steric access to its sequence-specific sites.

Hoeijmakers WA, Flueck C, Françoijs KJ, Smits AH, Wetzel J, Volz JC, Cowman AF, Voss T, Stunnenberg HG, Bártfai R.

Epigenetic makeup of the centromeres in the human malaria parasite, P. falciparum

Centromeres are essential for the faithful transmission of chromosomes to the next generation, therefore being essential in all eukaryotic organisms. The centromeres of Plasmodium falciparum, the causative agent of the most severe form of malaria, have been broadly mapped on most chromosomes, but their epigenetic composition remained undefined. Here, we reveal that the centromeric histone variant PfCENH3 occupies a 4-4.5 kb region on each P. falciparum chromosome, which is devoid of pericentric heterochromatin but harbours another histone variant, PfH2A.Z. These CENH3 covered regions pinpoint the exact position of the centromere on all chromosomes and revealed that all centromeric regions have similar size and sequence composition. Immunofluorescence assay of PfCENH3 strongly suggests that P. falciparum centromeres cluster to a single nuclear location prior to and during mitosis and cyt2inesis but dissociate soon after invasion. In summary, we reveal a dynamic association of Plasmodium centromeres, which bear a unique epigenetic signature and conform to a strict structure. These findings suggest that DNA-associated and epigenetic elements play an important role in centromere establishment in this important human pathogen.

Iannis Talianidis Biomedical Sciences Research Center Alexander Fleming, 16672 Vari, Greece

Epigenetic mechanisms regulating liver development, metabolism and disease

In the recent years chromatin structure alterations have emerged as critical components of gene regulation in higher eukaryotes. Histone or DNA modifications are considered as major determinants of epigenetic information for chromatin-templated processes such as transcription. In the liver, similar to other organs, specific histone modification patterns correlate with gene activity and represent important means of regulation of epigenetic states characteristic to different developmental stages, metabolic states or diseases.
However, histone or DNA modifications are not the only epigenetic signals that are responsible for the establishment, maintenance and reversal of metastable transcriptional states during liver development and various metabolic conditions. Trans-epigenetic signals, which eventually accumulate into complex gene networks and gradually emerging "promoter-marking" signals, that determine the potential of gene activation are equally important in the establishment of self-propagating transcription states.
The contribution of the above epigenetic mechanisms in the regulation of liver development and function will be discussed.

Ildikó Eszik, János Horváth, Titanilla Szögi, Géza Müller, Botond Penke, Viktor Szegedi

Epigenetic correlation of anxiety

Epigenetic changes are likely to underlie psychiatric diseases, like depression and anxiety disorder. However, this aspect of affective disorders is poorly studied, despite the fact that clinically effective antidepressants and anxiolytics require several weeks for exerting beneficial effects. Here, two inbred mouse strains displaying either anxiety- or non-anxiety related behaviour (AX and nAX, respectively) were analyzed for differences in levels of various histone acetylations. The Ac-H4K12 level was found to be higher in the nAX strain in the ventral hippocampus, but not in the prefrontal cortex nor in the dorsal hippocampus. Chronic treatment with Buspiron, a clinically effective anxiolytic increased measures of anxiety in the nAX, but decreased in the AX strain, as was assessed by elevated plus maze. These behavioral changes were correlated by changes of the Ac-H4K12 level in the ventral hippocampus and prefrontal cortex. These results suggest that epigenetic changes to2 place after chronic anxiolytic treatment, and certain epigenetic markers may correlate with level of anxiety.

Imre M. Boros
Department of Biochemistry and Molecular Biology, University of Szeged, Közép fasor 52, Szeged

BRIEF TOUR GUIDE TO THE DROSOPHILA CHROMATIN LANDSCAPE

New data on chromatin modifications are accumulating daily with accelerating speed. The driving force for new data acquisition is that posttranslational modifications (PTMs) of nucleosomal core histones play roles in basic biological processes via altering chromatin structure and creating target sites for proteins acting on chromatin. Several features make Drosophila a uniquely effective model for studying PTMs. Position effect variegation, polycomb repression, dosage compensation and several other processes extensively studied by the powerful tools of Drosophila genetics, as well as polytene chromosome cytology reveal information on the dynamic changes of histone PTMs and factors that deposit, remove and recognize these. Recent determination of the genome-wide distribution of tens of different histone PTM types has resulted in a highly detailed view of Drosophila chromatin landscape. The efforts of recent years invested into the exploration of chromatin structure have resulted striking new data in many respects. On the one hand, it seems that widely accepted traditional views on heterochromatin - euchromatin classification for example, should be reconsidered. On the other hand, we seem to have glimpsed in the histone code by recognizing for example correlations between the presence of specific histone marks at transcriptional initiator regions and expression of genes with different types of biological functions. In the lecture I will samples from the wealth of data that genome-wide and gene-targeted analyses have provided on the distribution and role of specific histone modifications and modifiers. These together shape the continuously changing yet stable chromatin landscape that serves as soil for epigenetics.

Izabella Bajusz, Frank Sauer, Martin Müller, Henrik Gyurkovics, László Sipos

The SET domain of Enhancer of zeste is involved in the recognition of chomatin domains to be inactivated

Polycomb group (PcG) proteins belong to the family of epigenetic repressors. Mutants of PcG genes cause ectopic expression of homeotic genes. The antagonistic Trithorax-group proteins act as anti-repressors maintaining the active transcriptional state. Although many details about the mechanism of epigenetic maintenance of gene expression patterns are available, it is still unclear how the initially active or inactive chromatin domains are recognized.
We have described an unusual dominant allele of a PcG gene, Enhancer of zeste, in Drosophila melanogaster. While loss of function E(z) mutations cause derepression, our mutation, named E(z)Trithorax mimic, results in ectopic inactivation of target genes mimicking the phenotype of loss-of-function mutations of trithorax-group genes. E(Z) is a catalytic subunit of the Polycomb Repressive Complex 2. The evolutionally conserved SET domain of E(Z) specifically trimethylates histone H3 lysine 27, a modification essential for Polycomb silencing. E(Z)Trithorax mimic contains a single-amino-acid substitution of arginine-741 to lysine within the SET domain. E(z)Trm mutants have an elevated level of H3K27me3 suggesting that this mutation increases the trimethylation efficiency of E(Z). In addition, the strong dominant trithorax-like phenotypes of our allele indicates that the SET domain of E(Z) plays a critical role in distinguishing between the active and the inactive chromatin domains. Very likely, the R741K mutant SET domain makes a mistake in this process that leads to the inappropriate silencing of Polycomb target genes in cells where they should stay normally active.
Genetic interactions, regulation of different transgenic reporters by E(z)Trm and biochemical studies of the mutant protein are discussed.

Lajos A. Réthy
National Institute for Childd Health Budapest HungaryLajos A. Réthy
National Institute for Childd Health Budapest Hungary

Tales on a clinical case and epigenetics with small and few introns - an old fashioned detective story

This is an old-fashioned detective story about the identification of epigenetic mechanisms in the pathogenesis of Niemann-Pick disease A (NPDA), by the simple evaluation of symptoms of a unique clinical case.
NPDA is characterized by the deficient production of acidic sphingomyelinase (ASM). The unique association of a decreased ASM activity and an overgrowth disorder, Beckwith-Wiedemann Syndrome (BWS) with hemihypertrophy and final BWS-associated embryonal tumor (rhabdomyosarcoma) was described at a 23 months old boy in a case report (Réthy et al)
The SMPD1 gene (responsible for the production of ASM) is located distal to BWS chromosomal region 2 (BWSCR2).The latter region is responsible for many of the BWS syndrome characteristics by an impaired regulation of genomic imprinting.
The comparison of some known characteristics of imprinted genes (Hurst) like few and small introns, Alu 1 repeat element, CC-rich regulatory region, alternative splicing, to the characteristics of SMPD1 (comparison done by the author), led to surprising similarities between the two sets.
The described hemihypertrophy of the referred clinical BWS-case was clinically distinctive to the involvement of the maternal allele of BWSCR2. This characteristic as well as the known maternal involvement of the loss of heterozygosity in BWS-associated tumors suggested the involvement of the maternal allelic regions in this case, including both BWSCR2 and SMPD1.
These clinical observations led to the prediction on the imprinted nature of SMPD1 gene.
According to this prediction, SMPD1 is an imprinted, maternally expressed, BWS- and apoptosis-related growth suppressor gene (Réthy). Simonaro et al proved this prediction of the imprinted nature of the SMPD1 gene by studying heteroallelic individuals with ASM-deficient NPD. They also verified that the SMPD1 gene was paternally imprinted and the expressed allele was maternally inherited.
This short story emphasises the importance of the synthesis of fine clinical signs in the search for novel epigenetic effects in rare clinical cases.
References:
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Simonaro CM et al. Am. J. Hum. Genet.2006;78: 865-870.

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Sequence and Strand Specific DNA Hydroxymethylation Analysis

DNA 5-Hydroxymethylcytosin (5-hmC) is a new epigenetic hallmark which was re-discovered recently. The exact function of this base is still not fully elucidated, but it is thought to regulate gene expression or prompt active DNA demethylation. The levels of 5-hmC in genomic DNA vary significantly depending on the cell type. The highest levels are found in neuronal cells of the central nervous system and increases with age, implying its importance in gene regulation of central nervous system. Different methods have been developed to profile 5-hmC at genomic scale. Most of them are enrichment based method using either antibodies to 5-hmC or its modified forms, or specific binding protein to glycosylated 5-hmC such as JBP1. However, these approaches usually require large amount genomic DNA input and have relatively low resolution. Although efforts have been made to detect 5hmC at single base resolution using single molecular real time sequencing technology (SMRT) or oxidative bisulfite sequencing, they still require DNA several micrograms DNA, can be expensive and complicated, which make it impractical for many applications. By combining modification-sensitive restriction enzyme with next generation sequencing approaches, we developed a novel method for genome wide 5-hmC mapping at single site resolution with much lower DNA input. Moreover, this method can detect strand specific 5-hmC modification and SNP at the same time. Data can be directly integrated and compared with base resolution DNA methylation data. Human brain DNA 5-hmC data generated by this method over-layered with DNA methylation profiling data shows some very unique brain DNA 5-hmC pattern. It is confirmed that some neuronal related gene loci, such as BDNF gene, are modified by 5-hmC. The new method will provide a powerful tool in helping our understanding the interplay of genetic and epigenetic in gene regulations.

László Imre, György Fenyőfalvi, Zoltán Simándi, László Nagy and Gábor Szabó

Nucleosome-DNA cohesion is highly sensitive to certain H3 modifications and to superhelical twist.

We have developed a sensitive and high throughput method, applying a laser scanning cytometer (LSC), for the analysis of histone mobility features. Our method offers sensitive means to determine, quantitatively and in a cell-cycle phase specific manner, a major component of global histone mobility: nucleosome-DNA cohesion. After salt elution of agarose-embedded isolated nuclei the remaining histone levels are determined by immunofluorescence labeling, using modification-specific antibodies. H3K4me3 modified histones were eluted from isolated nuclei at much lower salt concentration than H3K27me3 modified histones, in various cell types, including mouse embryonic stem (ES) cells and their differentiated counterparts (NPCs). Nucleosome-DNA cohesion appears to be highly sensitive also to superhelical twist. Nucleosomes containing these two kinds of modifications could also be distinguished based on a differential sensitivity to intercalators. The effect of these agents on superhelicity related features of chromatin loops were analysed simultaneously, using a highly sensitized, LSC-based nuclear halo assay.

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Towards the understanding of histone acetyl transferase complexes in transcription regulation and cellular differentiation

Towards the understanding of histone acetyl transferase complexes in transcription regulation and cellular differentiation

Gene expression is a tightly regulated process. Initiation of transcription by RNA polymerase II (Pol II) is believed to be the outcome of a number of sequential events beginning with the binding of specific activators to their cognate binding sites. This initial step will trigger the recruitment of coactivator complexes and general transcription factors at promoters to allow the loading of Pol II into the preinitiation complex (PIC) to achieve transcription initiation. In this process, coactivators play multiple crucial roles through enzymatic as well as non-enzymatic functions. GCN5 and PCAF are mutually exclusive histone acetlyl transferase (HAT) subunits of two functionally distinct, but related, multi-subunit coactivator complexes, the SAGA (Spt-Ada-Gcn5-Acetyltransferase) and the ATAC (Ada-Two-A-Containing) complexes. These complexes have been shown to differentially regulate both locus specific gene expression and global chromatin structure through their enzymatic activities (HAT, and histone deubiquitination).
I will describe how these human HAT complexes are targeted to different genomic loci representing functionally distinct regulatory elements both at broadly expressed and tissue specific genes. While SAGA can principally be found at promoters, ATAC is recruited to promoters and enhancers, yet only its enhancer binding is cell-type specific. Furthermore, I will show that ATAC functions at a set of enhancers that are not bound by p300, revealing a class of enhancers not yet identified. These findings demonstrate important functional differences between SAGA and ATAC coactivator complexes at the level of the genome and define a role for the ATAC HAT complex in the regulation of a set of enhancers.
Moreover, the role and the requirement of five different HAT complexes will be discussed in pluripotent embryonic stem cell and during differentiation to neuronal cells.

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Mutations altering epigenetic regulation in acute myeloid leukemia: isocitrate dehydrogenase 1 and 2

Mutations altering epigenetic regulation in acute myeloid leukemia: isocitrate dehydrogenase 1 and 2

In the molecular background of acute myeloid leukaemia (AML) novel recurrent somatic mutations have been recently identified influencing the chromatin regulation and the methylation state of haematopoietic progenitors. One type of these mutations alter isocitrate dehydrogenase 1 and 2 (IDH1 and 2) genes. The gain of function mutations result in the production of 2-hydroxy-glutarate (2HG) with consequent competitive inhibition of 2-oxoglutarate (2OG) dependent enzymes, causing global DNA hypermethylation and changes in the gene expression profile.

The aim of our study was to investigate the frequency, the clinical associations and the prognostic effect of IDH mutations in AML. 376 patients were screened by allele-specific PCR and/or high resolution melting confirmed by sequencing.

IDH1mut and IDH2mut were mutually exclusive, detected in 8.5% and 7.5% of cases respectively. IDH1/2mut associated with: older age ($p=0.001$), higher average platelet count ($p=0.001$), intermediate karyotype ($p<0.0001$), NPM1mut ($p=0.022$) and with lower mRNA expression level of ABCG2 gene ($p=0.006$). Overall survival (OS), remission and relapse rate were not different in IDH1mut or IDH2mut vs. IDHneg. Particular IDH1 and IDH2 mutations associated differently with NPM1; co-occurrence was observed in 14.3% of IDH1 R132C vs. 70% of R132H carriers ($p=0.02$) and in 47.4% of IDH2 R140Q vs. 0% R172K carriers ($p=0.02$). IDH1 R132H negatively influenced OS compared to IDHneg ($p=0.02$).

IDH mutations are frequent recurrent mutations in AML. Although a general common pathogenetic role is proposed, our results indicate that differences in clinical characteristics and treatment outcome may exist among distinct mutations of both genes.

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Circulating nucleic acids are biomarkers of colorectal diseases and TLR9dependent activators of immune and regenerative response, depending on the methylation status

Background: New whole genome sequencing techniques can analyse in detail quantitative disease related circulating free DNA(cfDNA) alterations in colorectal diseases. Toll like receptor9(TLR9) is not known to be receptor for self-DNA, only for non-methylated bacterial and viral sequences. TLR9 has increased density on immune and regenerative cells.
Aims: To identify colorectal cancer(CRC), adenoma and IBD related methylated/non-methylated cfDNA sequences in peripheral blood(PBL). Experimental investigation of a found segment in animal model and cell cultures for immun and regenerative response activation in methylated and non-methylated status.
Materials and methods: cfDNA was isolated from 5 normal, adenoma (>1cm), IBD, CRC patients. Whole genomic DNA sequencing was performed using the Solid ... sequencer. Bioinformatic analysis was performed for detection of disease specific patterns. A methylation specific PCR was used and tested for the CRC related SEPTIN9 promoter presence in PBL of 40 normal, colorectal adenoma, CRC patients after collecting 9 ml blood. 300 mg Septin9 methated/ nonmethyated sequence was applied in healthy and DSS induced animal model (mouses) by tail vein injection. PBL interleukin alterations(NFKB, TNFalfa, IL8, IL1), stem cell number(CD133) and TLR9 activation was determined in a time course. Applying methylated/nonmethylated cfDNA sequences on HT29 colorectal cel culteres in concentration of ... TLR9, MYD88,IL8,Il1 determinations were performed.
Results: Sequences from the whole human genome could be detected in cfDNA. CpG island copy numbers were found in less frequency, unless they were stabilised by methylation. The methylated septin9 promoter was found to be overpresented in adenoma and in CRC (6000x) as compared to IBD and normal cases. Septin9 was present in 95% and 50% of the CRC and adenoma patients PBL by PCR. Non-methylated CpG island proved to be immune andregeneration response activators after injection in tail veins of mouse by TLR9upregulation. Methylation changed and minimised this effect. Cultured HT29 cancer cells reacted with similar expression changes after treatment with methylated, nonmethylated artificially amplified cfDNA DNA sequences.
Conclusions: cfDNA presents local organ specific alterations in PBL by increased concentrations of organ-specific methylated CpG islands that can be used in PCR assays for screening and diagnostics. CpG island sequences from cfDNA are furthermore a physiological immune and regenerative response activator depending on the methylation status. TLR9 reacts on human non-methylated CpG DNA, as well.

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Genome evolution in animals: clues from the homeobox genes

In the 1980s, the fields of evolutionary biology and developmental biology underwent a revolution. The discovery of Hox gene clusters in very different animals paved the way for an integrated science in which the control of embryonic development could be compared between widely divergent evolutionary lineages. It is clear that early bilaterian animals possessed a Hox gene cluster involved in pattering the head-to-tail axis of the embryo; this may have been regulated by epigenetic processes that linked chromatin structure to embryonic development. Yet there are modifications – such as Hox cluster breakage, Hox cluster shrinkage, Hox cluster duplication, and Hox cluster expansion - and these differences between species pose questions for how genotype evolution relates to phenotype evolution, and how epigenetic mechanisms co-evolve with genomic change.

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Promoter-associated noncoding RNAs for the sequence-specific epigenetic alterations during mammalian development

Early mammalian embryos generally undergo DNA demethylation, which is thought to be a key to the acquisition of pluripotency. In the present study, we found that the expression of certain promoter-associated noncoding RNAs (pancRNAs) was accompanied by active demethylation of the corresponding promoter in early mouse embryos. At the Il17d locus, a pancRNA named panc-Il17d was upregulated in 2-cell-stage embryos while the corresponding Il17d promoter region became hypomethylated, in comparison with that in germ cells. These pancRNA expression/DNA demethylation processes occurred significantly later when artificial oocyte activation was triggered by SrCl2. Nonetheless, during the development of SrCl2-activated embryos, pancRNA expression was inversely correlated with DNA methylation. Knockdown of panc-Il17d by siRNA injection induced hypermethylation not only at the DNA-methyation consensus sequence CpG, but also at several non-CpG sites, indicating that panc-Il17d promotes hypomethylation of both CpG and non-CpG sites. Similar sequence-specific effects of pancRNA knockdown were observed at the promoter region of Cap2, which was normally hypermethylated at the 2-cell stage but aberrantly demethylated together with panc-Cap2 expression by artificial SrCl2. These findings provide evidence that pancRNAs with stage-dependent expression profiles have epigenetic abilities to mediate sequence-specific DNA demethylation following both normal and artificial oocyte activation. In this talk, I will also present genomic feature of pancRNA-bearing genes analyzed by deep sequencing.

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The interplay between transcription initiation codes and nucleosome positioning signals on core promoters during embryo development

The maternal to zygotic transition during early embryonic development represents the most dramatic shift in the transcription programme in vertebrate life cycle. This transition reflects the changes in composition of transcription initiation complexes between the mother and embryo and provides an ideal model to study promoter-associated sequence and epigenetic determinants of transcription start site (TSS) definition in vertebrate development. We addressed these determinants by studying the developmental dynamics of promoters in zebrafish at single nucleotide resolution by Cap Analysis of Gene Expression (CAGE). This genome wide analysis was complemented by in situ immunhistochemistry and ChIP sequencing to study the developmental regulation of H3K4me3 marked nucleosomes in zebrafish embryos. We show that the transition from maternal to zygotic transcriptome is characterised by a switch between two fundamentally different mechanisms for defining transcription initiation: a maternal specific W-box motif-dependent TSS definition is replaced with a C/G > A/T dinucleotide enrichment boundary aligned with +1 nucleosome prepositioned prior to transcription. The H3K4me3 occupied +1 nucleotide positioning is supported by intra- and inter-nucleosomal sequence preference signals associated with zygotic but not maternal TSS. Thus nucleosome positioning and initiation of H3K4me3 occupancy preceed and are broadly independent of activation of zygotic transcription and suggest instructive roles in TSS determination during early embryo development.

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PRMT1 produced arginine methylation and PRMT8 provide selective and coordinated retinoic acid-driven gene expression during the differentiation of mouse embryonic stem cells

Protein arginine methyltransferases (PRMT) have been shown to have important roles in transcriptional regulation. Previous studies have demonstrated that they act as co-regulators in nuclear receptor signaling. Here, we report that a novel mechanism integrates arginine methylation and retinoid signaling in the context of embryonic stem cell development. Embryonic stem cells differentiate into neurons upon retinoic acid treatment. This process is governed by retinoic acid receptors (RAR). PRMT1 is present and active in the early phase of this differentiation process. Our genome-wide comparison of wild-type and PRMT1 knockdown embryonic stem cells revealed a selectively altered retinoid responsiveness, primarily affecting the Hoxa and Hoxb clusters. Therefore, arginine methylation by PRMT1 may serve as a fine tuning regulatory mechanism for retinoic acid induced, Hox-gene mediated patterning. In addition we also found that PRMT8 induction is part of the retinoic acid induced gene expression program. Interestingly, this protein acts as a more general regulator of retinoic acid responsiveness in embryonic stem cells as loss of PRMT8 results a decreased retinoid responsiveness. These data reveal an integrated, cooperative interaction between PRMT 1 and 8 mediated arginine methylation and retinoid signaling in regulating selective gene expression, differentiation and developmental patterning.

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Blocking effect of 5-(hydroxy)methylcytosine on Taq DNA polymerase

Recently, Huang et al. reported that the bisulfite modified version of 5-hydroxymethylcytosine, cytosine-methylene-sulfonate hinders the amplification by Taq polymerase in an in vitro system using synthetic oligonucleotides. This stalling effect is particularly pronounced when two modified nucleotides are adjacent to one other or separated by a single nucleotide (e.g., CGC sequence). We observed similar stalling effects using plasmids as substrates even before bisulfite conversion.

Methylated and hydroxy-methylated cytosine sequences were introduced to a plasmid by cloning (hydroxy)methylated CMV region to pEGFP-1. The CMV–EGFP border region was tested where only one strand contained (hydroxy)methyl-C in a CGCG sequence. For comparison, SssI or HpaII methylated IL-33 promoter sequence was used, which was cloned into pCpGL, a CpG-less luciferase vector. Here the closest CG sequences were 10-15 bp apart. Purified plasmid samples were tested on LightCycler 480 System (Roche) using SYBR Green I master mix. Primers were designed for unmethylated regions around the CG sequences, a control PCR amplicon was generated at the EGFP or luciferase region which did not contain modified cytosine sequences. For quantification the delta-delta Ct method was used with the unmethylated sample serving as comparison.

The amplification efficiency of the methylated and hydroxymethylated CMV–EGFP plasmids was 2 orders of magnitude lower than the unmethylated plasmid, whereas in the IL-33-pCpGL construct no difference was observed between the methylated and unmethylated plasmids. We propose that dense CpG (hydroxy)methylation at specific genetic regions can be utilized in assessing methylation level by quantitative PCR method where the neighboring modified C sequences hinder Taq polymerase.

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How many is enough?
CpG dinucleotide methylation percentage estimation: models and accuracy applied to different techniques

DNA methylation plays a crucial role in gene silencing. In cancers the methylation level of specific genes have prognostic or even therapeutic values. The most generally used techniques to study DNA methylation are bisulfite treatment, coupled with either Polymerase Chain Reaction (PCR) and targeted sequencing (BSG) or Next Generation Sequencing (NGS) (Bis-seq).
As DNA methylation is variable within a cell population, several different sequences have to be analyzed to estimate the methylation level of a CpG dinucleotide. But how many sequences are enough? This depends on the accuracy needed. In general, a ±15% accuracy seems enough to have a good picture of the methylation profile. Several algorithms, based on binomial distribution, can be used to calculate this. They allow defining a confidence interval, around the measured methylation level.
We determined the amount of sequences to be analyzed, corresponding to the needed accuracy. Wilson's formula, a widely used equation based on normal distribution, needs at least 30 sequences to be analyzed, leading to ±17% accuracy. When using Bis-seq technique, a similar 30-fold coverage of each CpG dinucleotide is needed to determine the methylation level, requiring a 40-fold global genome coverage.

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DETECTION OF LOCALLY METHYLATED SEPTIN 9 OF COLORECTAL CANCER IN PERIPHERAL BLOOD FROM HEALTHY, ADENOMA AND COLON CANCER PATIENTS

INTRODUCTION: Colorectal cancer (CRC)is one of the most frequent causes of cancer-related death worldwide. Methylated Septin 9 (SEPT9) is a sensitivive and specific biomarker for colorectal cancer (CRC) from peripheral blood. However its detection from adenoma and tissue samples rarely studied.
AIMS&METHODS: 1) Compare SEPT9 methylation from matched plasma and tissue samples. 2) SEPT9 mRNA expression analysis in laser microdisseted samples. 3) Evaluation SEPT9 detection in health, adenoma and cancer samples. Plasma samples were collected from patients with no evidence of disease (NED), adenomas and CRC (n = 40 per class). Matching biopsy tissues were available from 33 NED, 30 adenoma and 36 CRC patients. Plasma samples were processed using the Epi proColon kit (Epigenomics AG, Berlin, Germany). Total DNA from tissue was prepared using QIAamp DNA extraction and High Pure PCR Template Preparation Kit followed by Epi proColon bisulfite treatment. Quantitative determination of total DNA and mSEPT9 was performed using the Epi proColon RT-PCR assay. SEPT9 was detected from double amount of plasma. For LMD mRNA expression microarray surgical samples were used from 6 histologically normal, 6 adenoma and 6 CRC tissues. Gene expression profile was evaluated using HGU133Plus2.0 microarrays.
RESULTS: SEPT9 PMR (percent methylation reference) values larger than 1% were detected in 27% (9/33) NED, 100% (30/30) adenoma and 97% (33/34) CRC biopsy tissues. In laser microdissected epithelial cells, septin 9 significantly underexpressed in CRC compared to healthy controls ($p<0,001$), but not in stroma samples. In plasma from the same patients, however, mSEPT9 PMR values larger than 0.01% were detected in 5% (2/40) NED, 25% (10/40) adenoma and 87,5% (35/40) CRC. SEPT9 methalylation was detected 50% (3/6) from double plasma amount of adenoma samples.
CONCLUSION: Healthy and cancer plasma and tissue samples showed strong correlation, however it was not maintain for adenoma samples. Laser microdissected mRNA assay showed an inverse correlation in epithelium samples. SEPT9 was confirmed as a highly sensitive biomarker for the detection of CRC in blood, but weak of adenoma cases. Sensitivity for adenoma samples was higher in case of evaluation of double amount plasma.

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CHARACTERISTIC DNA METHYLATION PATTERN IDENTIFIES SPORADIC COLORECTAL CANCER AND ITS PRECURSOR LESIONS

INTRODUCTION: BACKGROUND: Aberrant DNA hypermethylation has been described to play an important role in colorectal carcinogenesis, particularly in the proximal colon, where a distinct subtype (CpG island methylator phenotype, CIMP) was identified. Most studies addressing DNA methylation have so far focused on the analysis of few, rather than multiple genes.
AIMS&METHODS: Our primary aim was to study DNA methylation in 96 genes in different settings related to colorectal carcinogenesis, including lesions of adenoma-dysplasia-carcinoma sequence (ADCS) and field carcinogenesis. Our further aim was to analyse the effect of ageing and ulcerative colitis (UC) on DNA methylation. A total of 67 endoscopically removed colonic biopsy samples (10 healthy adult, 5 healthy children, 12 low-grade dysplasia (LGD), 10 high-grade dysplasia (HGD), 13 CRC (UICC stage I-III), 5 metastatic (UICC stage IV) CRC (MCRC), 4 normal adjacent tissue (NAT) and 8 ulcerative colitis (UC) were analysed for their DNA methylation status. DNA methylation percentages of 96 genes were determined using Methyl-Profiler PCR array system. 3 genes of interest were validated with bisulphite-PCR followed by high-resolution melting curve analysis. Immunohistochemistry for MLH1, MSH2 and MSH6 was performed on the samples to define their microsatellite status.
RESULTS: All samples but one (that derived from the proximal colon, showing CIMP-H and used as reference) were microsatellite stable (MSS). 8 genes were constantly hypermethylated in all 67 samples. DNA methylation levels of 6 genes, including that of ALDH1A3 (a novel finding in CRC) have shown highest levels in LGD, then gradually decreased along ADCS, being the lowest in MCRC. This panel significantly distinguished LGD, HGD and CRC from UC and healthy tissue ($p<0.05$). Compared to CIMP-H, distal CRC and its precursors showed fewer methylated genes (65 vs 30±5, $p<0.05$). Comparing methylation profiles between CRC and NAT revealed that hypermethylation occurred only in cancer but not in its field. No UC- and age-related methylated gene was identified.
CONCLUSION: Sporadic, distal, MSS CRC shows a decreased level of DNA methylation as compared to CIMP-H, but has a characteristic methylation pattern that can contribute to colorectal carcinogenesis. Detection of the identified genes could facilitate earlier detection of CRC, whereas decreasing levels of DNA methylation towards MCRC might indicate prognostic relevance of this epigenetic alteration in CRC.

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Expression of ABCB1 transporters is regulated by several different mechanisms in drug resistant rat hepatoma cells

MDR1 (Abcb1) is an energy-dependent transporter that is able to extrude cytotoxic agents from the cell. In the presence of these drugs MDR1 expression is up-regulated by different mechanisms, though the molecular background of increased MDR expression is mostly unknown. Recent studies suggested that epigenetic modifications might play an important role in this process.
The aim of our study was to reveal epigenetic modifications responsible for the increased MDR1 level in multidrug resistant cell lines.
The cell lines we used in our experiments were a drug sensitive parental rat hepatoma cell line (D12), a medium (col500) and a highly (col1000) drug-resistant variant of it.
Rodents have two MDR1 isoforms: Abcb1a and Abcb1b. First, we determined the expression of these genes and found that the mRNA levels of both Abcb1a and Abcb1b were increased in the drug resistant cell lines compared to the parental D12.
Next we treated the cells with histone deacetylase inhibitors (HDACi) to maintain the acetylated state of histones. Surprisingly, Abcb1a and Abcb1b genes responded to the treatment in an opposite way: the expression of Abcb1a was decreased, while the expression of Abcb1b was increased in cells treated with HDACi. After the treatment, H3K9 and H3K14 acetylation increased in all tested regions of both genes, contrary that, their expression changed in opposite directions.
In conclusion, our data suggest that elevated Abcb1 gene expression is not always coupled to histone acetylation changes and conversely, the H3K9 and H3K14 acetylation levels do not necessarily predict the expression level of the Abcb1 genes.

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Characterization of epigenetic changes, gene expression pattern and protease production in tumor-associated myofibroblast

The tumor microenvironment is an important factor in cancer development and progression. In the stroma of various epithelial tumors the predominant cell types are myofibroblasts differentiated from various progenitors. Compared to normal tissue, the number of myofibroblasts is increased in the tumor stroma and the shape and distribution of the cells are altered as well. Epigenetic regulation has been implicated in myofibroblast differentiation accompanied by activation of gene expression. However, direct analysis of DNA-methylation or histone modification has not been systematically undertaken.
In order to compare epigenetic characteristics and gene expression pattern of tumor-associated and normal myofibroblasts, we used primary myofibroblast cultures obtained from the gastrointestinal tumor stroma or from healthy tissues near the tumor margins. We studied the level of histone acetylation, methylation and DNA methylation as well. Results have indicated that no significant changes could be observed in histone modifications at global level in tumor-associated myofibroblasts, but DNA methylation might play more important role in regulation of tumor-associated myofibroblast. To characterize the differences in the gene expression profile of tumor-associated versus control myofibroblast partial transcriptome analysis was performed. Based on the results, we concluded that the TGF-ß signaling pathway is altered, less extracellular matrix (ECM) components are produced, more ECM modifying enzymes (matrix metalloproteinases, MMPs) are expressed in tumor-associated myofibroblasts. We have also performed substrate zymographies to detect the activity of MMP-1, 2, 3 and 9. The results indicated strong MMP activities in the samples, and the tumor-associated myofibroblasts secreted more MMP-2 enzymes to the extracellular space.

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ABCC6 expression is regulated by a primate-specific sequence located in the first intron of the gene

Pseudoxanthoma elasticum (PXE), a rare recessive genetic disease causing skin, eye and cardiovascular lesions is characterized by the calcification of elastic fibers. The disorder is due to loss-of-function mutations of the ABCC6 gene but the pathophysiology of the disease is still not understood. We investigated the transcriptional regulation of the gene, using DNase I hypersensitivity assay followed by luciferase reporter gene assays and chromatin immunoprecipitation (ChIP). We identified DNase I hypersensitive sites (HS) specific to cell lines expressing ABCC6. These sites are located in the proximal promoter and in the first intron of the gene. We observed Hepatocyte Nuclear Factor 4α (HNF4α) and CCAAT/Enhancer binding protein ß (C/EBPß) binding to the proximal promoter and the primate-specific second intronic HS, respectively. We also showed that C/EBPß interacts with the proximal promoter of the gene and propose that it forms a complex with other regulatory proteins including the previously identified regulatory factor HNF4α. Our data indicate that this complex, which would account for the tissue-specific expression of the gene, is under the negative control of ERK1/2 kinases. ChIP using anti-HNF4α antibodies show a rapid loss of HNF4α binding on the ABCC6 proximal promoter upon treatment with an ERK1/2 activator. Likewise, acetylated forms of histone H3 (Lys 9 and Lys 27) are found enriched in the ABCC6 proximal promoter, this acetylation being lost after stimulation of ERK1/2 activity. Our results point toward a better understanding of the transcriptional regulation of ABCC6.

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5-HYDROXYMETHYLCITOSINE DETECTION BY MASS SPECTROSCOPY AND IMMUNOFLUORESCENCE IN DIFFERENT GENOMIC CONTEXTS

5-hydroxymethylcytosine (5-hmC) is a recently discovered epigenetic modification, which appears to have an important role during mammalian development, especially within the nervous system. Produced by oxidation of 5-methylcytosine (5-mC) by the TET family of enzymes, it has been suggested to participate in the process of DNA demethylation. DNA demethylation erases epigenetic marks from the DNA, allowing gene expression reprogramming and remodelling of the chromatin structure. Detection techniques for 5-hmC are quickly developing and there is an increasing body of evidence indicating its biological significance in different organisms at different stages of development.
In our lab, we have set up two different detection systems for 5-hmC. On one side, the mass spectrometry (LC-MS/MS) has allowed us to determine total levels of 5-mC and 5-hmC in different cell types under various conditions, compared to adequate standards. This led us to determine the rapid turnover of DNA methylation in human cells. On the other side, immunofluorescent detection of both molecules on fixed cells shows their spatial arrangement on the intact nuclei and chromosomes. These two complementary approaches have provided us information on 5-hmC status of chicken DT-40 cells, human lymphoblast and HepG2 hepatoblastoma cells under various stress conditions.

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Trans-acting siRNA producing genes in plants evolve from protein coding genes

There is a diverse set of small non-coding regulatory RNAs in plants. MicroRNAs (miRNAs) and trans-acting siRNAs (ta-siRNAs) regulate gene expression by recognising and cleaving specific mRNAs. While plant miRNAs are generally accepted to derive from protein coding genes through inverted duplication and subsequent deletions, currently there is no proposed model for ta-siRNA evolution. Here we identified eight ta-siRNA producing TAS loci in tomato and found that two of them were similar to the known Arabidopsis TAS3 and TAS4 loci. We also show that the regulation and function of tomato TAS4 is similar to the Arabidopsis TAS4. In addition we identified six TAS loci that are related to NB-LRR genes and produce ta-siRNAs from the conserved P-loop region. These are down-regulated during pathogen infection suggesting that a large number of resistance genes are potentially regulated by ta-siRNAs. Finally, we propose that TAS genes are also evolved from protein coding genes and provide examples for various stages of evolution.

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MicroRNA expression patterns and microRNA-mediated molecular pathways in adrenal tumors

Introduction: MicroRNAs are major epigenetic factors in the posttranscriptional regulation of gene expression. Altered microRNA expression patterns were noted in tumors, and microRNAs can be exploited as biomarkers of malignancy or prognosis, and might even represent therapeutic targets. Our research group was among the first to study microRNA expression in adrenocortical and adrenomedullary tumors.
Methods: Total RNA was isolated from adrenocortical and pheochromocytoma samples. Taqman TLDA cards and Agilent 8x1.5K arrays were used for microRNA profiling of adrenocortical tumors and pheochromocytomas, respectively. mRNA expression profiling was performed in adrenocortical tumors on Agilent 4x44K arrays. GeneSpring, Gene Set Enrichment and Ingenuity Pathway Analysis were used for bioinformatics analysis.
Results: Significant differences in microRNA expression were noted in different groups of adrenocortical and adrenomedullary tumors. The expressional difference of miR-503 and miR-511 was the best marker of malignancy in adrenocortical tumors. By using parallel gene expression profiling, we have developed a novel tissue specific microRNA target prediction algorithm. Damage of cell cycle G2-M checkpoint turned out to be the major microRNA-affected pathway. Similar results were obtained in our meta-analysis on adrenocortical tumor genomics data. The overexpression of miR-1225-3p was noted in recurring pheochromocytomas. Pathway analysis revealed the possible involvement of Notch-signaling in pheochromocytoma recurrence.
Conclusions: Significant differences in microRNA expression of adrenocortical and adrenomedullary tumors were found. Since the histological diagnosis of adrenocortical tumors is difficult and there have been no markers of pheochromocytoma recurrence before, these findings may have diagnostic relevance. The identified pathways might even represent potentially druggable molecular targets.

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Why twins are different?

The investigation of twins have provided an opportunity to determine role of genetic and environmental factors in case of given phenotypic trait. The monozygotic (MZ) twins who share the same genotype, may be observed significant phenotypic differences, such as differences in susceptibilities to disease. Beside environmental factors the epigenetic variation can contribute to different phenotype in monozygotic twin pairs. In Hungary during the period 1920-2010 the multiple births was ~2.37% (range 1.82-3.75%) of total births, this means that ~2% of hungarian population is multiple births, that is ~200 000 multiple births. In Hungary the cancer is the second leading cause of death, ~67 000 cases of cancer are recognized annually. As ~2% of population is multiple birth, this means that among 67 000 persons with cancer may be found ~1 340 multiple birth. Our aim is to find such monozygotic twin pairs with cancer, who has differences in occurence of cancer (one of MZ twin pair is cancerous and other is healthy). We plan to examine the role of epigenetic modifications in the given cancer types.

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MicroRNAs: biogenesis, regulation of gene expression in carcinogenesis, clinical applications

MicroRNAs (miRNAs) are a class of non-coding RNAs that regulate gene expression at posttranscriptional level. The 19-24nt mature form is the result of a processing progress consisting of several sequential steps in both the cell nucleus and the cytoplasm.
Emerging amount of evidence confirms the role of miRNAs in carcinogenesis. Among their target genes, numerous regulators of cell cycle, cell proliferation, DNA repair and apoptosis can be found, therefore miRNAs are implicated in the initiation and progression of cancer, in tissue invasion and in metastasis formation as well. They can act as oncogenes (oncomiRs) and tumorsuppressors (anti-oncomiRs) by targeting tumorsuppressor genes and oncogenes, respectively. MiRNA profiles supply information about the origin and differentiation state of tumours, moreover, their altered levels often can be detected in premalignant conditions.
MiRNAs have key role in fine-tuning diverse cell functions. Many of them are involved in the regulation of haemopoiesis. Altered miRNA expression levels were observed in different types of leukemia, but their exact role in the pathogenesis has not been elucidated. However, there are successful attempts to apply miRNAs in the diagnosis and prognosis of acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Measurement of the expression levels may help clinicians to choose the most suitable therapy, or even to predict the success of administering different kinds of chemotherapeutic drugs. MiRNAs are regarded as promising therapeutic targets: antitumour effect can be achieved by increasing or decreasing the level of them, which can also contribute to improved results of other therapeutic strategies.

András Szántó, Éva Hegedűs, Alain Nicolas, Gábor Szabó and Lóránt Székvölgyi

Genome-wide mapping single- and double strand DNA breaks with chromatin immunoprecipitation on Chip

Chromosomal DNA is a highly fragile structure, in which single- or double strand breaks may develop during the cell cycle. These lesions may result from physiological events, such as isotype switching of the immunoglobulin genes or meiotic homologous recombination, but they might as well be caused by a variety of pathological, (pre)carcinogenic processes. Based on the above it is evident that the accurate repair of DNA strand breaks is crucial to cell viability and the efficient mapping of vulnerable genomic regions can be of clinical significance.

In the work presented herein we have developed two chromatin immunoprecipitation-based assays called "Nick ChIP" and "RPA ChIP", with which the presence and localization of single- and double strand DNA breaks can be detected throughout the whole genome. For the inspection of single-strand breaks, we have used „Nick-ChIP" capturing nicks by limited in situ nick translation. With the aid of this method, we incorporated biotin-dUTP molecules at the discontinuities to detect them by anti-biotin antibodies. „RPA-ChIP", used to trace double strand breaks, specifically detects the Rfa1 subunit of replication protein A (RPA) with anti-Rfa1 antibodies. RPA covers resected DSB ends being processed by MRX (Mre11/Rad50/Xrs2) complex. In the final step we have hybridized the Nick/RPA ChIP samples to whole genomic microarrays (ChIP-Chip), which enabled us to precisely localize the DNA discontinuities along the chromosomes.

Our method was developed in S. cerevisiae due to its relatively small genome (12 Mbp) that can be covered by a single microarray, but in the long run we would like to adapt this technique to human cells and clinical samples: precise mapping of DNA breaks can help us achieve a deeper understanding of the processes leading to cancer formation, making Nick/RPA-ChIP an important part of the future's cancer diagnostics.

László Imre, György Fenyőfalvi, Zoltán Simándi, László Nagy and Gábor Szabó

Nucleosome-DNA cohesion is highly sensitive to certain H3 modifications and to superhelical twist.

We have developed a sensitive and high throughput method, applying a laser scanning cytometer (LSC), for the analysis of histone mobility features. Our method offers sensitive means to determine, quantitatively and in a cell-cycle phase specific manner, a major component of global histone mobility: nucleosome-DNA cohesion. After salt elution of agarose-embedded isolated nuclei the remaining histone levels are determined by immunofluorescence labeling, using modification-specific antibodies. H3K4me3 modified histones were eluted from isolated nuclei at much lower salt concentration than H3K27me3 modified histones, in various cell types, including mouse embryonic stem (ES) cells and their differentiated counterparts (NPCs). Nucleosome-DNA cohesion appears to be highly sensitive also to superhelical twist. Nucleosomes containing these two kinds of modifications could also be distinguished based on a differential sensitivity to intercalators. The effect of these agents on superhelicity related features of chromatin loops were analysed simultaneously, using a highly sensitized, LSC-based nuclear halo assay.

Ildiko Stier and Antal Kiss

Cytosine-to-uracil deamination by SssI DNA methyltransferase

A few pr2aryotic DNA(cytosine-5]methyltransferases [C5-MTase] catalyze C to U deamination of the target cytosine in the absence of the methyl donor S-adenosyl-methionine {SAM) [e. g. M.Hpall, Cell 1992, 71: 1073-1080). The reaction appears to be dependent on transient covalent bond formation between the active site cysteine thiol and carbon 6 of the cytosine leading to a 5,6-dihydrocytosine intermediate with an enhanced tendency for deamination. It remains to be determined how general this feature is for C5-MTases. One of these enzymes (M.SssI) shares the specificity [CG] of eukaryotic DNA MTases, and thus has special importance as an experimental tool in the study of eukaryotic DNA methylation. The possibility to use M.SssI as a CG-specific cytosine deaminase would greatly increase the value of this enzyme in epigenetics research. There are conflicting results in the literature with regard to the ability of M.SssI to deaminate cytosine, therefore we started to re-investigate this phenomenon for M.SssI using a sensitive genetic reversion assay in which a C to U transition in a mutant kanamycine resistance gene restores kanamycine resistant phenotype.

We found that incubation with M.SssI in vitro in the absence of SAM led to a 3-fold increase of cytosine deamination over the spontaneous rate. The SAM analog 5'-aminoadenosine increased M.SssI-catalyzed deamination 100-fold. A mutant enzyme M.SssI(G19D) presumably impaired in SAM binding exhibited high cytosine-deamination activity in vivo in the presence of SAM, acting as a mutator enyme. Experiments aimed to determine whether M.SssI can deaminate m5C to T are in progress.

György Purebl MD, PhD

How stress gets under the skin?
Epigenetic mechanism in the background of psychosocial risk factors

Psychosocial factors carry significant health risk in many frequent public health concerns, and increase the overall risk of morbidity and mortality. Whilst numerous studies suggest some hints about the possible mechanisms linking psychosocial events and health/disease, yet science lacks a more comprehensive, general model that could incorporate all aspects of the interactions between these disorders and their risk factors.
Epigenetic models are promising candidates for the setup such models and could efficiently contribute to our knowledge of these interactions, suggesting some preventive measures at the same time. The growing number of epigenetic studies emerge broader perspectives: not focusing only recent life events, acute and chronic stress, but also highlighting early life and prenatal effects. The presentation summarizes the most important studies on the field.

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Generation of molecular hybrids to determine the complex specificity of two adaptor proteins in Drosophila melanogaster

In the majority of the eukaryotic cells the Gcn5 (general control nonderepressed 5) represents the catalytic component of several histone acetyltransferase complexes (HATs) that also share ADA2 (alteration/deficiency in activation 2) adaptor proteins. In Drosophila melanogaster structurally related but functionally distinct ADA2 proteins are complex specific subunits of two GCN5 (general control nonderepressed 5) containing HAT complexes. dADA2b is present in the SAGA complex, which targets histone H3 K9, and K14 for acetylation. dADA2a is a component of the ATAC complex, which acetylates lysine K5, K12 and K16 of histone H4. Both dADA2a and dADA2b are essential for the HAT catalytic activity of their cognate complex. Using domain swap chimeras we examined which part of the Drosophila ADA2 proteins determine their specific incorporation into SAGA or ATAC complexes. We tested hybrid ADA2 protein functions in vivo, by determining the phenotypic rescue and histone modifying abilities of HAT complexes formed with ADA2a/ADA2b chimeras in Ada2a and Ada2b mutant background. We found that hybrid ADA2 proteins were capable of partial phenotypic rescue and restoration of lost acetylation of one or the other Ada2 mutant. Significantly, the functional characteristics of hybrid ADA2 containing HAT complexes depended on the C-terminal region of ADA2 subunit they contained. Our finding demonstrate that the ADA2 C-terminal regions play important role in the specific incorporation of ADA2 into SAGA or ATAC type complexes, what in turn determines H3 or H4 specific histone targeting.

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Mapping the STAT6 cistrome in human primary macrophages

Primary human macrophages play critical roles in the host defense and exert sometimes opposing roles. This plasticity is brought about by the cyt2ine milieu, which induce their differentiation into multi-faceted cell populations with divergent functions that the host at the given "situation" requires. For example, classically activated macrophages are pro-inflammatory, while alternatively activated macrophages that differentiate upon encountering IL-4 play anti-inflammatory roles. Cyt2ines induce transcription factors that regulate gene expression to shape the cells' function. Interleukin (IL) -4 activates Signal Transducer and Activator of Transcription (STAT) 6, a transcription factor known to be responsible for Th2 cell differentiation. On the other hand, it is also known that STAT6 regulates alternative activation markers in mouse macrophages and serves as a facilitator of the Peroxisome Proliferator Activated Receptor (PPAR) gamma mediated gene expression changes in alternatively activated macrophages.
In order to understand how transcription factors shape cell specificity it is important to learn which genes they regulate in a specific subset of cells. For this purpose, we performed chromatin immunoprecipitation with STAT6 specific antibodies and sequenced the enriched DNA from primary human macrophages that were left untreated or were treated with Rosiglitazone and IL-4. We used the known promoter element within the SOCS1 promoter to validate the ChIP assays and performed various bioinformatics analysis on the resulting pool of STAT6 binding sites.
At the end of these studies we hope to better understand how STAT6 contributes to shaping the specific functions of alternatively activated human macrophages.

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New diagnostic possibilities: epigenetic alteration of cfDNA in colorectal cancer patients

INTRODUCTION: DNA methylation is well known epigenetic regulation of genes functions and its alteration can be observed during cancer development.
AIMS&METHODS: Our aim was to detect methylation pattern changes of cfDNA isolated from peripheral blood of 50-50 patients from healthy, IBD, colorectal adenoma and carcinoma group. We investigated cfDNA of plasma samples was isolated with QIAamp Circulating Nucleic Acid Kit (Qiagen). From each sample 50-100 ng extracted DNA was assembled in each groups and pooled DNA was applicated for further sequencial analysis (SOLiD sequencing). After matching native plasma samples to the reference genome, coverage and pile up diagrams were defined.
RESULTS: Region between 92075 and 92115 derived from SEPT9 gene has shown 6200 times higher number of reads in CRC samples in 50-50% from reverse and forward direction to ensure that is not artifact. This position is exactly matched with region detected by EpiProColon Kit (Epigenomics), a method based on the enrichment of methylated fragments. It is already proven that methylated SEPT9 (mSEPT9) is present 94,7% in CRC patients. The coverage of mSEPT9 was 245 times higher in adenoma and only 8 times higher in healthy patients plasma sample. SFRP1, SFRP2, MAL and PRIMA1 genes proceeds the same result.
CONCLUSION: Our previous study investigated the kinetics of methylated DNA elimination, and showed difference from non-methylated fragments, which may result the enrichment of methylated regions in plasma. The detection of these tumour characteristic epigenetic changes from peripheral blood can be important step to develop screening and diagnostic assays.

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Analysis of circulating free DNA release and methylation dependent degradation in mouse colorectal cancer xenograft model

INTRODUCTION: Circulating free DNA (cfDNA) was first detected in human plasma in the 1940s. In many cancer patients increased cfDNA concentration was measured compared to healthy control.
AIMS: Our primary aim was to investigate the rate of cfDNA's release in SHO mouse/HT-29 colorectal adenocarcinoma cell line xenograft model. Our further aim was to define the decay of in vitro methylated and non-methylated DNA fragments in C57BL/6 bloodstream.
METHODS: SHO mice were xenografted with human HT-29 cells, than blood samples were collected over 2 months. After separating plasma fraction, DNA was isolated, and then quantified by real-time PCR with highly specific genomic and mitochondrial human and mouse primer sets. This method permitted to define the ratio of human/mouse DNA. To assess the degradation rate of cfDNA, 3000 bp sized methylated and non-methylated DNA fragments were injected into healthy and C38 tumour cells vaccinated C57BL/6 mouse's bloodstream. The decay of amplicons was measured with 19 PCR assays.
RESULTS: The amount of human DNA showed continuous growth from the third week, and reached 18.26% for the 8th week. Moreover, it was found that in healthy animals the non-methylated DNA disappears from the plasma after 6 hours, while the methylated fragment was detectable after 24 hours. In animals with tumour both amplicons were detectable after 24 hours.
CONCLUSION: Methylated DNA showed longer presence in plasma, and in the malignant samples both methylated and non-methylated fragments could be characterized with slower degradation compared to healthy status.

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Dissimilar methylation influence on somatostatin (SST) production in juvenile and malignant cell proliferation in colonic mucosa

Background: Molecular background of controlled and uncontrolled cell proliferation in colonic mucosa is unknown. SST production is unexamined in colonic mucosa during ageing and colorectal carcinogenesis which has anti-proliferative and pro-apoptotic effects.
Aims: Our aims were the determination and comparison of mitotic index (MI) in healthy colonic samples from children, adults and colorectal cancers (CRCs), the analysis of SST expression on mRNA and protein levels, and the examination of methylation profile of SST gene in these sample groups.
Materials and methods: Proliferation was detected by Ki-67 immunohistochemistry and SST producing cells with polyclonal antibody on colonic biopsy from healthy children (n1=14; n1=14), adults (n2=10; n2=20) and CRCs (n3=10; n3=23). MI and ratio of SST producing cells were determined in colonic mucosa. Colonic samples were collected for the analysis of SST gene expression (n1=6; n2=41; n3=34), using HGU133plus2.0 microarrays; results were validated on dependent and independent samples (n1=6; n2=6; n3=6) with real-time PCR. DNA methylation percentage was defined using methylation-sensitive restriction enzyme digestion followed by fluorescence real-time PCR (n1=5; n2=5; n3=10).
Results: MI were significantly higher in children colonic samples (0,34±0,07) and CRC samples (0,42±0,11) compared to healthy adults (0,15±0,06) ($p<0,05$). Ratio of SST producing cells was significantly higher in children (0,70%±0,79%) compared to CRCs (0%±0%) (<0,05). mRNA expression of SST did not alter during ageing in healthy mucosa, but decreased during carcinogenesis ($p\leq0,05$). SST showed significantly higher methylation level in tumor samples (27,3%±14,3%) compared healthy young individuals (3,5%±1,9%) ($p<0,05$).
Conclusions: Colonic samples from children and CRC can be characterized with increased proliferative activity compared to healthy colonic samples from adults, although it is a well controlled process in childhood contrary to CRC. Local SST production decrease during colorectal carcinogenesis and it can contribute to the unregulated cell proliferation in CRC. Possible cause of the reduced SST expression in CRC can be the increased DNA methylation in promoter region.

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Human DNA from HT-29 cells can activate TLR9 depending on the methylation status

Background: Toll-like receptor 9 (TLR9) can be activated by frequently expressed motifs, by (unmethylated) cytosin-guanine dinucleotides frequently expressed in bacteria, fungi, pr2aryotes and viruses), immunoglobulin-DNA complexes and synthetic oligodeoxynucleotide (ODN) sequences. There are no published data whether the human DNA can also be recognised by Toll-like receptor 9 (TLR9).
Aims: To prove that human DNA isolated from a human cancer cell line can activate TLR9.
Materials and methods: Genomic DNA for the treatment was isolated from 6x107 HT29 cells. Subsequently, 1x106 HT29 cells were treated with 15µg of DNA in 2 ml RPMI 1640 without BSA. After 6 hours cells were harvested and total RNA was isolated both before and after DNA treatment using Qiagen RNeasy Mini Kit. Expression levels of genes on TLR9 pathway were examined by quantitative RT-PCR. From the treated (by unmethylated, non-fragmented DNA) and untreated samples immunocytochemistry was performed DNA methyltransferases (DNMT1, DNMT3a, DNMT3b), proliferation and differentiation factors (CDX2, CK).
Results: mTLR9 RP11 showed overexpression after treatment by unmethylated, non-fragmented DNA (average dct of control: 27.80, StD: 0.3702; average dct of treated sample: 26.76, StD: 0.4002; p=0.0298) and unmethylated, fragmented DNA (average dct of control: 27.29, StD: 0.1838; average dct of treated sample: 26.19, StD: 0.1850; p=0.0072). Altered mRNA expression was found at key adaptor molecules in TLR9 pathway. mTRAF6 and mMYD88A showed overexpression at unmethylated, fragmented and underexpression at unmethylated non-fragmented samples (mTRAF6 p=0.0106; p=0.0060), (mMYD88A p=0.0304; p=0.0033). Methylated, non-fragmented DNA treatment resulted no effect on TLR9 pathway, but overexpression of NFkB (p=0.0010) and mIL8 (p=0.0002). Immunocytochemistry showed overexpression at DNMT3a and CK in sample treated by unmethylated non-fragmented DNA.
Conclusion: TLR9 pathway downstream analysis showed that TLR9 can be activated by human DNA containing unmethylated CpG sequences.

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UVB and inhibition of histone de-acetylation result in superinduction of MMP genes in human keratinocytes

UVB radiation is an important environmental agent that affects cutaneous carcinogenesis, photoaging, inflammation and immune suppression. Like other cellular stressors, UVB influences the transcription of many genes through the activation of distinct signaling pathways which in turn determine the cellular response for the damage. UV radiation also causes global changes in histone posttranscriptional modifications and as well in gene-specific histone acetylation. Here we report that genes encoding ATF3 and COX2 are upregulated in UVB response of HKerE6SFM human keratinocyte cell line. Histone deacetylase inhibitor trichostatin A (TSA) influenced the UVB dependent activation of ATF3 and COX2 and caused superinduction of these genes. An analysis of the alterations in the global transcriptome of HKerE6SFM cell upon UVB and TSA revealed the induction of members of the MMP gene cluster – MMP13, MMP12, MMP3, MMP1 and MMP10 – at 11q22.3 region. Surprisingly, the expression of MMP9 was not altered significantly. Results of chromatin immunoprecipitation indicated that polymerase II enrichment at the initiation regions of genes of the MMP cluster paralleled the expression pattern of the genes. Curiously, the Pol II density was also high at the MMP9 promoter in UVB treated keratinocytes. The level of acetylated histone H3K9 was also elevated upon UVB irradiation except at the MMP10 and MMP9 promoters in correlation with the mild and no-significant alteration in the expression level of these genes, respectively. These findings suggest that acetylation of histoneH3 at lysine 9 plays important role in the UVB response of MMP genes in the 11q22.3 region.

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PRIMA1 is hypermethylated in colon cancer

Changes in promoter methylation of certain genes are common features of malignant transformation that may accompany the silencing of tumor suppressor genes or re-expression of oncogenes. Colon cancer is one of the most frequent tumors world wide. The early diagnosis is crucial for its efficient treatment, thus, development of new diagnostic methods is needed.

The aim of the present study was to identify genes with mRNA expression related methylation changes in colorectal adenoma-dysplasia-carcinoma sequence. The mRNA expression profile of 49 normal, 49 adenoma and 49 colorectal cancer biopsies was investigated by microarray analysis. Genes showing highest changes in colorectal adenoma-dysplasia-carcinoma sequence and possess CpG islands in their promoter were selected for subsequent DNA methylation analysis. The methylation level was detected by bisulfite sequencing PCR, combined with high-resolution melting analysis (HRM) or pyrosequencing.

Expression of thrombospondin 2 (THBS2) was upregulated in 18% of adenomas and 84% of carcinomas, while proline rich membrane anchor 1 (PRIMA1) was downregulated in 88% of adenomas and 84% of carcinomas. Upregulation of THBS2 proved to be independent of promoter methylation. PRIMA1 was non-methylated in normal biopsy samples, but a methylated subpopulation appeared in a number of carcinomas by HRM analysis. The increase in PRIMA1 promoter methylation was confirmed by pyrosequencing results.

Our results suggests that epigenetical inhibition of PRIMA1 that is known to have tumor suppressor activity can be a common feature of colorectal cancers. We show for the first, that hypermethylation of PRIMA1 gene promoter can be a promising tumor marker.

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Chronic HDAC inhibitor treatment alters levels of anxiety in two mouse strains

Among certain genetic predisposition, the risk factors of anxiety disorders also include contribution from environmental factors, which might precipitate in abnormal gene expression patterns governed by epigenetic changes. Acetylation of core histones is the most well studied epigenetic mechanism, which is regulated by two opposing class of enzymes - histone deacetylases (HDACs) and histone acetyltransferases (HATs). Two inbred mouse strains, having extremes in anxiety related behaviors (AX and nAX), have been developed in our laboratory and were investigated for differences in histone acetylation levels of anxiety-related brain parts. The Ac-H4K12 level was found to be higher in the nAX strain in the ventral hippocampus, but not in the prefrontal cortex nor in the dorsal hippocampus. We hypothesized that increasing the level of Ac-histones might result in a less anxious phenotype.
Chronic treatment with HDAC inhibitors sodium butyrate (SB) or valproic acid (VPA) increased Ac-H4K12 level in all of the brain parts investigated. Treatment increased measures of anxiety in the nAX, but decreased that of the AX strain as was assessed by elevated plus maze. We propose that there is an optimum Ac-histone level in the brain for normal anxiety-related behavior.

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The associations of cellular aging and psychosocial factors: an epigenetic link

Background: Telomere length and rate of telomere shortening are indicators of mitotic cell age. Telomere shortness in humans is a prognostic marker of disease risk, progression, and premature mortality.
Aim of the study: We conducted a pilot study to investigate the psychosocial determinants of celullar aging. In this study we focused on religiousness, spirituality and life meaning.
Sample: 40 full time working women (n=20) and men (n=20) participated in our study. The age range was between 40 and 70.
Methods: Telomere length was determined in peripheral blood mononuclear cells (PBMCs). Telomere PNA Kit/FITC for Flow Cytometry was used for detection of the telomeric sequences which allows the estimation of the telomere length. Religiousness and spirituality dimensions were assessed by four items derived from the Brief Multidimensional Measure of Religiousness/Spirituality (BMMRS). We used the eight-item version of the Brief Stress and Coping Inventory's Life Meaning Subscale (BSCI-LM) to measure person's sense that life is meaningul. Organizational religiousness was assessed by two separate items measured by the frequency of religious service attendance and the form of religious practice. We used partial correlation analyses to determine the associaton of psychosocial variables and telomere length. Age and gender was used as covariates.
Results: Religiousness and spirituality assessed by BMMRS positively associated with telomere length (r=0.34; p=0.03). A moderate positive relationship was found between meaning in life measured by BSCI-LM and the indicator of cellular age (r=0.42; p=0.01). Those who attended religious services more frequently, had longer telomere (r=0.37; p=0.02). The form of the religious practice also significantly associated with telomere length (r=0.41; p=0.01).
Conclusions: This pilot study showed that there were associations between cellular age and religiousness, spirituality and meaning in life, independently from age and gender. The results suggest that these psychosocial factors have protective role to avoid early health deterioration and premature mortality. Since the sample was small, further investigations on larger representative samples are needed to confirm the findings of this study.

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Evaluation of DNA methylation by bisulfite-PCR (BS-PCR), high-resolution melting (HRM) and pyrosequencing analyses to assess epigenetic biomarkers in the colorectal adenoma-carcinoma sequence model

Background: Today, an increasing number of predictive and prognostic biomarkers have been identified that are of tremendous significance in the prevention of colorectal cancer. Altered DNA methylation subsequently resulting in aberrant protein expression is an early step in carcinogenesis. Thus, accurate detection and quantification of heterogeneous DNA methylation characteristic to biological samples is a fundamental task. Inspection of the melting curves of bisulfite-treated genomic DNA amplicons by high-resolution melting analysis allows both quantitative and qualitative estimation of DNA methylation. Bisulfite pyrosequencing has also been used to detect heterogeneous methylation by quantifying methylation levels present at individual CpG sites.
Materials and Methods: DNA was isolated (High Pure PCR Template Kit, Roche), bisulfite converted (EZ DNA Methylation-Direct Kit, Zymo) from 50 endoscopic distal colon (sigmoid and rectum) biopsies (2nd Department of Internal Medicine, Semmelweis University and 1st Department of Medicine, Sopron), and analyzed on LightCycler 480 (Roche) and PyroMark Q24 (Qiagen). Samples were from normal (n=5), normal adjacent to carcinoma (n=5), adenoma with low-grade (n=5) or high-grade (n=5) dysplasia, adenoma adjacent to carcinoma (n=7), carcinoma (n=22), carcinoma with CpG island methylator phenotype (n=1) tissues.
Results: Evaluating CpG island methylation of previously identified marker genes SFRP1, SFRP2, MAL and SDC2 may serve as a reliable tool to identify various stages of colorectal adenoma-carcinoma sequence.
Conclusion: Sequentially combining all methodologies above provides us with momentous and reliable data on DNA methylation status. Laser capture microdissection of biological specimens prior to DNA isolation and bisulfite treatment can further clarify the results obtained.

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Hypermethylated secreted frizzled-related protein 1 (SFRP1) originates from myofibroblast in colorectal adenoma and cancer

Background: The Wingless (Wnt) pathway plays a central role in the maintance of proliferative capacity of stem cells. SFRP1 has been postulated as an intercellular inhibitory regulator of Wnt pathway secreted by α-smooth muscle actin (α-SMA) positive myofibroblasts. This inhibitory effect becomes dysregulated in colorectal adenoma (AD) and carcinoma (CRC). Our aims were to analyze the methylation status, mRNA and protein expression of SFRP1 in myofibroblasts during colorectal adenoma carcinoma sequence (ACS).

Materials and Methods: Methylation status and mRNA expression of SFRP1 in laser microdissected samples (normal, n=10; AD, n=10; CRC, n=10) from colonic lamina propria were examined with bisulfite-PCR high-resolution melting (BS-PCR HRM) and reverse transcription PCR (RT-PCR). SFRP1 protein expression in myofibroblasts (normal, n=5; AD, n=5; CRC, n=5) was studied with α-SMA/SFRP1 double immunofluorescence staining.

Results: SFRP1 expressing cells were identified as α-SMA+ myofibroblasts. These cells showed reduced SFRP1 mRNA expression and increased hypermethylation during ACS. SFRP1 protein expression decreased in line with the decreased mRNA levels. In normal samples, 99,4±0,1% of all α-SMA+ cells showed moderate or strong SFRP1 protein expression, while SFRP1 protein expression and the ratio of α-SMA+/SFRP1+ cells significantly ($p<0.05$) decreased in AD (23,92±6,22%) and CRC (19,7±4,28%).

Conclusion: In AD and CRC α-SMA+ myofibroblasts showed reduced expression of SFRP1 at both mRNA and protein level, possibly caused by hypermethylation. During ACS, the decreased SFRP1 expression secreted by myofibroblasts may contribute to increased proliferation of cancer stem cells via uncontrolled Wnt signaling.

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Colorectal cancer epigenetics: lost in translation?

Colorectal cancer (CRC) has served as a prototype for multistep carcinogenesis and has been at the forefront of basic and translational cancer research. Epigenetics has expanded our understanding of this complex disease and the traditional model has recently been revisited. CRC is regarded as a molecularly and clinically heterogeneous disease. It is estimated that there are at least 4 different molecular pathways that lead to colorectal cancer, although most of these have not been associated with a particular clinical behavior.
A distinct subtype abundant in hypermethylated genes, termed CpG island methlyator phenotype (CIMP), however, has a unique molecular phenotype with characteristic clinical features. These CRCs arise from distinct precursor (serrated) lesions in the proximal colon and tend to occur in elderly women. It has also been shown that this subtype is intimately linked to certain environmental factors (eg. sm2ing, famine) and considered to be a poor prognostic factor.
Recent epigenome-wide studies have revealed that thousands of gene promoters might be hypermethylated in CRC (exceeding the number of genetic alterations), including many tumor suppressor genes, however it is currently vaguely known which of these drive carcinogenesis and which are merely consequences of this process, therefore targeted epigenetic therapy is still in its infancy.
DNA methylation-based prescreening biomarkers are more sensitive and more specific to select patients for screening colonoscopy. Next-generation sequencing studies will provide a vast amount of data with potential new biomarkers, that need to be translated in clinical studies to yield clinically useful diagnostic, prognostic and predictive biomarkers.

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miR-17 and miR-21- are involved in tissue regeneration following renal ischemia-reperfusion injury

Acute tubular necrosis (ATN) is a main cause of acute kidney injury. Reperfusion paradoxically increases the injury through apoptosis. We investigated miRNA expression profiles to elucidate their role in ATN.
After lethal and sublethal renal ischemia of C57BL/6J mice, renal function (urea, NGAL) and morphology were assessed at different reperfusion time-points. MiRNA expression profile after ischemia (Luminex multiplex assay) and during reperfusion (real-time PCR) was evaluated. Renal cell proliferation was evaluated by cyclinD1 immunohistochemistry on tissue microarray slides. Three miRNAs (miR-21, miR-17-5p and miR-106a) significantly elevated following ischemia. During reperfusion, these miRNAs started to elevate after 24 hours (h) further increasing at 48 hours (miR-21:2.3 fold, miR-17:2.2 fold, miR-106a:1.9 fold). Renal histology was pathologic already at 1h, blood urea was elevated at 3h and renal NGAL mRNA at 6h post-ischemia. CyclinD1 nuclear staining decreased significantly after 1h (0.5 fold), but returned to control values at 48h of reperfusion. After sublethal ischemia kidney function and miRNA levels have been restored on the 4th day of reperfusion. Kidney damage markers positively correlated with miR-17-5p and miR-21 expression.
Our results suggest that besides miR-21, the miR-17 family is involved in IR injury. We demonstrated that miR-21 and miR-17-5p expression increased after kidney damage markers, but before amelioration of renal tubular cell proliferation. Validated targets of the identified miRNAs have pro-apoptotic effects. Thus, our results suggest that these miRNAs may be involved in the regeneration processes and could represent possible therapeutic targets in the treatment of ATN.

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The role of epigenetics in assisted reproduction

The association between assisted reproduction technologies (ART) and genomic imprinting has been recognized, however the significance of this risk still remains unkown. There is a growing body of evidence that artificial culture systems that used in assisted reproduction affect the epigenetic makeup of embryos. The composition of culture media and oxygen concentration during embryo culture are already linked to epigenetic changes, which raises concern over the usage of assisted reproduction techniques. ART includes the isolation, handling and culture of gametes and early embryos at times when imprinted genes are likely to be particularly vulnerable to external influences may lead certain changes. Different imprinting syndromes have been identified but current evidence links ART with only three (Beckwith-Wiedermann syndrome, Angelman syndrome, maternal hypomethylation syndrome). Clinical reports suggest that imprinting changes can be associated with hyper- or hypomethylation of certain impriting regions among other possibilities. Another interesting field in epigenetics and assisted reproduction is the occurence of epigenetic mutatitons in infertile population. For obvious reasons, there is a more extensive literature evaluating epimutations in infertile males. Both imprinted and non-imprinted has been shown to have altered epigenetic profile on infertile males compared to fertile controls.
The aim of this presentation is to shed light of the current state of the filed of epigenetics and assisted reproduction and looking for directions for new studies.

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Start	End	Presenter	Title	Time
12:00	12:45	2012.09.20.	Registration desk: OPEN	0:45
12:45	13:00		Opening of the conference	0:15
13:00	13:30	Andràs Pàldi	Epigenetics: a new paradigm of what?	0:30
			TRANSCRIPTION REGULATION AND HISTONE TAILS	
13:30	14:00	Làszlò Tora	Towards the understanding of histone acetyl transferase complexes in transcription regulation and cellular differentiation	0:30
14:00	14:30	Imre M. Boros	BRIEF TOUR GUIDE TO THE DROSOPHILA CHROMATIN LANDSCAPE	0:30
14:30	15:30		COFFEE AND POSTERS, Registration desk: OPEN	1:00
15:30	16:00	Laszlo Nagy	PRMT1 produced arginine methylation and PRMT8 provide selective and coordinated retinoic acid-driven gene expression during the differentiation of mouse embryonic stem cells	0:30
16:00	16:15	Izabella Bajusz	The SET domain of Enhancer of zeste is involved in the recognition of chomatin domains to be inactivated	0:15
16:15	16:30	Ferenc Müller	The interplay between transcription initiation codes and nucleosome positioning signals on core promoters during embryo development	0:15
16:30	17:00	Gábor Szabó	Nucleosome-DNA cohesion is highly sensitive to certain H3 modifications and to superhelical twist.	0:30
18:30	22:30		GALA DINNER	4:00