SIBBM Seminar
“Frontiers in Molecular Biology”
Padua, 3-5 June 2010

Programme & Abstracts

Sponsors

Thursday, 3 June

14:30-14:45 **Welcome**

Session I » Non coding RNA – *Chair: Valerio Orlando*

14:45-15:30 **Keynote Lecture:** *Ruggero De Maria (Roma)* From biomarkers to biotherapeutics: the new frontier of microRNAs in cancer

15:30-15:55 *Luigi Naldini (Roma)* Exploiting and antagonizing microRNA regulation in gene therapy

15:55-16:15 **Coffee break**

16:15-17:25 **Poster Session I**

17:25-17:50 *Stefano Volinia (Ferrara)* Reprogramming of miRNA networks in cancer and leukemia

17:50-18:15 *Claudio Schneider(Trieste)* The stromal microenviroment, its precursor cells and role in serous ovarian carcinoma

18:15-18:30 *Silvia Barabino (Milano)* Genotoxic stress modulates alternative pre-mRNA splicing by modifying the intracellular distribution of SRPK2

18:30-18:45 *Maria Cristina Onorati(Palermo)* Functional interaction between chromatin remodelers and non-coding RNA’s

Friday, 4 June

Session II » Genomic stability, plasticity and differentiation – *Chair: Giulia Piaggio*

9:00-9:45 **Keynote Lecture:** *Marco Foiani (Milano)* Mechanisms controlling genome integrity

9:45-10:10 *Maria Pia Longhese (Milano)* Regulating processing of accidental and specialized chromosome ends

10:10-10:35 *Enrico Avvedimento (Napoli)* Transcription driven by oxidation: chromatin loops

10:35-11:00 *Claudio Brancolini (Udine)* Isopeptidases in the control of apoptosis: exploring new therapeutic potentials

11:00-11:20 Coffee break

11:20-11:35 *Chiara Lanzuolo (Roma)* Maintenance of PcG mediated epigenetic signatures through S phase in *Drosophila melanogaster*

11:35-11:50 *Elena Longobardi (Milano)* Expression and function of the TALE proteins in mouse mammary gland stem cell and progenitors

11:50-12:05 *Simona Pilotto (Pavia)* Design and development of new inhibitors of the histone demethylases LSD1 and LSD2

Session III » Signaling pathways – *Chair: Giannino Del Sal*

12:05-12:50 **Keynote Lecture:** *Pier Paolo Di Fiore (Milano)* Decoding the endocytic matrix

12:50-13:50 Lunch

13:50-14:15 *Alberto Gulino (Roma)* Regulation of Hedgehog/Gli function in neural stem/progenitor and medulloblastoma cells

14:15-14:40 *Francesca Granucci (Milano)* CD14 regulates the dendritic cell life cycle through NFAT activation following LPS encounter

14:40-14:55 *Barbara Stecca (Firenze)* Hedgehog-Gli signaling controls neural stem cell and tumor cell numbers

14:55-15:10 *Fiamma Mantovani (Trieste)* BRD7 is a candidate tumor suppressor gene required for p53 function

15:10-15:25 *Fabiana Aceto (Campobasso)* Mechanism of retinoic acid-induced transcription: epigenetic changes, DNA oxidation and formation of chromatin loops

15:25-16:35 Poster Session II

16:35-16:55 Coffee break

Session IV » Signaling pathways as therapeutic targets – *Chair: Francesco Cecconi*

16:55-17:40 **Key Lecture:** *Alberto Bardelli (Candiolo)* Cancer mutations and targeted therapies in cells, mice and patients

17:40-18:05 *Giovanni Blandino (Roma)* Therapeutic targets in breast cancer

18:05-18:30 *Antonello Pietrangelo (Modena)* Targeting the hepcidin signalling pathway to cure human iron disorders

18:30-18:45 *Anna Montesano (Milano)* Resveratrol promotes myogenesis and hypertrophy in murine myoblasts

18:45-19:00 *Alessio Valletti (Bari)* Identification of tumor-associated alternative splicing events in human cancer through EST-based computational prediction and experimental validation

19:00-19:15 *Simona Pedrotti (Roma)* The RNA-binding protein Sam68 regulates SMN2 exon 7 alternative splicing in spinal muscular atrophy

20:30 Social dinner

Saturday, 5 June

Session V » Cell biology & cell therapy – *Chair: Enrico Avvedimento*

9:00-9:45 **Key Lecture:** *Rosario Rizzuto (Padova)* Mitochondria, calcium, and cell death

9:45-10:10 *Francesco Cecconi (Roma)* Autophagy regulation in cell death and survival

10:10-10:35 *Michele De Luca (Modena)* Epithelial stem cells and regenerative medicine

10:35-10:55 Coffee break

10:55-11:20 *Luca Scorrano (Ginevra)* Size matters: mitochondrial elongation is a conserved and protective step of autophagy

11:20-11:35 *Paolo Grumati (Padova)* Autophagy is defective in dystrophic muscle and its reactivation rescues myofiber degeneration

11:35-11:50 *Marco Tripodi (Roma)* Epithelial differentiation and liver zonation of hepatocyte imply coordinate activation and repression of HNF4α target genes

11:50-12:55 Chiara D'Onofrio "Giovani" Award

12:55 Final remarks

Oral Presentations

(in alphabetical order of presenting author)

Mechanism of retinoic acid-induced transcription: epigenetic changes, DNA oxidation and formation of chromatin loops

F. Aceto[1], C. Zuchegna[1], A. Bertoni[2], V.E. Avvedimento[2], A. Porcellini[1]
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Retinoic Acid binds its cognate receptor and assembles the transcription initiation complex on specific DNA sites (RARE). We find that upon activation of RA receptor, on the RARE-promoter chromatin of *caspase 9* gene, histone H3 lysine 9 undergoes demethylation, catalyzed by lysine specific demethylase, LSD1 and JMJ-domain containing demethylase, D2A. The action of the oxidase (LSD1) and dioxygenase and Fe^{++} (JMJD2A) sets off an oxidation wave, that modifies the DNA locally and recruits the enzymes, involved in base excision repair (BER). These events are essential for the formation of chromatin loop(s) juxtaposing the RARE with the 5' transcription start site and the 3' end of the genes. The receptor bound on the RARE governs the 5' and 3' end selection, directing the productive transcription cycle of RNA polymerase.

Genotoxic stress modulates alternative pre-mRNA splicing by modifying the intracellular distribution of SRPK2

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Pre-mRNA splicing is an essential step of gene expression for the vast majority of metazoan genes. Pre-mRNA splicing has emerged in recent years as a major mechanism for generating proteomic complexity. Like transcription, splicing can be regulated both on a global level and in a gene-specific manner by cell signaling during the cell cycle and in response to cellular stress leading to the phosphorylation of target splicing.
The SR proteins, named for their C-terminal region enriched in alternating serines (S) and arginines (R), are a family of essential pre-mRNA splicing factors. Both the subcellular location and the activity of SR proteins in constitutive and alternative splicing depend on phosphorylation status of their RS-rich domain. Despite its functional importance, little is known about how SR protein phosphorylation might be regulated in the cell and how a specific signal might be transduced to control RNA processing via modulation of SR protein phosphorylation. Here we show that SR protein–specific kinase 2 (SRPK2) modulates alternative splicing in response to genotoxic stress. Treatment of neuroblastoma cells with paraquat (1,1'-dimethyl-4,4'-bipyridinium, a potent ROS generator) or with DNA-damaging agents such as cisplatin, promotes (i) the relocalization of SRPK2 from the cytoplasm to the nucleus, (ii) the phosphorylation of SR proteins leading to the their accumulation in nuclear speckles, and (iii) changes in the alternative splicing pattern of endogenous genes involved cell cycle regulation and DNA damage response, as well as of the E1A minigene splicing reporter. Moreover, mutational analysis shows that the phosphorylation of a specific serine residue (S581) is sufficient to promote nuclear localization of SRPK2. The chacterization of the kinase responsible for S581 phosphorylation is currently ongoing.

Autophagy is defective in dystrophic muscle and its reactivation rescues myofiber degeneration

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Autophagy is an evolutionarily conserved process crucial in the turnover of cell components. Clearance of damaged organelles by autophagy is essential for tissue homeostasis. Defects of this degradative system play a role in various diseases, but little is known about the involvement of autophagy in muscular dystrophies.
Congenital muscular dystrophies linked to collagen VI are characterized by the presence of dysfunctional mitochondria and spontaneous apoptosis, which ultimate lead to myofiber degeneration. We found that persistence of abnormal organelles and apoptosis are caused by defective autophagy. Skeletal muscles of collagen VI knockout (Col6a1−/−) mice displayed decreased LC3 lipidation both in basal state and after starvation. The reduction of LC3 lipidation is due to a strong delay in autophagic flux, and Col6a1−/− muscles seem unable to activate correctly the autophagic process.
Autophagy is regulated by a group of highly conserved genes and by the mTOR pathway. In Col6a1−/− muscles the lack of autophagosomes after starvation was paralleled by lower levels of Beclin1 and by the constitutive activation of mTOR pathway. Beclin1 is essential for autophagosome formation and suppression of the mTOR pathway is critical for autophagy induction. The constitutive Akt activation is also involved in the presence of altered mitochondria since Akt acts negatively on Bnip3 transcription. Bnip3 is crucial for the elimination of mitochondria through mitophagy. Reactivation of autophagy in Col6a1−/− mice by forced expression of Beclin1, prolonged starvation or low-protein diet restored myofiber survival and ameliorated the dystrophic phenotype of Col6a1−/− muscles.
Analysis of muscle biopsies from patients affected by Bethlem myopathy or UCMD showed lower levels of Beclin1 and Bnip3 proteins.
These findings indicate that defective activation of the autophagic machinery plays a key pathogenic role in congenital muscular dystrophies linked to collagen VI deficiency.

Maintenance of PcG mediated epigenetic signatures through S phase in Drosophila melanogaster

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The polycomb group (PcG) of proteins is part of a conserved cell memory system in higher eukaryotes that conveys epigenetic inheritance of repressed transcriptional states through several rounds of cell division by regulating multiple levels of chromatin structure. In Drosophila, PcG function is mediated by specialized epigenetic DNA modules, called Polycomb response elements (PREs). A remarkable features of the PcG-repressive system is inheriting patterns of gene expression by daughter cells. However, the time when PcG system establishes cell memory is not known. Althoug some models have been proposed after in vitro experiment or analysis done on exogenous systems, to date models from direct in vivo evidence are still missing. In particular, timing of when the parental marks are imposed and how tightly the process of PcG epigenetic inheritance is coupled to replication has not yet been determined.
To address this question we analysed the dynamic of PcG mediated epigenetic marks in the repressed Bithorax Complex (BX-C) during S phase in synchronized Drosophila S2 cells.
Our experiments allowed us to identifiy a specific time window, the early S phase, in which PcG proteins and the repressive mark H3K27me3 interact at high frequency with their targets, preceding PREs replication. Further, by 3C technology we show that Polycomb-mediated BX-C higher order interactions are also S-phase regulated, suggesting that during S-phase progression PRE-promoters interactions are disrupted whereas core PRE-PRE interactions are maintained.
We propose that a specific chromatin environment is established by PcG proteins at their target sites in early S phase suggesting a mechanism in which the uploading of Polycomb complexes and H3K27me3 would occur before their targets replication preventing dilution due to redistribution on daughter strands. Further, we suggest that Polycomb proteins binding and H3K27me3 enrichment are uncoupled from DNA replication.

Expression and function of the TALE proteins in mous mammary gland stem cell and progenitors

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The Meinox (Prep and Meis) and PBC (Pbxs) families, belonging to the TALE (Three Aminoacid Loop Extention) superfamily of homeodomain transcription factors, play a basic role in many biological processes, such as development, tumorigenesis, cell cycle, apoptosis and transcriptional regulation. Prep1, Meis1 and Pbx act developmentally by regulating gene expression and apoptosis during embryogenesis. In addition, Pbx and Meis1 have oncogenic properties while Prep1 has been shown to be a tumor suppressor, both in human and mice thus enlightening a novel fuction for this member of the high homology Meinox family. Prep1 hypomorphic mice, indeed, develop spontaneous tumors, including mammary cancer and Prep1 haploinsufficiency accelerates the development of EμMyc lymphomas. Breast cancer is the most common cancer in women representing 16% of all female cancers. Despite the huge data accumulated in the last years no or few information is available on the role of the TALE proteins in mammary gland biology and cancer. Mammary glands (MG) develop after birth from a small number of ectoderm derived cells. These cells generate a series of branching ducts that terminate in sac-like lobules embedded in stromal tissue. The MG also contains proliferating progenitor cells as well as low proliferating mammary gland stem cell, from which the entire mammary gland can be reconstituted. Both progenitor and stem cells can be easily isolated by the “mammosphere” technology. In the present work for the first time we show that all the eight members of the TALE family, Prep1,2, Meis1-3, and Pbx1-3 are expressed in mouse mammospheres, differently localize between stem and progenitor compartments, show a different nuclear and cytoplasmatic localization and that at least one member of the family is essential for proper in vitro mammosphere formation and in vivo mammary gland development.

BRD7 is a candidate tumor suppressor gene required for p53 function

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Oncogene-induced senescence (OIS) is a p53-dependent defense mechanism against uncontrolled proliferation. Consequently, many human tumors harbor p53 mutations while others show a dysfunctional p53 pathway, frequently by unknown mechanisms. We identified BRD7, a bromodomain-containing protein whose inhibition allows full neoplastic transformation in the presence of wild-type p53. Intriguingly, in human breast tumors harboring wild-type, but not mutant p53, the BRD7 gene locus was frequently deleted and low BRD7 expression was found in a subgroup of tumors. Functionally, BRD7 is required for efficient p53-mediated transcription of a subset of target genes. BRD7 interacts with p53 and p300, and is recruited to target gene promoters, affecting histone acetylation, p53 acetylation, and promoter activity. Thus, BRD7 suppresses tumorigenicity by serving as a p53 cofactor required for efficient induction of p53-dependent OIS.

Resveratrol promotes myogenesis and hypertrophy in murine myoblasts

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Many different conditions, such as muscular dystrophies or cancer, are associated with skeletal muscle wasting and weakness. Skeletal muscle development requires the expression of myogenic regulator factors MRFs (MyoD, Myf5, Myostatin and Myf6) and myosin heavy chain (MyHC). Resveratrol (RSV), a natural polyphenolic compound that is found in grapes and red wine, has anti inflammatory and antioxidant properties. Aim of this work is to study the effect of RSV during the late phase of muscle differentiation in C2C12 myoblast cells after 72 hours of differentiation.
We first examined the dose- and time-dependent effects of RSV. By Western Blotting and Immunofluorescence studies, we investigated the muscle proteosynthesis and morphologic features in C2C12 myotubes that were exposed to 0.1 or 25 μM of RSV for 5, 30 minutes (min) and 8, 24 hours (h). We used a negative control in which RSV was not added to the medium.
RSV induced an early peak activation of AKT/p70S6 and ERK Kinase pathways at 5 min which gradually decreased up to 4h. A second phase of activation was observed at 8h. The treatment with the highest dose of RSV (25 μM) caused also an increase of the AMPK protein content. Then, RSV might control the cell cycle progression trough p21/p27 cycline. Subsequently, we showed the positive effects of RSV on differentiation and hypertrophy process. RSV increased Myf6, Myostatin and MyHC, N-Caderin, Actin and alfa-Tubulin protein content, inducing also hypertrophic morphological changes in neo-formed myotubes. Our data suggest that RSV promotes myogenesis and hypertrophy process, opening a novel field of application of RSV in clinical conditions characterized by chronic functional and morphological muscle impairment.

Functional interaction between chromatin remodelers and non-coding RNA's

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The Hsr-ω gene is developmentally expressed in almost all cells types of *D.melanogaster* and is one of the most strongly induced heat shock genes in flies. The Hsr-ω locus encodes multiple non-coding RNAs (ncRNA) and the large nuclear species (Hsr-ω-n) is essential for the assembly and organization of hnRNP-containing omega speckles. These special nuclear compartments are thought to play essential roles in the storage/sequestration of members of hnRNP family and other proteins playing important roles in RNA maturation. ISWI is an evolutionarily conserved ATP-dependent chromatin remodeler playing essential roles in chromosome condensation, gene expression and DNA replication and is also involved in a variety of nuclear functions including telomere silencing, stem cell self-renewal, neural morphogenesis and epigenetic reprogramming.

Using an in vivo assay to identify factors that antagonize ISWI activity, we recovered a genetic interaction between *ISWI* and *Hsr-ω*. Loss of *Hsr-ω* function results in a strong suppression of eye morphology and chromosome condensation defects caused by loss of *ISWI* activity. Moreover, the organization of the omega speckles in *ISWI* mutant cells is profoundly altered when compared to wild type cells. Interestingly, immuno-FRISH analysis revealed a significant number of sites in the nucleus where the chromatin-bound ISWI protein overlaps with the omega speckles-associated Hsr-ω-n ncRNA. Remarkably, RNA-immunoprecipitation assays, conducted in salivary glands extracts, revealed a physical interaction between ISWI and the Hsr-ω-n ncRNA.

A functional homolog of Hsr-ω exists in mammals (*Sat III transcript*). Nuclear speckles and their associated ncRNAs are emerging as new and exciting players in gene regulatory networks, and their deregulation may underlie or be a marker for many complex diseases. Our data strongly suggest that ISWI could act as a functional bridging factor between chromosomes and nuclear speckle compartment.

The RNA-binding protein Sam68 regulates SMN2 exon 7 alternative splicing in spinal muscular atrophy

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Spinal Muscular Atrophy (SMA) is caused by degeneration of α-motor neurons, as a consequence of null mutations in the Survival of Motor Neuron-1 (SMN1) gene. SMA is the leading genetic cause of death in infancy, urging the development of novel therapeutic approaches. Humans carry a highly homologous duplicated copy of the gene, named SMN2, that potentially encodes for the same protein. However, the majority of the SMN2 transcripts lack exon 7 because a C-to-T transition determines the skipping of the exon during splicing, thereby leading to production of a non functional SMN protein. For this reason, much of the research efforts have been oriented at correcting this splicing event. Many splicing factors that modulate exon 7 inclusion or skipping have been described. Among them, we have identified Sam68 as a crucial splicing inhibitor, whose binding to SMN2 pre-mRNA triggers exon 7 skipping, potentially concurring to the SMA phenotype (Pedrotti et al., EMBO J. 2010). RNA pull-down, UV-crosslinking and EMSA experiments demonstrated that Sam68 binds to a consensus site created by the C-to-T transition in SMN2 exon 7 (UUUUA), whose integrity is absolutely required for Sam68-dependent exon 7 skipping. Dominant-negative mutants of Sam68 that interfere with its RNA-binding activity, or suppress its association with the splicing repressor hnRNP A1, abrogate Sam68-induced SMN2 exon 7 skipping. Retroviral infection of SMA fibroblast with these Sam68 mutants rescued SMN protein levels and its functional assembly in nuclear gems. Moreover, since the splicing activity of Sam68 is regulated by post-translational modification, like phosphorylation by Src-family and ERK kinases, the contribution of different signaling pathways on Sam68-dependent SMN2 exon 7 splicing in SMA fibroblast was investigated. Our results demonstrate that Sam68 is a novel, crucial regulator of SMN2 splicing and provide new tools that may correct this aberrant splicing and ameliorate the SMA phenotype.

Design and development of new inhibitors of the histone demethylases LSD1 and LSD2

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LSD1 and LSD2 histone demethylases are implicated in a number of physiological and pathological processes, ranging from tumorigenesis to herpes virus infection. A comprehensive structural, biochemical, and cellular study is presented here to probe the potential of these enzymes for epigenetic therapies. This approch employs tranylcypromine as chemical scaffold for the design of novel demethylase inhibitors. This drug is a clinically validated antidepressant known to target monoamine oxidases A and B, two flavoenzymes structurally related to LSD1 and LSD2. Mechanistic and crystallographic studies of tranylcypromine inhibition reveal a lack of selectivity and differing covalent modifications of the FAD cofactor depending on the enantiomeric form. A large set of tranylcypromine analogues were synthesized and screened for inhibitory activities. A few compounds with partial enzyme selectivity were identified. The biological activity of one of these new inhibitors was evaluated with a cellular model of acute promyelocytic leukemia chosen since its pathogenesis includes aberrant activities of several chromatin modifiers. Marked effects on cell differentiation and an unprecedented synergistic activity with anti-leukemia drugs were observed. These data demonstrate that these LSD1/2 inhibitors are of potential relevance for the treatment of promyelocytic leukemia and, more generally, as tools to alter chromatin state with promise of a block of tumor progression.

Hedgehog-Gli signaling controls neural stem cell and tumor cell numbers

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It is not known how strict controls of cell numbers are achieved during development and how they are deregulated in cancer. The Hedgehog-Gli (Hh-Gli) signaling pathway plays important roles in development, homeostasis and cancer. During early brain development Hh-Gli modulates the proliferation of neural precursors in the dorsal brain, while later it regulates the behavior of neural stem cells in neurogenic niches. It has been also involved in the genesis of tumors, including those of the brain, and in controlling self-renewal of brain cancer stem cells. However, it remains unclear if the zinc finger transcription factor Gli1, the final mediator of Hh signaling, controls stem cell number and how its activity is normally restricted. We show that GLI1 expression increases stem cell numbers in vivo and in vitro. In contrast, p53, the major tumor suppressor, inhibits GLI1-driven neural stem cell self-renewal, proliferation and tumor growth. p53 inhibits GLI1 and, in turn, GLI1 represses p53, establishing a negative regulatory loop that is central to control neural stem cell numbers and to prevent GLI1-driven tumorigenesis.

Epithelial differentiation and liver zonation of hepatocyte imply coordinate activation and repression of HNF4α target genes

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The concept that terminal differentiation allowing cellular tissue-specific functions is stably maintained once development is complete is questioned by numerous observations. Regarding the liver, it has been shown as a differentiated hepatic epithelium dynamically undergo i) an Epithelial-to-Mesenchymal Transition (EMT) and ii) a post-differentiation program leading to the metabolic liver zonation; in each hepatocyte, the specific repertoire of gene expression is influenced by its exact location along the portocentrovenular axis of the hepatic lobule causing a functional compartmentalization defined "zonation."
First, we showed that the HNF4α pivotal role for induction of hepatocyte differentiation is based on its capacity to counteract EMT phenotypic transitions (1): HNF4α inhibits transcription of the "EMT master genes" Snail, Slug and HMGA2, through the direct in vivo binding to their promoters, together with its corepressor NCoR. Notably, we also provided evidence that the HNF4α–mediated repression of EMT genes induces a Mesenchymal to Epithelial Transition (MET) in hepatomas and is required for the maintenance of the epithelial phenotype.
Secondly, we showed that HNF4α controls also the liver zonation. We demonstrated that Wnt signalling converges on the HNF4α-driven transcription (2): the Wnt-induced activation of PV genes and the repression of PP genes correlates with a HNF4α dynamic recruitment.
Overall, our data highlight a complex role for HNF4α in driving differentiation and post-differentiation programs through differential activation and repression of gene expression.

(1) Santangelo L., et al "Epithelial differentiation implies EMT repression: a dual role for Hepatocyte Nuclear Factor 4α" submitted
(2) Colletti M., et al "Convergence of Wnt Signaling on the HNF4α-Driven Transcription in Controlling Liver Zonation" Gastoenterology 137:660-672 (2009)

Identification of tumor-associated alternative splicing events in human cancer through EST-based computational prediction and experimental validation

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Alternative splicing (AS) is a key mechanism for expanding the transcriptome and proteome complexity in eukaryotes. Indeed, > 90% of multi-exon genes undergo AS. Alternative splice sites can be specifically recognized depending on tissue specificity, developmental stage, external stimuli, cellular stress or even pathological conditions, including cancer. So the presence or the expression level of specific splice variants can be indicative of a physiological or pathological condition or even trigger a disease.

The identification of a robust association between cancer and specific splicing patterns could be very useful to define signatures that can be used as diagnostic or prognostic indicators, and contribute to the discovery of therapeutic targets.

We undertook a systematic analysis of AS of human genes, developing a reliable strategy to identify cancer-specific splicing events determining the inclusion/skipping of specific exons (cassette exons). In particular, we performed a computational analysis of normal and cancer ESTs, considering all data collected in our ASPicDB[1] including over 18,000 genes and 300,000 alternative transcripts. Using a suitable statistical methodology, this dataset allowed us to predict normal/cancer-specific genes, splice sites and cassette exons. The condition association of some of the predicted cassette exons was experimentally verified by RT-qPCR assays, carried out on commercial RNAs derived from the same type of tissue where they were predicted. Moreover, we validated some of these AS events in a set of glioblastomas samples. The results confirm the effectiveness of our computational analysis, suggesting that the relative ratio between alternative transcripts may represent a promising tumor biomarker.

Our methodology can be adapted to manage exon array and NGS data, opening new opportunities for a thorough investigation of the expression pattern in human and other organisms.

[1]Castrignanò et al, 2008, Bioinformatics, 24(10):1300-4

Poster Abstracts

P1

HCV Modulates lipid matabolism through LXR dependent genes

S. Petta[1], M. Arciello[2,3], F. Pacifici[3], B. Conti[3], S. Anticoli[2,3], V. Leoni[4], C. Balsano[2,3]
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Liver steatosis is observed in about 70% of chronic hepatitis C patients thus is considered as a typical histological feature of these subjects. Metabolic cofactors and direct viral activities on lipid pathways are only a part of the multiple mechanisms involved in steatosis onset. Several studies suggest a direct role of HCV in inducing this pathology. Oxysterols, peroxidation products of cholesterol, are considered reliable markers of oxidative stress (OS) in vivo and are potentially involved in liver damage progression. These molecules, in fact, are able to directly activate nuclear Liver X Receptor (LXR), which, through the regulation of several genes (e.g. SREBP1c, FAS, ACC1), is able to modulate lipid metabolism. OS is a common feature of HCV infection, thus we investigated the possible link between OS, steatosis and HCV-related chronic hepatitis in 90 patients.
Estimation of oxysterols was performed by GC/MS technique in the culture media of HepG2 polyclones, constitutively expressing the entire (WT) or partial (only Core) HCV genome, and in plasma samples collected from patients. HBV- and NAFLD-related hepatitis have been considered as controls. At the same time we performed comparative RT-PCR for ACC1, SREBP1c and FAS in HepG2 polyclones.
Plasma concentration of oxysterols (7-β-hydroxycholesterol and 7-ketocholesterol) resulted statistically higher in HCV than in the control groups. HCV enhances oxysterols levels also in both polyclonal models. RT-PCR investigations showed that the expression of HCV viral proteins up-regulate, at least of two fold, the expression levels of LXR-dependent ACC1, FAS, SREBP1c genes. LXR activation in polyclonal cells by ChIP assays is under investigation.
These data suggest a key role for HCV in modulating the expression of LXR-depending genes involved in lipid metabolism, thus playing an important role in inducing hepatic steatosis, through two different mechanisms: directly by viral proteins and indirectly by oxysterols.

P2

New insights into the molecular and functional properties of LSD1/KDM1 neuronal mini-exon

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A variety of chromatin remodeling complexes are thought to orchestrate transcriptional programs that lead neuronal precursors from earliest commitment to terminal differentiation. We showed that mammalian neurons have a specialized chromatin remodeling complex arising from a neurospecific splice variant of LSD1/KDM1 - Histone Lysine Specific Demethylase 1- whose demethylase activity on Lys4 of histone H3 has been related to gene repression (Zibetti et al. 2010). We found that alternatively spliced LSD1 mammalian transcripts generate four isoforms. Two isoforms retain E8a, an unusually short exon four amino acids-long, internal to the amine oxidase domain, whose inclusion within transcripts is restricted to the nervous system.
Remarkably, their expression is dynamically regulated throughout brain development, particularly during perinatal stages. While inclusion of exon E8a within LSD1 proteins does not affect the *in vitro* biochemical properties nor the interaction with known co-repressor partners, *in vivo* the mini-exon E8a enables LSD1 to pace-make early neurite morphogenesis.

Our goal is now to understand the molecular mechanisms by which the mini-exon E8a confers a neurospecific function to LSD1 epigenetic activity. Structural studies show that the mini-exon E8a residues form a sort of protrusion from the amino oxidase domain that emerges from the main body of the protein without causing any local conformational change into LSD1. Such a feature indicates that these residues could easily generate a docking site for other protein partners as well as they can be targeted by post-translational modifications (PTM). Functional assays based on these two possibilities might provide a strategy to uncover *in vivo* biochemical differences.
We will present data relative to the physiological PTM pattern of immuno-isolated LSD1 from rat brain (analyzed by mass spectrometry) and to the functional role of such PTM in the acquisition and maintenance of normal neuronal phenotype.

P3

Novel microRNAs regulating the p53 pathway

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A vast body of evidence from clinical and basic research studies has demonstrated that the p53 pathway acts as an essential barrier in preventing cancer onset and development. p53 receives and integrates a wide variety of cytotoxic and genotoxic stress signals from upstream sensors translating them into different cellular outcomes, ranging from apoptosis, cell cycle arrest, senescence, DNA repair or other tumor-suppressive responses. The crucial role of the p53 pathway in tumor suppression is highlighted by the fact that almost all tumors select for its functional inactivation, either by directly mutating the p53 gene or by altering the expression and functions of key p53 regulators and effectors. Therefore, identification of cellular factors that modulate this pathway and that could be altered in cancer cells, thus allowing to evade p53 control, is crucial for understanding cancer development and for designing novel effective therapeutic approaches. In this context, the aberrant expression or function of microRNAs - small non coding RNAs that finely regulate gene expression by binding the 3’UTR of their target mRNAs thus altering their translation, stability and localization - might be highly relevant. Here, we present the identification of novel miRNAs able to regulate p53 activation and functions. In particular, these miRNAs were tested for their ability to modulate p53-dependent apoptosis, cell-cycle arrest and senescence, and to impair p53 transcriptional activity. We speculate that these microRNAs will provide a better understanding of the molecular mechanisms regulating functions and dysfunctions of the p53 pathway in cancer, and will represent new candidate targets for a more effective cancer therapy.

P4

Global chromatin analyses to understand the 3D network of interactions orchestrated by Polycomb complex during myogenic differentiation

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Basic nuclear functions such as transcription and replication are structurally integrated within the nucleus of eukaryotic cells. Higher order structures define the spatial organization of genes and their regulatory elements. In this context genome 3-D organization becomes a key epigenetic feature that controls the stability of cell programs through cell division.
Polycomb group proteins (PcG) are epigenetic regulators that prevent changes in transcription programmes and maintain the memory of silent gene states by controlling higher order structures. However, little is known about putative PcG function in regulating topological organization of their target genes in the nuclear space.
We addressed these questions in differentiating mouse skeletal muscle cells (C2C12). We found specific dynamics of proteins composing the PRC2 complex in myoblasts and in myotubes (i.e. PRC2-Ezh1 and PRC2-Ezh2); indeed, Ezh1 and Suz12 proteins are required for myogenic differentiation. Moreover, PRC2-Ezh2 and PRC2-Ezh1 complexes differentially regulate muscle specific genes (early differentiation versus late differentiation genes). To globally understand the role of PRC2 complex in muscle differentiation we applied different genome-wide analyses, namely C3C (ChiP-3C), ChiP seq and RNA-CAGE profiles. We are currently generating a set of integrated databases where dynamic changes in transcription profile, Polycomb binding sites and long-range chromatin interactions mediated by Polycomb are analysed by deep-sequencing in myoblasts and myotubes. We focused our attention on SUZ12 and Ezh1, and H3K27 tri-methylation histone mark. These approaches could represent powerful tools to achieve a full comprehension of the molecular mechanisms that dynamically regulate cell identity, differentiation and reprogramming and acquire a great relevance as an innovative point of view to understand the molecular basis of diseases in humans.

P5

Protein kinase PAK4 is involved in keratinocyte differentiation and modulates transcription factor p63

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The epidermis is a multilayered, stratified epithelium continuously regenerated by keratinocytes through a terminal differentiation process called cornification. Recently, it has been shown that in epithelial cells PAK4 interacts with Keratinocyte Growth Factor (KGF) Receptor, and is activated following KGF or UV exposure. PAK4 is a member of the PAK family of serine/threonine kinases, originally identified as an effector protein of the RhoGTPase CDC42. While PAK4 is expressed at low levels in most adult tissues, it is found overexpressed in tumor cell lines and primary tumors, and its locus amplification is recurrent in many cancers, including squamous cell carcinoma. Considering that PAK4 is involved in many cellular processes necessary for proper development and homeostasis of epidermis, like cell adhesion, motility and control of cell death, and that its expression is increased during keratinocyte differentiation, in this work we evaluated PAK4 role in signalling mechanisms that control differentiation and survival in human keratinocytes. Using the RNA interference technique, we first showed that PAK4 down-regulation sensitizes HaCaT cells to UV induced apoptosis, confirming its pro-survival role also in keratinocytes. Secondly, we could demonstrate that PAK4 is necessary for the differentiation process induced by confluence growth: PAK4 knock-down cells show a loss of Keratin1 induction and impaired down-regulation of transcription factor p63, a master regulator of epithelial tissue differentiation. We then evaluated activated PAK4 effects on p63 stability and function. Our data show that active PAK4 can both modulate protein levels of the α and β isoforms of DNp63, and inhibit its trancriptional activity. Our results show that PAK4 plays a relevant role in differentiation of human keratinocytes, and suggest a novel p63 regulatory network that could be implicated in epithelial tissues pathologies.

P6

Activation-iduced T cell death (AICD) depends on mitochondrial shape changes and on autophagy

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Mitochondria are highly dynamic organelles that continuously move, divide and fuse in a highly regulated fashion. The balance between the opposing processes of mitochondrial fusion and fission is controlled by a growing family of “mitochondria-shaping” proteins (i.e. OPA1 and DRP1). Evidence is accumulating on the role of these proteins in several physiological functions, from apoptosis to development. We recently unraveled an unexpected role for mitochondrial dynamics in the immune system where it controls migration of T cells. This prompted us to investigate whether mitochondrial shape participates in other crucial processes of T cells. Early in the process of activation-induced cell death (AICD) mitochondria undergo fragmentation. This is not caused by changes in the levels of mitochondria shaping proteins, but results from the activation of signaling cascades impinging on the fusion/fission machinery. Strikingly, the changes in mitochondrial shape are accompanied by an unexpected increase in autophagy. The combination of the two processes of mitochondria fragmentation and autophagy orchestrates the TCR-dependent death in lymphocytes.

P7

Impaired expression of p66Shc, a novel regulator of B-cell survival, in chronic lymphocytic leukemia

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B-cell chronic lymphocytic leukemia (B-CLL) is the most frequent adult leukemia in Europe and USA, with a highly variable clinical course and problematic prognosis. However the common denominator of the disease seems to be the extended survival and accumulation of mature CD5+ B-cells. p66Shc is an adaptor molecule expressed in T-cells that acts as inhibitor of mitogenic signaling and apoptosis promoter. Here we demonstrate that p66Shc is also expressed in B-cells, where it appears to subserve a similar dual function. After BCR stimulation Ramos B-cell line overexpressing p66Shc show a strong impairment in the survival pathway and an enhanced susceptibility to apoptosis, in contrast to B-cells from p66Shc$^{-/-}$ mice. Because of apoptosis defects are believed to be primarily responsible for the extended lifespan of CLL B-cells, we have quantified the expression of p66Shc and other members of the Bcl-2 family in B-cells from a subset CLL patients (IGHV mutated or unmutated). We have observed not only an impairment in p66Shc expression in all CLL patients, but also a concomitant reduction in Bax and Bak (pro-apoptotic) and an increase in the levels of Bcl-2 and Bcl-XL (anti-apoptotic), compared to healthy donors. Moreover we have demonstrated that p66Shc expression is causally related to the alteration in the balance among Bcl-2 family members by quantifying the expression of these mRNAs also in B-cells from p66Shc$^{-/-}$ mice and by reintroducing the expression of p66Shc in CLL B-cells. Collectively, these findings support the notion that p66Shc promotes apoptosis by shifting the Bcl-2 family balance in favor of the pro-apoptotic members and suggest that the imbalance towards the anti-apoptotic members observed in CLL may result from the defect in p66Shc expression.

P8

The transcriptional regulator p100/SND1 interacts with Sam68 and regulates CD44 alternative splicing in prostate cancer cells

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Alternative splicing (AS) is a mechanism that generates protein diversity from a limited number of genes. Through the differential assortment of exons, AS allows the production of different mRNAs from most human genes, thereby increasing genome complexity. Splicing abnormalities have profound impact on several human diseases, including cancer. A growing body of evidence suggests that the splicing machinery is an important target for misregulation in cancer, and that altered expression of several splicing factors correlates with cancer development and progression. Sam68 is an RNA-binding protein involved in alternative splicing regulation. Sam68 has a pro-oncogenic function and it is frequently up-regulated in human cancer, including prostate cancer (PCa), wherein Sam68 supports cell proliferation, migration and survival. In the present work, we have analyzed by mass spectrometry proteins that co-immunoprecipitate with endogenous Sam68 from PCa cells and we identified p100/SND1 as a novel Sam68-interacting protein. P100/SND1 is a transcriptional co-activator, which also interacts with components of the spliceosome, suggesting a role in coupling transcription and splicing. We found that p100/SND1 is over-expressed in PCa cells like Sam68. Sedimentation assays and co-immunoprecipitation experiments showed that p100/SND1 and Sam68 form a complex. Moreover, the functional relevance of this interaction was demonstrated by splicing assays using a CD44 minigene, a well established target of Sam68 splicing activity. Up-regulation of p100/SND1 enhances Sam68-dependent exon v5 inclusion in CD44 mRNA, which is known to correlate with increased proliferation and invasiveness of cancer cells. In line with these observations, depletion of p100/SND1 by RNAi reduced migration of PCa cells, similarly to what observed with Sam68 depletion. These results strongly suggest that p100/SND1 is a novel positive regulator of Sam68 splicing activity that promotes PCa cell growth and survival.

P9

Role of nitric oxide on gene expression and MAP kinase activation during Ciona Histone content dictates nucleosomal occupancy on yeast and mammalian genomes

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The basic unit of genome packaging is the nucleosome; the number of nucleosomes is often tacitly considered a fixed parameter, although variations in histone content among different tissues have long been known. We found that mammalian cells lacking High Mobility Group Box 1 protein (HMGB1) contain a reduced amount of core, linker and variant histones, and a correspondingly reduced number of nucleosomes. HMGB1 is a highly abundant and evolutionarily conserved chromatin component involved in many fundamental nuclear processes, including nucleosome sliding, transcription, replication, V(D)J recombination and DNA transposition. Yeast *nhp6* mutants, lacking NHP6A and –B proteins, which are related to HMGB1, also have a reduced amount of histones and fewer nucleosomes. By high-resolution nucleosome mapping we show that although more than one nucleosome in four is missing in *nhp6* cells, the location of nucleosomes is conserved, whereas nucleosomal occupancy varies extensively. Our results can be modelled assuming that different nucleosomal sites compete for the available histones: sites with high affinity are almost always packaged into nucleosomes in both strains, whereas sites with low affinity are less frequently packaged in *nhp6* cells. Nucleosome limitation increases the sensitivity of DNA to radiation damage, affects global transcription moderately, and the expression of about 10% of genes more substantially. We suggest that by modulating the occupancy of selected nucleosomes, and the expression of subsets of genes, histone availability may constitute a novel layer of epigenetic regulation.

P10

H4K16 acetylation: an epigenetic link between genome stability, transcription and replication

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In *S. cerevisiae* the ribosomal locus (rDNA) represents a region in which DNA replication, transcription and recombination meet both physically and functionally. The locus is a single gene cluster, consisting of approximately 150 repeated units. Each repeat contains the 35S and 5S genes, transcribed by RNA Polymerase I and III, and a non transcribed spacer (NTS). Despite its name, the latter sequence could be transcribed by RNA polymerase II to produce non coding RNAs (ncRNAs). ncRNAs transcription may locally displace cohesins leading to rDNA copy number variation and extrachromosomal ribosomal circles (ERCs), markers of genome instability and aging in yeast. Recently it has been shown that also the replication efficiency is essential for rDNA recombination rate. Each unit contains an ARS element (rARS) but only 20% of these origins are active in a single cell cycle. Once a rARS has fired, replication proceeds bidirectionally but the leftward-moving fork is blocked at the RFB (replication fork barrier) site. DSBs originating at the stalled forks may lead to ERCs formation and copy number variation. Interestingly, mutant strains of the NAD-dependent histone deacetylase Sir2p increase their replication efficiency, recombination rate and ncRNA production, suggesting that an epigenetic link between these processes exists. Our results indicate that other sirtuins mutants (*hst3* and *4*), as well as the inhibition of their deacetylation activity, increase ERCs and ncRNA levels. These two phenotypes are always associated with histone H4 hyperacetylation in the NTS. We have also found that the only hyperacetylation state of lysine 16 of histone H4 (H4K16) is sufficient to create a chromatin structure in which recombination and transcription controls are lost. We are now assessing if H4K16 is involved in rDNA replication efficiency to demonstrate its role in coordinating the main DNA transactions within this region.

P11

Role of the RNA-binding protein Sam68 in alternative splicing in neural stem cells and its impact on the severity of Spinal Muscular Atrophy

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Alternative splicing is differentially modulated in neuronal and non-neuronal cells of the nervous system. The relevance of alternative splicing in neurons is highlighted by neurodegenerative diseases arising from defects in this step of gene expression regulation. A clear example is provided by the pathological condition known as Spinal Muscular Atrophy (SMA). SMA is caused by loss of function mutations in the *SMN1* gene, which encodes for the Survival of Motor Neuron (SMN) protein. Notably, severity of the SMA disease in patients depends on the functional amount of SMN protein resulting from the activity of the almost identical *SMN2* gene. Indeed, although *SMN2* potentially encodes for an identical protein, a C-to-T transition in exon 7 causes skipping of this exon and production of a truncated and unstable protein that is rapidly degraded. We have recently shown that the RNA-binding protein Sam68 regulates this splicing event and promotes exon 7 skipping in heterologous cells and in fibroblasts derived from SMA patients. On the other hand, no much information on the expression and function of Sam68 in spinal cord motor neurons, the cells affected in SMA patients, is currently available. Herein, we have investigated the expression and function of Sam68, and its related Slm-1 and Slm-2 homologous proteins, in the nervous system. We observed that although they are all expressed in the brain, Sam68, Slm-1 and Slm-2 expression levels show different patterns in developing mice. Splicing assays indicated that Sam68 is a stronger regulator of *SMN2* splicing than Slm-1 and Slm-2. Moreover, immunofluorescence analysis showed that Sam68 protein is strongly expressed in α-motor neurons of mouse spinal cord. Notably, loss of function of *Sam68* in mice is critical during differentiation of neural stem cells (NSCs). In particular, ablation of *Sam68* enhances differentiation of neurons whereas it slightly reduces differentiation of other neuronal and non-neuronal cell lineages. Our study now aims at determining the role played by Sam68 in neurogenic splicing regulation and how its function has an impact on the pathology of SMA in mouse models of the disease.

P12

FGFR2 (Fibroblast Growth Factor Receptor 2) is a novel molecular partner of the MLL-AF4 leukemic oncoprotein

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The mixed-lineage leukemia (MLL) gene is involved in chromosomal aberrations associated with human leukemia. In ALLs, a balanced translocation fuses in-frame the N-terminal portion of MLL with the C-terminal of AF4.The resulting fusion gene encodes a MLL-AF4 chimeric protein with oncongenic activity. Human MLL-rearranged ALLs could be distinguisched from other ALLs by the H3-K79 methilation profiles.The H3K79 methilation is important for maintenance of MLL-AF4-driven gene expression.There are evidences that MLL-AF4 cellular oncogenic pathways involve some receptor tyrosine kinases(RTKs).We expressed recombinant MLL-AF4 in HEK293 cells and found that it interacts with a RTK, namely FGFR2 (Fibroblast Growth Factor Receptor 2), wich we previously demonstrated to be a molecular partner of AF4. Moreover, in this system we identified by Real-Time PCR, that also GRB2 and FGFR2 gene transcripts were over-expressed. Grb2 links FGFR2 phosphotyrosines and is able to stimolate downstream pathways to propagate mitogenic signals. Noteworthy we found an increase of Akt phosphorilation in HEK293 cells that express recombinant MLL-AF4. We hipotisize a potential role of FGFR2 in cellular pathways activated by MLL-AF4 chimeras.

P13

MicroRNA-based, p53 dependent post-transcriptional circuits: mechanisms, targets and inter-individual variation

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The tumor suppressor p53 is a sequence specific transcription factor that regulates the expression of many target genes linked, among others, to the control of cell cycle, apoptosis and DNA repair. Recent studies identified direct p53 regulation of miRNAs and related regulatory circuits.
Using bioinformatics approaches, we identified an additional group of candidate miRNAs for direct p53 transcriptional control. Furthermore, some of those miRNAs can be predicted to target mRNAs in genes relevant to p53-mediated responses. Notably, we found examples of miRNA seed binding sequences at target 3'UTRs that contain SNPs predicted to modulate miRNA binding. Our work aims at the validation of p53-mediated control of the newly predicted miRNA genes and related circuitries that would provide additional negative and/or positive feedback loops for p53 regulation. To validate p53-responsiveness of 15 miRNA promoters not previously described to be under control of this family of transcription factors, we initially evaluated the potential for wild type p53, p63 and p73 to transactivate the predicted p53 response elements (REs).in those miRNA promoters. For these experiments we developed in the model system S. cerevisiae a panel of isogenic reporter strains harboring the chosen p53 REs upstream of the firefly luciferase reporter gene. 11 REs (including miR10b, 23b, 106a, 151, 191, 198, 202, 221, 320) were responsive to p53 of which 7 were also responsive to p63 or p73. Next we developed RT-qPCR and ChIP assays in human cell lines where p53 proteins could be ectopically expressed or induced by genotoxic stress. In general, results confirmed p53-dependent transcriptional regulation of the studied miRNAs, although cell line differences were observed. Finally, to establish miRNA targeting of selected mRNAs and the functional impact of SNPs at the miRNA binding sites we developed 3'UTRs reporter assays. Specific examples of p53-directed post-transcriptional circuits will be presented.

P14

A genome-scale protein interaction pro?le of Drosophila p53 uncovers additional nodes of the human p53 network

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The genome of the fruitfly Drosophila melanogaster contains a single p53-like protein, phylogenetically related to the ancestor of the mammalian p53 family of tumor suppressors. We reasoned that a comprehensive map of the protein interaction profile of Drosophila p53 (Dmp53) might help identify conserved interactions of the entire p53 family in man. Using a genome-scale in vitro expression cloning approach, we identified 91 previously unreported Dmp53 interactors, considerably expanding the current Drosophila p53 interactome. Looking for evolutionary conservation of these interactions, we tested 41 mammalian orthologs and found that 37 bound to one or more p53-family members when overexpressed in human cells. An RNAi-based functional assay for modulation of the p53 pathway returned five positive hits, validating the biological relevance of these interactions. One p53 interactor is GTPBP4, a nucleolar protein involved in 60S ribosome biogenesis. We demonstrate that GTPBP4 knockdown induces p53 accumulation and activation in the absence of nucleolar disruption. In breast tumors with wild-type p53, increased expression of GTPBP4 correlates with reduced patient survival, emphasizing a potential relevance of this regulatory axis in cancer.

P15

Activation of Erk1/2 MAPK by cannabinoid receptors in mouse germ cells

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The endocannabinoid system (ECS) is a versatile system widely expressed in many cell types that is modulated during cell proliferation, differentiation and apoptosis through alterations of the expression levels of cannabinoid receptors CB1 and CB2, and of the enzymes involved in the biosynthesis and degradation of the two endocannabinoids: anandamide (AEA) and 2-arachidonoylglycerol (2-AG).
We have previously demonstrated that male mouse germ cells possess an active and complete ECS and that the cannabinoid receptor CB2 is expressed, in the testis, in all germ cell differentiation stages with decreasing levels from spermatogonia to spermatids. In addition CB1 receptor is expressed specifically in round spermatids and in spermatozoa. Spermatogonia possess higher levels of the endocannabinoid 2-AG, which decreases in spermatocytes and spermatids. This endocannabinoid likely plays a pivotal role in promoting spermatogenesis via CB2 receptor activation in spermatogonia, stimulation of the Erk1/2 MAPK phosphorylation cascade and subsequent progression of these cells towards meiosis (Grimaldi et al. PNAS, 2009).
In this study we investigated the presence of a functional CB2 receptor in purified germ cell fraction of spermatocytes and spermatids by analyzing the activation of MAPK and PI3K pathways after stimulation with the selective CB2 agonist JWH133. Spermatocytes and spermatids were purified from adult testes by elutriation with a purity of about 90%. Our results show that treatment with the CB2-specific agonist did not stimulate the phosphorylation of Erk1/2 MAPK, p38 MAPK and Akt in these cells, thus indicating that apparently only spermatogonia possess a functional CB2 receptor. Interestingly, since spermatids express mainly CB1 receptor, we found that treatment of these cells with the CB1 specific agonist ACEA stimulated the phoshorylation Erk1/2 MAPK, thus suggesting a role of this receptor in the haploid phase of spermatogenesis.

P16

Gene expression profile in liver transplantation and the influence of gene dysregulation occurring in deceased donor grafts

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Brain dead patients are the main source of organs for transplants. Brain death causes changes in peripheral organs. We define modificationss of gene expression in specific pathways occurring in donor livers and their influence on gene expression profile of livers after transplant.
Methods
We compared gene expression profile of both deceased donor livers and transplanted livers to gene expression data of liver tissue, retrieved from Array Express database, used as control. All expression data were obtained by microarray analysis.
The expression of about 33,000 genes has been compared in liver samples from three groups: deceased donor livers, transplanted livers two hours after reperfusion, and control livers. We found that about 900 genes are dysregulated in deceased donor versus control livers. Up-regulated genes are mainly involved in apoptosis, immune response and inflammation. Down-regulated genes are mostly involved in metabolism and electron transport. We also re-evaluated a group of genes that in a previous study were found dysregulated in transplanted livers when compared to donor livers. Most of these genes, but not all, were dysregulated also when compared to control livers. Moreover 317 additional genes, dysregulated after liver transplant, were identified in this study; they were undetectable in the previous study because. they had the same dysregulation both in donor and in transplanted livers.
Understanding molecular mechanisms that in the donor compromise graft function is crucial in order to discriminate between basal graft damages and ischemia-reperfusion injuries and therefore to identify therapeutic targets aiming to improve liver transplantation performances.

P17

microRNA-221 and microRNA-222 modulate differentiation and maturation of skeletal muscle cells

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MicroRNAs (miRNAs) are a class of small non-coding RNAs that have recently emerged as important regulators of gene expression. They negatively regulate gene expression post-transcriptionally by translational repression and target mRNA degradation. miRNAs have been shown to play crucial roles in muscle development and in regulation of muscle cell proliferation and differentiation. By comparing miRNA expression profiling of proliferating myoblasts versus differentiated myotubes, a number of modulated miRNAs, not previously implicated in regulation of myogenic differentiation, were identified. Among these, miR-221 and miR-222 were strongly down-regulated upon differentiation of both primary and established myogenic cells. Conversely, miR-221 and miR-222 expression was restored in post-mitotic, terminally differentiated myotubes subjected to Src tyrosine kinase activation. By the use of specific inhibitors we provide evidence that expression of miR-221 and miR-222 is under the control of the Ras-MAPK pathway. Both in myoblasts and in myotubes, levels of the cell cycle inhibitor p27 inversely correlated with miR-221 and miR-222 expression, and indeed we show that p27 mRNA is a direct target of these miRNAs in myogenic cells. Ectopic expression of miR-221 and miR-222 in myoblasts undergoing differentiation induced a delay in withdrawal from the cell cycle and in myogenin expression, followed by inhibition of sarcomeric protein accumulation. When miR-221 and miR-222 were expressed in myotubes undergoing maturation, a profound alteration of myofibrillar organization was observed. In conclusions, we found that miR-221 and miR-222 are modulated during myogenesis and play a role both in the progression from myoblasts to myocytes and in the achievement of the fully differentiated phenotype. Identification of miRNAs modulating muscle gene expression is crucial for the understanding of the circuits controlling skeletal muscle differentiation and maintenance.

P18

Citron-k controls the completion of cytokinesis functionally interacting with Anillin and RhoA

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Cytokinesis is the process by which a cell divides after the completion of mitosis. In eukaryotic cells, the tight regulation of mitotic and cytokinetic events is essential to ensure the genetic stability of a cell lineage. A long-standing hypothesis on tumorigenesis is that cytokinesis failure, generating genetically unstable tetraploid cells, facilitates the development of aneuploid malignancies.
The small GTPase RhoA is well known to play a critical role in cytokinesis initiation, progression and completion through its binding to a complex network of specific effectors. Citron kinase (Citron-k) is a conserved RhoA-binding protein required for cytokinesis from insects to mammals. However, it is still not well understood in which stages of cytokinesis and by which mechanisms this protein deploys its function.
In this study, we demonstrate that both Citron-k depletion and overexpression specifically affect abscission, the latest event of cytokinesis. Moreover, we show that these defects can be explained mechanistically by the functional interaction of Citron-k with RhoA and with the scaffold protein Anillin.
Although Citron-k is commonly considered a downstream effector of RhoA, our data are rather consistent with the idea that Citron-k could be an upstream regulator of RhoA during late stages of cytokinesis. Indeed, Citron-k depletion leads to a loss of both Anillin and RhoA from the midbody. Conversely, Citron-k overexpression leads to an increase of RhoA levels, especially localized to the midbody. In addition, while RhoA activity is required to keep Anillin at the midbody, Citron-k localizes properly to this structure in a RhoA independent manner. Finally, we demonstrate that the inhibition of RhoA in cells that are completing cytokinesis is sufficient to reverse the Citron-k dependent abscission delay phenotype.
In conclusion, we propose that Citron-k stabilizes Anillin and RhoA at the midbody and that, by doing so, it plays a very important role in the regulation of abscission timing.

P19

MDM2 and Fbw7 cooperate to induce p63 protein degradation following DNA damage an cell differentiation

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Tight control of p63 protein levels must be achieved under differentiation or apoptotic conditions. Here, we describe a new regulatory pathway for the ΔNp63α protein. We found that MDM2 binds ΔNp63α in the nucleus promoting its translocation to the cytoplasm. The MDM2 Nuclear Localization Signal is required for ΔNp63α nuclear export and subsequent degradation, while the MDM2 ring finger domain is dispensable. Once exported to the cytoplasm by MDM2, p63 is targeted for degradation by the Fbw7 E3-ubiquitin ligase. Efficient degradation of ΔNp63α by Fbw7 requires GSK3 kinase activity. By deletion and point mutations analysis we have identified a phosphodegron located in the α and β tail of p63 that is required for degradation. Furthermore, we show that MDM2 or Fbw7 depletion inhibits degradation of endogenous ΔNp63α in cells exposed to UV irradiation, adriamycin and upon keratinocyte differentiation. Our findings suggest that following DNA damage and cellular differentiation MDM2 and Fbw7 can cooperate to regulate the levels of the pro-proliferative ΔNp63α protein.

P20

CYP2E1 VNTR polymorphisms and hepatocarcinoma: a gender-specific correlation

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Cytochrome P450 (CYP2E1) is often associate to susceptibility to alcohol-related diseases and various cancers, because of its role in the metabolism of multiple environmental xenobiotics.
In the 5'- flanking region of the human CYP2E1 gene there are restriction fragment length polymorphism which are involved in the transcriptional regulation of the CYP2E1 gene. Recently a tandem repeat polymorphism (VNTR) in the 5'-flanking region of CYP2E1 was found. Because cytochrome P450 2E1 catalyzes the metabolic activation of pro-carcinogen and cytotoxic compound, we value the genetic distribution of this tandem repeat polymorphism in a healthy population, and in patients with hepatocellular carcinoma living in same country, in order to found a correlation between CYP2E1 VNTR genotype and neoplasia.
DNA was isolated from spit sample of 108 control subject and from the peripheral lymphocytes of 35 HCC patients. The 5' flanking region of the CYP2E1 gene was amplified by polymerase chain reaction and examined for tandem repeat polymorphism using Nla IV restriction map.
This study reports that only four of the ten possible genotype were found in all subjects. The modal genotype, found in both analyzed populations, is A2/A2. Interestingly, in a gender-based analysis of data this genotype was found more frequent in woman with disease that in control ones.
These preliminary findings represent a first report of a gender-specific correlation between CYP2E1 VNTR polymorphism and hepatocarcinoma.

P21

Activation of Erk1/2 MAPK by cannabinoid receptors in mouse germ cells

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The endocannabinoid system (ECS) is a versatile system widely expressed in many cell types that is modulated during cell proliferation, differentiation and apoptosis through alterations of the expression levels of cannabinoid receptors CB1 and CB2, and of the enzymes involved in the biosynthesis and degradation of the two endocannabinoids: anandamide (AEA) and 2-arachidonoylglycerol (2-AG).
We have previously demonstrated that male mouse germ cells possess an active and complete ECS and that the cannabinoid receptor CB2 is expressed, in the testis, in all germ cell differentiation stages with decreasing levels from spermatogonia to spermatids. In addition CB1 receptor is expressed specifically in round spermatids and in spermatozoa. Spermatogonia possess higher levels of the endocannabinoid 2-AG, which decreases in spermatocytes and spermatids. This endocannabinoid likely plays a pivotal role in promoting spermatogenesis via CB2 receptor activation in spermatogonia, stimulation of the Erk1/2 MAPK phosphorylation cascade and subsequent progression of these cells towards meiosis (Grimaldi et al. PNAS, 2009).
In this study we investigated the presence of a functional CB2 receptor in purified germ cell fraction of spermatocytes and spermatids by analyzing the activation of MAPK and PI3K pathways after stimulation with the selective CB2 agonist JWH133. Spermatocytes and spermatids were purified from adult testes by elutriation with a purity of about 90%. Our results show that treatment with the CB2-specific agonist did not stimulate the phosphorylation of Erk1/2 MAPK, p38 MAPK and Akt in these cells, thus indicating that apparently only spermatogonia possess a functional CB2 receptor. Interestingly, since spermatids express mainly CB1 receptor, we found that treatment of these cells with the CB1 specific agonist ACEA stimulated the phoshorylation Erk1/2 MAPK, thus suggesting a role of this receptor in the haploid phase of spermatogenesis.

P22

Pathogenic role of microRNA in smoke induced lung diseases

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Cigarette smoke is a main carcinogen for humans. However its mechanism of action at molecular level still remain to be defined. Using microarray we demonstrated that smoke induces in lung early alteration of microRNA expression mainly oriented towards downregulation in rodents. This situation results in the overexpression of a large number of gene and a selected number of protein bearing both adaptive and damage-inducing activities (1). Whenever smoke exposure goes on for few months, microRNA alterations become irreversible and mainly oriented towards dysregulation of damage-inducing activities. This situation results in the activation of function involved in inflammation, angiogenesis, DNA damage, inhibition of tumor suppressor genes, and activation of oncogenes. These microRNA alteration leads finally to the development of adenoma and, later on, of malignant carcinoma. MicroRNA profiling is able to distinguish in lung biopsies among the occurrence of smoke-related pathological condition including pneumonia, adenoma, and carcinoma thus having diagnostic potential. Smoke-induced carcinoma detected are very aggressive as predicted by their microRNA profile demonstrating the activation of genes involved in drug detoxification, metastatisation, and angiogenesis. Furthermore, microRNA analysis indicate that younger organism are more sensitive to smoke as demonstrating comparing smoke induced microRNA alteration induced during the first month of life and in adult mice (2). This finding explains the potent carcinogenicity of smoke whenever exposure starts immediately after birth in animal models.
MicroRNA also rapresent a possibile target for the prevention of smoke-induced lung diseases. Alterations of microRNA profile may be attenuated by using chemopreventive agents thus identifying those drugs characterised by an adequate safety and efficacy (3). The identification of selective downregulation of miRNA expression, as those occurring for let-7 microRNAs, may be tentatively corrected by microRNA delivery with the goal of attenuating smoke adverse effects in still healthy orgniasms thus counteracting cancer appearance.
Therefore, on the basis of our experimental data, microRNA are an important tool for both detection and prevention of lung cancer as induced by cigarette smoke.

P23

A novel mammalian flavin-dependent histone demethylase

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Methylation of Lys residues on histone proteins is a well known and extensively characterized epigenetic mark. The recent discovery of lysine-specific demethylase 1 (LSD1) demonstrated that lysine methylation can be dynamically controlled. Among histone demethylases so far identified, LSD1 has the unique feature of functioning through a flavin-dependent amine oxidation reaction. Data base analysis reveals that mammalian genomes contain a gene (AOF1, for amine-oxidase flavin-containing domain 1) that is homologous to the LSD1-coding gene. Here, we demonstrate that the protein encoded by AOF1 represents a second mammalian flavin-dependent histone demethylase, named LSD2. The new demethylase is strictly specific for mono- and dimethylated Lys4 of histone H3, recognizes a long stretch of the H3 N-terminal tail, senses the presence of additional epigenetic marks on the histone substrate, and is covalently inhibited by tranylcypromine. As opposed to LSD1, LSD2 does not form a biochemically stable complex with the C-terminal domain of the corepressor CoREST. Furthermore, LSD2 contains a CW-type zinc finger motif with potential zinc binding sites that are not present in LSD1. We conclude that mammalian LSD2 represents a new flavin-dependent H3-Lys4 demethylase that features substrate specificity properties highly similar to those of LSD1 but is very likely to be part of chromatin-remodeling complexes that are distinct from those involving LSD1.

P24

Mitochondrial dysfunction affects transcription and pre mRNA maturation of genes related to the axon guidance pathway

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Amyotrophic lateral sclerosis (ALS), is a late-onset progressive neurodegenerative disease affecting motor neurons. The causes of this disorder remain unknown, however pathogenesis is partially understood in familial ALS (fALS) where some cases are caused by mutations in Cu/Zn superoxide dismutase 1 (SOD1), a powerful antioxidant. Mitochondrial dysfunction, protein misfolding, axonal transport disruption, among others have been suggested as ALS causes. Interestingly aberrant mRNA alternative splicing has also been observed in ALS patients and in ALS murine models. With the aim to identify alterations in (i) gene transcription, and of (ii) pre-mRNA Alternative Splicing (AS) induced by mitochondrial dysfunction, we performed a whole-genome analysis using Exon 1.0 ST GeneChips (Affymetrix), which allow the definition of both transcription patterns and alternative pre-mRNA maturation events. We analyzed two cellular model of neurodegeneration: SH-SY5Y neuroblastoma cells 1) untreated, or treated with paraquat (PQ), a toxic herbicide that impairs mitochondrial function, and 2) stably expressing either wild type SOD1 or a mutant SOD1 protein carrying the G93A fALS mutation. We then combined the data set from the two experiments in order to discover genes whose expression was significantly altered in both. These genes were analyzed for their functions and involved pathways using the Ingenuity Pathways Analysis (IPA7) software. Among them, 28 were found to be associated to neurological disorders, and 8 to axonal guidance signaling. Moreover, additional genes involved in neuritogenesis were differentially expressed only in one of the two dataset. We confirmed the microarray data using real time qPCR and RT-PCR. Further characterization of alterations in axon guidance signaling was performed analyzing spinal cords from transgenic mice carrying the SOD1(G93A) mutation. On the whole, our data suggest that mitochondrial damage affects axon guidance signaling in the studied models.

P25

MicroRNA-based, p53 dependent post-transcriptional circuits: mechanisms, targets and inter-individual variation

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The tumor suppressor p53 is a sequence specific transcription factor that regulates the expression of many target genes linked, among others, to the control of cell cycle, apoptosis and DNA repair. Recent studies identified direct p53 regulation of miRNAs and related regulatory circuits.
Using bioinformatics approaches, we identified an additional group of candidate miRNAs for direct p53 transcriptional control. Furthermore, some of those miRNAs can be predicted to target mRNAs in genes relevant to p53-mediated responses. Notably, we found examples of miRNA seed binding sequences at target 3'UTRs that contain SNPs predicted to modulate miRNA binding. Our work aims at the validation of p53-mediated control of the newly predicted miRNA genes and related circuitries that would provide additional negative and/or positive feedback loops for p53 regulation. To validate p53-responsiveness of 15 miRNA promoters not previously described to be under control of this family of transcription factors, we initially evaluated the potential for wild type p53, p63 and p73 to transactivate the predicted p53 response elements (REs).in those miRNA promoters. For these experiments we developed in the model system S. cerevisiae a panel of isogenic reporter strains harboring the chosen p53 REs upstream of the firefly luciferase reporter gene. 11 REs (including miR10b, 23b, 106a, 151, 191, 198, 202, 221, 320) were responsive to p53 of which 7 were also responsive to p63 or p73. Next we developed RT-qPCR and ChIP assays in human cell lines where p53 proteins could be ectopically expressed or induced by genotoxic stress. In general, results confirmed p53-dependent transcriptional regulation of the studied miRNAs, although cell line differences were observed. Finally, to establish miRNA targeting of selected mRNAs and the functional impact of SNPs at the miRNA binding sites we developed 3'UTRs reporter assays. Specific examples of p53-directed post-transcriptional circuits will be presented.

P26

MLK4, a novel oncogenic kinase in the RAS pathway

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Activating KRAS mutations are present in some 20% of all human tumors and are particularly prevalent in pancreatic, lung, and colon cancers. KRAS mutated tumors are characterized by poor prognosis and lack of response to therapies. Targeting oncogenic KRAS is one of the most challenging tasks in cancer research as attempts to generate pharmacological inhibitors of KRAS have failed. We find that Mixed Lineage Kinase 4 (MLK4) is a novel human oncogene whose inactivation impairs the growth of cancer cells carrying mutated KRAS. MLK4 is somatically mutated in colorectal cancers (CRC) and glioblastomas and expressed at the invasive front of primary CRC and of the corresponding metastasis. Mutated MLK4 alleles show increased kinase activity and cooperate with oncogenic KRAS to promote transformation and invasiveness *in vitro* and drive metastatic formation *in vivo*. The knockout or knockdown of the MLK4 gene impairs the tumorigenic and invasive potential of KRAS mutated cells by reducing MAPK- ERK signaling. Expression of mutated MLK4 rescues the tumorigenicity of cancer cells in which KRAS was genetically ablated. Similar to oncogenic KRAS, mutated MLK4 confers resistance to anti EGFR therapy in cancer cells. Our results identify the MLK4 kinase as a key effector of KRAS, the most frequently mutated and presently undruggable human oncogene. Pharmacological inhibition of MLK4 is amenable and may lead to novel anticancer therapies for cancers harboring oncogenic KRAS.

P27

Role of the p53 oncosuppressor gene family members in the expression regulation of genes involved in the intracellular transduction of growth signals

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The expression study of genes involved in growth hormone/insulin-like growth factors (GH/IGFs) axis seems to have a particular relevance in understanding growth failure in patients with normal GH secretion. A preliminary study conducted in our laboratory has highlighted that many genes involved in the signal transduction pathways of GH and IGF-1 contain at least one responsive element (RE) of p53 family members in their regulatory regions. The p53 oncosuppressor gene family is composed by three members: p53, p63 and p73. Recently, we have demonstrated that these three proteins play different roles in the control of cell proliferation. In particular, p63 and p73, differently from p53, are directly involved in cell proliferation, since they activate genes promoting the transition from G1 to S phase in cell cycle.
The aim of the present study is to verify whether the p53 gene family members, in particular p63 and p73, regulate the expression of genes involved in signal transduction of GH and insulin-like growth factors. Among these genes, IGFBP3 plays a key role, because transports more than 75% of serum IGFs, extends IGF's half-life and modulates their biological effects. In addition, IGFBP3 also has IGFs-independent effect, including cell growth inhibition and induction of apoptosis. The literature reports that IGFBP3 is a direct target of p53 gene with two p53REs in intron 1 and in intron 2. We have identified an additional p53RE in intron 1.
Our preliminary results demonstrated that the IGFBP3 expression is regulated by p73 and p63 in addition to p53. Interestingly we found that the p53RE we identified is exclusively activated by p63 and p73 and not by p53, suggesting that the p53 family members regulate the expression of the IGFBP3 by interacting differentially with the three p53REs present in the gene.

P28

A new proposed role for apo(a) as LPS neutralizing molecule in CNS cells

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Introduction: The athero-thrombogenic lipoprotein(a) [Lp(a)] is an LDL-like lipoparticle having the exclusive multi-kringle apolipoprotein(a) [apo(a)]. Due to its binding with oxidized phospholipids (OxPLs), apo(a) exerts pro-inflammatory roles1. Since OxPLs may also act as negative regulators of endotoxemia through competitive inhibition of LPS signalling, Lp(a) may acquire a new function as LPS neutralizing molecule.
We previously demonstrated that Lp(a) may be present in the cerebrospinal fluid of neuroinflammatory patients2. In this study we investigated the effect of apo(a) on LPS-induced inflammatory response in CNS cells.
Methods: We investigated whether recombinant apolipoprotein(a) A10K [r-apo(a)] (175-5.5 nM) can stimulate IL-6 secretion or interfere with LPS induction (100 and 10 ng/ml) of IL-6 in human astrocytoma U373 cells. IL-6 levels were evaluated in culture supernatants by immunoblotting.
Results: r-apo(a) does not have agonistic activity in IL-6 secretion by U373 cells. It causes dose-dependent inhibition of IL-6 secretion in LPS activated cells (p:0.023,one-way ANOVA). Pre-conditioning of cells with r-apo(a), washing and subsequent LPS activation did not reverted the inhibition of IL-6 secretion (p:0.0134,one-way ANOVA), suggesting interaction of r-apo(a) with cellular components of LPS signalling. Saturation of lysine binding sites (LBS) of r-apo(a) with the lysine-analogue EACA (1:2000) showed that this interaction is not LBS-mediated. Experiments with sCD14 (2-0.05μg/ml) showed a quantitative competition between LPS and r-apo(a) for binding to sCD14 (p:0.0119,one-way ANOVA).
Conclusions: We demonstrated that apo(a) inhibits LPS-induced IL-6 secretion in U373 cells, supporting the hypothesis of apo(a) as a natural regulator of the inflammatory response. apo(a) exerts this role by interfering with cellular and serum components of LPS signalling.

1) Edelstein C et al. J Biol Chem 2003. 278: 52841-7
2) Pepe G et al. Clin Chem 2006; 52: 2043-8.

P29

Novel insights into the pathogenesis of AEC syndrome revealed by the characterization of a knock-in mouse model carrying a clinically relevant mutation in the p63 gene

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AEC syndrome (or Hay-Wells syndrome) is a rare autosomal dominant disorder characterized by cleft palate, ectodermal dysplasia, and severe skin defects. The disorder is caused by missense mutations in the p63 gene, which encodes a tetrameric transcription factor essential for epidermal development. Although p63 null mice have contributed significantly to elucidation of the function of p63 in the epidermis, little is known about the pathogenesis of AEC syndrome. To dissect the molecular defects underlying AEC syndrome, we generated a unique knock-in mouse model (p63+/L514F) that carries a clinically relevant point mutation in the SAM (sterile-alpha-motif) domain of the p63 alpha isoform.
p63+/L514F mice die within 12 hours after birth, and exhibit severe cleft palate, skin defects and ectodermal dysplasia, but no limb abnormalities, thus faithfully recapitulating the defects observed in AEC syndrome. In mutant embryos the palatal shelves initiate and elevate properly, although there are unable to meet to form the secondary palate. An aberrant hypoplastic epithelium is observed, although cell proliferation appears normal.
Similarly, p63+/L514F epidermis and hair follicles are hypoplastic in spite of normal cell proliferation, terminal differentiation, and cell survival rate. Interestingly, a reduced number of stem cells accounts for the hypoproliferative phenotype. At the molecular level, only a small subset of genes is affected in the AEC mouse mutant epidermis, suggesting a selective effect of the SAM domain mutation on p63 function.
Taken together these data indicate that molecular defects in AEC syndrome are likely to involve as-yet poorly explored cellular mechanisms downstream of p63, involving a specific subset of p63 target genes.

P30

Loss of ATM impairs hypoxia-mediated HIF-1 induction

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Hypoxia Inducible Factor-1 (HIF-1) is a heterodimeric transcription factor that has a central role in cellular adaptation to hypoxia. ATM is a cellular damage sensor that coordinates DNA damage-response checkpoints and DNA repair. Moreover a role for ATM in other metabolic pathways has been described. In order to understand if the cellular response to hypoxia is altered in the absence of ATM, we subjected Epstein Barr-virus transformed lymphoblastoid L6 cells, derived from an A-T patient, or ATM reconstituted L6 cells, L6-ATM cells, to hypoxia or to treatment with the hypoxic mimetic drug desferoxamine, DFO. We measured the level of hypoxia inducible transcripts, by quantitative reverse PCR, and HIF-1α protein accumulation, by western blot analysis. Unexpectedly, the transcriptional response of L6 cells to hypoxia was much reduced in comparison to L6-ATM cells. Instead, DFO treatment similarly induced a number of hypoxia responsive mRNAs in L6 and L6-ATM cells. This finding is not limited to these specific cell lines, because a number of similarly immortalized lymphoblastoid lines derived from different A-T patients, fail to accumulate HIF-1 α protein in hypoxia and consequently have a reduced transcriptional response to hypoxia. Moreover, RNAi-mediated down-regulation of ATM in HeLa and 293T cells also resulted in impaired HIF-1α protein stabilization in hypoxia. We do not have presently a molecular explanation for this defective regulation of HIF-1α in ATM negative cells. However ROS are known regulator of HIF-1α stability and we found that ROS generation in response to hypoxia is blunted in ATM negative cells. Other possible mechanisms will be discussed.

P31

PDGF receptor as therapeutic target in scleroderma

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Background: scleroderma (SSc) is characterized by fibrosis of skin and visceral organs. The serum of SSc patients contains PDGF receptor (PDGFR)-stimulatory auto-antibodies (auto-abs) eliciting HaRas-ERK1/2 signaling and collagen production in human fibroblasts (Baroni et al, NEJM 2006). A recent study (Olson et al, Dev Cell 2009) confirmed the role of increased PDGFR signaling in driving fibrosis *in vivo* in transgenic mice.
Objectives: to identify the regions of PDGFR extracellular domain bound by stimulatory auto-abs in order to generate PDGFR selective inhibitors.
Methods and preliminary results: IgG-positive memory B cells from blood of SSc patients were immortalized by EBV. Supernatants of B cell clones were screened for the ability to react with F alpha cells (mouse fibroblasts expressing the human PDGFR?alpha), but not with F-/- cells (mock-transfected, PDGFR-null mouse fibroblasts) by flow cytometry. Positive clones were screened for the production of agonistic antibodies (abs) inducing reactive oxygen species (ROS) in normal human fibroblasts. mRNA was obtained from such double positive B cell clones for sequencing and cloning of ab variable regions into a human IgG expression vector. Some of these recombinant (rec.) monoclonal antibodies (mabs) display the features of total IgG pools of SSc patients, since they bind to PDGFR and induce ROS, p-ERK and collagen gene in normal human fibroblasts. Molecular docking simulation indicates that stimulatory and non-stimulatory mabs bind to different epitopes. This was confirmed by binding competition experiments using a rec. PDGFR immobilized on a biosensor. A peptide library is under construction to complete the PDGFR epitope mapping.
Conclusions: we identified the specific epitopes involved in PDGFR signaling by agonistic PDGFR auto-abs. This is shedding light on the structure of active PDGFR domains, on SSc pathogenesis, and can be used to devise new therapeutic strategies to block PDGFR signaling and SSc progression.

P32

Characterization of prostate cancer stem cells isolated from TRAMP murine model

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Prostate adenocarcinoma is the most frequent visceral neoplasia and the second cause of death in men after lung tumors. The mouse model of the pathology is the TRAMP (TRansgenic Adenocarcinoma of the Mouse Prostate) model that was generated by introducing in the mouse genome 426 base pairs belonging to the probasin gene promoter, followed by the gene for T and t (Tag) antigen of SV40. Starting at 12 weeks of life, TRAMP mice develop a lesion called PIN (Prostatic Intraepithelial Neoplasia) that evolves in adenocarcinoma (AD) between 24-30 weeks. An androgen-independent (AI) adenocarcinoma develops in 5% of mice. By applying the in vitro neurosphere assay, we established prostate cancer stem cells (CSCs) lines from different stages of TRAMP tumor progression. For each tumor stage, one or more tumor cell lines were established. We characterized putative CSCs based on CSC definition criteria: self-renewal, multipotency and tumorigenicity. All lines were able to proliferate, to extensively self-renew and expressed stemness markers (i.e. CD133, Sca-1, CD49f). After differentiation by DHT, CSC lines expressed markers, typical of mature prostate cells, such as basal (P63, K5, K14 and CD44), luminal (AR, K8, and K18) and neuroendocrine (synaptophysin) cell markers. Thus, these prostate cancer stem cells (PCSCs) proved to be multipotent. Finally, PCSCs showed to possess evident in vivo tumorigenic potential after subcutaneous inoculation in immunodeficient mice, even in a serial transplantation context.

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A Pin1 / mutant p53 axis fosters aggressiveness in breast cancer

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Alterations in the p53 gene are found in approximately 50% of human tumours. More than 75% are missense mutations, leading to the expression of mutant proteins defective for wild type function, which accumulate to high levels in tumour cells. Furthermore mutant p53 proteins acquire novel gain of function properties and influence the progression of malignancy in a different manner than loss of p53 does. Despite compelling evidences showing that mutant p53 promotes tumour aggressiveness and metastasis, the mechanistic basis for this remains poorly understood.
Here we report that the oncogenic role of mutant p53 is aggravated by the prolyl-isomerase Pin1 and that these proteins contribute to develop tumour aggressiveness by promoting cell migration and invasion. We found that Pin1 enhances tumourigenesis in a Li-Fraumeni mouse model affecting both tumour-free survival and tumour spectrum and cooperates with mutant p53 in Ras-induced cell transformation. In human breast cancer cells, we observed that the ability of mutant p53 to promote migration and invasion is amplified by the catalytic activity of Pin1 and the concerted action of Pin1 and mutant p53 regulates reprogramming of gene expression to favour a specific transcriptional program that supports tumour aggressiveness. In breast cancer patients, we found that the prognostic value of p53 mutation results markedly influenced by Pin1 levels. Cases stratification on the base of p53 status and Pin1 levels allows identification of patients with different clinical outcome and response to adjuvant chemotherapy.
Collectively, our results demonstrate the existence of a Pin1 / mutant p53 axis which has a profound effect on tumour aggressiveness in breast cancer.

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Alternative splicing of ribosomal protein genes regulated by AS-NMD association

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By generating mRNA containing a premature termination codon (PTC), alternative splicing (AS) can quantitatively regulate the expression of genes that are degraded by nonsense-mediated mRNA decay (NMD) (1). We previously demonstrated that AS-induced retention of part of intron 3 of rpL3 pre-mRNA produces an mRNA isoform that contains a PTC and is targeted for decay by NMD. We also demonstrated that overexpression of rpL3 downregulates canonical splicing and upregulates the alternative splicing of its pre-mRNA (2). We are currently investigating the molecular mechanism underlying rpL3 autoregulation. Here we report that the heterogeneous nuclear ribonucleoprotein (hnRNP) H1 is a transacting factor able to interact in vitro and in vivo with rpL3 and with intron 3 of the rpL3 gene. We are now investigating the role played by hnRNP H1 in the modulation of the alternative splicing event

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P35

MicroRNAs and cancer progression in breast tumours

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microRNAs (miRNAs) acts as crucial regulators in gene expression and aberrant miR expression can contribute to cancer formation and progression. The aim of our work is the identification and characterization of miRNAs specifically involved in cancer progression. We performed a microarray miRNA expression analysis on 77 ductal breast carcinoma biopsies and 18 mammoplasties using the Human Agilent platform (V2). 242 out of 723 miRNAs turned out to be expressed in our samples and differentially expressed miRNAs were identified using SAM two classes analysis. 20 miRNAs discriminated patients with or without disease relapse within 72 months from surgery; 24 miRNAs correlated with lymph node positive versus negative status; 50 miRNAs separated ER+ versus ER- tumors. A significant association with survival was found analyzing relapse or lymph node associated signatures. In addition, most of the miRNAs present in lymph node or ER signatures associated with the clinico-pathological features of the tumors even considering one miRNA at the time. Comparisons between normal and tumor samples are ongoing. First, we investigated the biological functions of relapse-associated miRNAs using a computational method. The predicted target genes of the entire signature (over 6000) was obtained from miRecords System and used to perform Gene Ontology and Pathway analyses. A significant enrichment (Fisher test, $p<0.05$) for intracellular signal transduction pathways, such as Apoptosis or ERK/MAP or Neuregulin signalling was found and we are now validating some miRNA targets biologically. Our data favour a correlation between miRNA expression and primary breast cancer prognosis suggesting a potential prognostic value for miRNA expression analysis. In order to investigate the potential role of selected miRNAs in tumour progression, we are currently performing biological assays by gain of function or knock down approaches.

P36

HCV and ethanol induce aneuploidy through different intracellular pathways

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Hepatocellular carcinoma (HCC) is considered the fifth most frequent malignancy and because of its poor prognosis, the third leading cause of cancer death. This tumor is characterized by several etiologic factors. Persistent infection with HCV has been considered a major risk for development of HCC, as well as heavy alcohol abuse, which has been linked with earlier progression to HCC in chronic hepatitis C patients. During progression of HCC, liver cells accumulate chromosomal alterations, including aneuploidy. Aneuploidy often occurs as a result of mitosis dysregulation and both HCV proteins and ethanol are known to be involved in its induction in HCC; however, the mechanism are still unknown. We have already reported the ability of HCV core protein to lead mitotic arrest depending on unconventional activation of PKR. Thus, we investigated mitosis dysregulation in HepG2 polyclonal cells stably expressing all HCV proteins or HCV core protein alone. In addition, we analyzed mitosis dysregulation in HCV polyclonal cells treated with ethanol. Our results indicate that HCV proteins (especially core) cause a delayed exit from mitosis and alter expression of spindle-associated molecules (e.g. cyclin B1, cdk1, Aurora A, survivin) by a mechanism strictly dependent on PKR expression. HCV core protein leads to mitotic arrest, inducing nuclear localization of the cyclin B1-cdk1-PKR complex. Ethanol treatment alters the expression of the same mitotic molecules targeted by HCV, but this phenomenon seems to be PKR-independent. In conclusion, our study demonstrates that HCV proteins and alcohol synergistically alter mitotic apparatus, using different intracellular pathways; furthermore, we have identified new molecular mechanisms associated with HCV- and alcohol dependent mitotic abnormalities. Our findings provide important new insights into HCV and alcohol-associated hepatocarcinogenesis providing a good starting point to develop innovative combined therapeutic strategies.

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New insights into the molecular and functional properties of LSD1/KDM1 neuronal mini-exon

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A variety of chromatin remodeling complexes are thought to orchestrate transcriptional programs that lead neuronal precursors from earliest commitment to terminal differentiation. We showed that mammalian neurons have a specialized chromatin remodeling complex arising from a neurospecific splice variant of LSD1/KDM1 - Histone Lysine Specific Demethylase 1- whose demethylase activity on Lys4 of histone H3 has been related to gene repression (Zibetti et al. 2010). We found that alternatively spliced LSD1 mammalian transcripts generate four isoforms. Two isoforms retain E8a, an unusually short exon four amino acids-long, internal to the amine oxidase domain, whose inclusion within transcripts is restricted to the nervous system.
Remarkably, their expression is dynamically regulated throughout brain development, particularly during perinatal stages. While inclusion of exon E8a within LSD1 proteins does not affect the *in vitro* biochemical properties nor the interaction with known co-repressor partners, *in vivo* the mini-exon E8a enables LSD1 to pace-make early neurite morphogenesis.

Our goal is now to understand the molecular mechanisms by which the mini-exon E8a confers a neurospecific function to LSD1 epigenetic activity. Structural studies show that the mini-exon E8a residues form a sort of protrusion from the amino oxidase domain that emerges from the main body of the protein without causing any local conformational change into LSD1. Such a feature indicates that these residues could easily form a docking site for other protein partners as well as they can be targeted by post-translational modifications (PTM). Functional assays based on these two possibilities might provide a strategy to uncover *in vivo* biochemical differences.
We will present data relative to the physiological PTM pattern of immuno-isolated LSD1 from rat brain (analyzed by mass spectrometry) and to the functional role of such PTM in the acquisition and maintenance of normal neuronal phenotype.

P38

MageA2 is Recruited to Promyelocytic Leukemia-Nuclear Bodies and Impairs PML3-induced Senescence

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MAGE-A genes are a subfamily of the Melanoma Antigen Genes (MAGE), whose expression is restricted to a variety of tumor cells of different origin and to normal tissues of the human germ line. Although the specific function of individual MAGE-A proteins is being currently explored, compelling evidence suggest their involvement in the regulation of different pathways related to tumor progression. Previously, we have reported that MageA2 associates with HDAC3 and represses p53-dependent apoptosis in response to chemotherapeutic drugs. Here we demonstrate that MageA2 becomes re-localized to the PML-NBs through direct association with PML3. Localization of MageA2/HDAC3 complexes at PML-NBs results in decreased PML3 acetylation, a process reverted by HDAC inhibitors. In addition, expression of MageA2 interferes with p53 acetylation at the PML-NBs and with PML3-dependent activation of p53. As a result, we show that MageA2 expression leads to impairment of PML3-induced cellular senescence in normal human fibroblasts. All these data suggest that MageA2 could be functional in the progression to malignancy, interfering with the PML3-p53 axis, thereby blocking the senescence program, a critical barrier against cell transformation.

P39

Role of ubiquitination in DNA damage response: identification and characterization of RNF168, a novel histone ubiquitin ligase

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Maintenance of genome stability is critical to avoid abnormalities and tumorigenesis. As soon as DNA damage occurs, either upon genotoxic stress or during DNA replication, cells activate a well-timed cascade of events that culminates in cell cycle arrest to allow the repair of damage or, alternatively, to activate the apoptotic program. This chain of events is initiated by the recruitment to the site of lesion of a number of proteins that function as sensors, transducers and effectors of the DNA damage response (DDR). Post-translational modifications represent an important level of regulation of DDR. In fact, depending on the cell cycle phase and on the type of lesion generated, the cell induces a specific phosphorylation cascade through the activation of a family of Ser/Thr kinases, including ATM, ATR and DNA-PK. A growing set of evidences suggests a pivotal role for ubiquitination in DDR, involving both the enzymatic activities of the E2 conjugating enzymes and E3 ubiquitin ligases, and the docking abilities of protein modules (known as ubiquitin binding domains or UBDs) that recognize ubiquitinated proteins, establishing a bona fide ubiquitin-based signalling system.
We recently identified a novel E3 ubiquitin ligase, named RNF168, involved in the DNA damage response. We found that, upon formation of DNA lesions, RNF168 ubiquitinates histones H2A and H2AX, in a RING finger-dependent manner, which allows relaxation of the chromatin structure and recruitment of important downstream effectors, such as 53BP1. Important for the proper localization/activity of RNF168 is the presence of two UBDs named MIU1 and MIU2, although we have data showing the involvement of additional mechanisms. Here we identify and characterize a novel UBD present in RNF168, called UMI. Simultaneous inactivation of UMI and MIUs completely abolishes RNF168-dependent events following formation of DSBs in term of histone ubiquitination and 53BP1 recruitment.

P40

Regulation of clusterin expression in the endometrial proliferative diseases

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Clusterin (CLU) is a nearly ubiquitous multifunctional protein synthesized in different functionally divergent isoforms. Among the different CLU splice variants, sCLU and nCLU play a crucial role probably by keeping a balance between cell proliferation and cell death. Over-expression of CLU mRNA and protein has been reported in several human cancers such as prostate, breast and thyroid. In this light we decided to study in vivo the CLU expression in normal and diseased endometrial tissues, by Northern and Western analysis. Moreover gene transcriptional activity was also measured by nuclear Run-on experiment. Our results have shown an increase of CLU mRNA level both in hyperplastic and neoplastic endometrial tissues compared to the proliferative endometrial tissues. We have also shown an increase of CLU mRNA level in the differentiated physiological phases of the endometrial tissue, i.e. secretive and atrophic phase. Moreover, we investigated by PCR experiment, the specific CLU isoforms expression, among the different ASPicDB (http://t.caspur.it/ASPicDB/index.php) predicted transcripts, to possibly establish a specific association between the CLU isoform/s and the endometrial physio-pathological stage. The CLU protein content in neoplastic endometrial tissues shows an increase directly correlated to the mRNA level. Furthermore the transcriptional activity of CLU is up-regulated in the hyperplastic and neoplastic tissues, thus suggesting that CLU transcriptional activity may be responsible for the increase of the CLU mRNA level in both diseases. These results suggest the existence of a complex mechanism of regulation of CLU gene expression during progression from normal to invasive endometrial cancer, possibly contributing to the etiopatogenesis of endometrial proliferative diseases such as hyperplasia and adenocarcinoma. Moreover these results provide the first circumstantial evidence for the potential use of CLU as a new therapeutic target for endometrial cancer.

P41

TFAM binding to mtDNA is influenced by aging and calorie restriction (CR) diet in rat brain

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Mitochondrial transcription factor A (TFAM) is essential for the maintenance of mtDNA. It plays critical roles in multiple aspects of mtDNA functionality: transcription, replication, nucleoid formation, damage sensing and DNA repair. According to the mitochondrial theory of aging, mitochondria are deeply involved in origin and development of aging through an endogenously generated oxidative stress. Among the aging-related organelle's processes are the progressive dysfunction of the respiratory chain complexes and the accumulation of mutations in mtDNA. Due to its close relationship to mtDNA, TFAM might be relevant also in aging and its expression has been analyzed in aged rat liver, heart, cerebellum, kidney and different muscles of hind-limb. On the contrary, there are no data about TFAM expression in animals treated with the calorie restriction (CR) diet, which efficaciously prevents or delays various aging-related alterations. Recently, it has been demonstrated a differential binding of the Lon protease along mtDNA depending on a chemically-induced oxidative stress. Therefore, we decided to test TFAM binding to mtDNA in the physiological oxidative stress condition of aged rat brain and also to evaluate if the CR could affect TFAM-binding. We assayed some regions along the mtDNA molecule performing a semiquantitative PCR analysis with the mtDNA immune-precipitated by TFAM and we found a different binding of TFAM with aging and CR treatment.

P42

Role of histone phosphorylation in controlling polycomb function and nuclear reprogramming

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A key question in contemporary biology is how cell can acquire new identities via reprogramming of their original transcriptional program and cell memory. Polycomb group genes (PcG) control gene silencing and are master regulators of chromatin structure and cell memory. We got interested in signaling pathways that modulate PcG activity and could be therefore implicated in controlling stability and plasticity of the memory system. This might be relevant for cell reprogramming and could have a great medical potential, especially as possible pharmacological applications in regenerative medicine.
Our work: Ectopic expression of defined transcription factors can reprogram somatic cells to induce pluripotent stem (iPS) cells, but the utility of iPS cells is hampered by the use of viral delivery systems. Small molecules offer an alternative to replace virally transduced transcription factors with chemical signaling cues responsible for reprogramming. We are examining the associated changes in the chromatin to gain further insight into this reprogramming event. Although much is known about processes regulated by Polycomb (PcG) group proteins, mechanism that control their activity and the recruitment on chromatin are still poorly understood. We are investigating the role of phosphorylation of the histone H3 at Serine 28 (H3S28p), mediated by MSK1 kinase activity, in the control of gene activation and PcG chromatin association. Drug inhibition of MSK1, as well as knockdown of Msk1, impairs PcG-dependent epigenetic silencing, opening a particular temporal window in which it becomes possible to reprogam somatic cells. This overall change of epigenetic information may be crucial for resetting the genome for eventual acquisition of totipotency. So, the application of such molecules ultimately may lead to useful new therapeutics for treating disease.

P43

p63 acetylation is impaired in natural p63 mutants

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The p63 transcription factor, homolog to the p53 tumor suppressor, plays an important role in epidermal differentiation and limb development. Mutations in the p63 gene are associated with several human syndromes: Ectodactyl-Ectodermal displasia-Clefting (ECC), Ankyloblepharon-Ectodermal displasia- (AEC), Limb-Mammary Syndrome (LMS) and Split-Hand/Split-Footh Malformations (SHFM) type IV.
Little is known on the post-translational modifications that regulate p63 activities. On the other hand, it's well known that p53 activation is dependent on acetylation by the p300 acetyltransferase. Recently a new site for p300 acetylation was identyfied in p53 (K164). This lysine is well conserved in p63 and p73 and it corresponds to K193 in the ΔNp63α isoform. Natural mutations of this lysine (K193E) are associated to the SHFM-IV syndrome.
We have evidences that ΔNp63αK193 is acetylated by p300 and acetylation is associated with increased ΔNp63α stability. Mutations of K193 into glutamic acid (E) as well as into arginine (R) abolished p300 mediated stabilization.
In addition, p300 cotransfection enhances p63 transactivation potential on the Dlx5 promoter, while the ΔNp63αK193E and K193R mutants were not sensitive to p300 regulation. Interestingly, the K193E natural mutant is less active on development related promoters (Dlx5 and Dlx6) while it has normal activities on a cell-cycle regulated promoter (p57kip2).
Taken together our results suggest that p300 acetylates ΔNp63α on K193 and the importance of this post-translational regulation is underlined by the fact that this lysine is found mutated in SHFM-IV patients.

(Tang Y., Zhao W., Chen Y., Zhao Y. and Gu W. Acetylation Is Indispensabile for p53 Activation. Cell 2008 133, 612-626)

P44

Genome wide identification of ISWI binding and nucleosome spacing activity

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ATP-dependent nucleosome remodeling enzymes and covalent modifiers of chromatin set the functional state of chromatin. ISWI is the catalytic subunit of several ATP-dependent nuclesome remodeling complexes, highly conserved during evolution and essential for cell viability. ISWI complexes are thought to play central roles in DNA replication, gene expression and chromosome organization. Indeed, in Drosophila loss of ISWI function causes global transcription defects and leads to the dramatic alterations of higher-order chromatin structure. In order to understand if chromatin condensation and gene expression defects, observed in ISWI mutants, are directly correlated with ISWI nucleosome spacing activity, we conducted a genome–wide analysis of ISWI binding and nucleosome spacing activity in wild type and ISWI mutant chromatin. Our data revealed that ISWI has ~1200 high affinity chromatin binding sites, in genic and intergenic regions. Remarkably, we found that ISWI binds genes near their Transcription Start Site (TSS) causing alterations in nucleosome positioning especially within promoter regions. Interestingly, differences in nucleosome positioning, between wild type and ISWI mutant chromatin, tend to accumulate on the X chromosome for all ISWI-bound genes analyzed. Indeed, we found a significant enrichment of ISWI binding on genes mapping the X chromosome. In particular, we found that ISWI is highly active on dosage compensated genes in order to keep their TSS nucleosome-free. Our study reveals how higher eukaryote transcription and chromosome organization is regulated genome-wide by the activity of the chromatin remodeling factor ISWI.

P45

Molecular and functional analysis of adult human stem cell compartment of chronic myelogenous leukemia reveals the presence of a CD34- cell population with intrinsic resistance to imatinib treatment

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We show the molecular and functional characterization of a novel population of lineage-negative CD34-negative (Lin-CD34-) hematopoietic stem cells from chronic myelogenous leukemia (CML) patients at diagnosis. Molecular karyotyping and quantitative analysis of BCR-ABL transcript demonstrated that about one third of CD34- cells are leukemic. CML Lin-CD34- cells showed kinetic quiescence and limited clonogenic capacity. However, stroma-dependent cultures induced CD34 expression on some cells, cell cycling, acquisition of clonogenic activity and increased expression of BCR-ABL transcript. Lin-CD34- cells showed hematopoietic cell engraftment rate in immunodeficient mice similar to Lin-CD34+ cells whereas endothelial cell engraftment was significantly higher. Gene expression profiling revealed the down-regulation of cell cycle arrest genes, genes involved in antigen presentation and processing, while the expression of genes related to tumor progression, such as angiogenic factors, was strongly up-regulated when compared to normal counterparts. Flow cytometry analysis confirmed the significant down-regulation of HLA class I and II molecules in CML Lin-CD34-cells. Imatinib mesilate did not reduce fusion transcript levels, BCR-ABL kinase activity and clonogenic efficiency of CML Lin CD34- cells in vitro. Moreover, leukemic CD34- cells survived to BCR-ABL inhibitors in vivo. Thus, we identified a novel CD34- leukemic stem cell subset in CML with peculiar molecular and functional characteristics.

P46

Effect of betaine on myogenesis in murine myoblasts

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Betaine (BET) is found widely in various microorganisms, plant, and animals. The biological function of BET is considering acting as an osmolyte to protect cells stressed by a catabolic source of methyl groups. As a methyl donor, BET participates in the methionine cycle, primarily in liver and kidneys, and it is a precursor of S-adenosylmethionine (SAM). SAM is a direct methyl donor in proteins synthesis and DNA methylation. Skeletal muscle differentiation requires the coordinated activity of transcription factors and DNA methylation pattern modifications. Previous studies have hypothesized that BET supplementation may improve muscle endurance in human. However, BET effect on myogenesis remains unknown.
In this study the effects of BET on the late phase of muscle differentiation were investigated, using an in vitro skeletal muscle differentiation cell system (C2C12 cell line). After 72 hours of differentiation, C2C12 were treated with different concentration of BET (1, 5 or 10 mM) for different time (5, 10, 20, 30 min and 4, 8, 24 hours). BET was not added to medium in basal control. First, we performed a quantitative real-time-PCR gene expression analysis of 80 genes involved in insulin pathway: BET increased the mRNA content of IGF-1 pathway proteins in a dose-dependent manner. By Western Blot analysis, we observed that BET activates the Akt/p70S6 and ERK Kinase pathways, dose dependently. Furthermore, BET treatment increased the content of skeletal muscle protein Myosin Heavy Chain (MyHC) and induced morphological changes indicating the start of hypertrophy process.
Our results demonstrate that BET influences the late phase of myogenesis by activation of IGF1 and AKT/p70S6 kinase pathways. The BET action on myogenesis might provide the clue for a possible new drug/integrator strategy for clinical conditions characterized by muscle function impairment.

P47

Role of the prolyl-isomerase Pin1 in regulating the transcription-independent apoptotic activity of p53

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The major tumor suppressive activity of p53 is the induction of apoptosis in response to stress, relying on both regulation of transcription and on direct roles at the mitochondria. In the nucleus p53 is able to induce the expression of key proapoptotic genes in response to genotoxic stress. A key regulator of this pathway is the prolyl-isomerase Pin1, which is able to transduce phosphorylation of p53 into conformational changes in order to trigger dissociation of p53 from the E3-ubiquitin ligase MDM2 and the inhibitor iASPP with a consequent increase of proapoptotic transcriptional activity of p53. At the mitochondria p53 induces outer membrane permeabilization. We hypothesize that Pin1 might regulate also the mitochondrial apoptotic activity of p53, given the fact that the prolyl-isomerase has been previously shown to regulate other apoptotic proteins acting at the mitochondria, such as BIMEL, Bcl2 and p66 Shc. Here we show that Pin1 is necessary to p53-mediated transcription-independent apoptosis. Furthermore, we demonstrate that Pin1 is necessary for efficient localization of p53 to mitochondria and that it modulates the ability of p53 to interact with BclXL

P48

Abl regulates a novel signalling pathway required for Met-triggered cell survival and tumorigenesis

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Mutant forms of c-Abl play an important role in hematopoietic malignancies. However, the implication of c-Abl downstream of receptor tyrosine kinases (RTK) in solid tumours has been overlooked. We report here that c-Abl engages a novel signalling pathway to regulate cell survival and tumorigenesis triggered by Met RTK. In particular, we show that c-Abl activation by Met triggers p53 phosphorylation on Ser392, which in turn drives the transcriptional up-regulation of Mdm2 and ensure cell survival. Finally, we uncovered a clinical correlation between aberrant Met activation, wild-type p53 phosphorylation on Ser392 and Mdm2 expression in human hepatocellular carcinoma. Our findings elucidate a molecular mechanism by which wild-type p53 can participate to cancer development and disclose a novel signaling signature to identify patients whose treatment may benefit from c-Abl inhibition.

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Improved resistance to exercise in mice treated with metformin

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Hormones, like insulin, GH-IGF-1, glucocorticoids, induce structural changes leading to muscle hypotrophy, muscle fibers changes and muscle weakness. Skeletal muscle differentiation is a process in which proliferative myoblasts break free from the cell cycle and fuse to form multinucleated myotubes. These events are orchestrated by early myogenic regulator factors (MyoD, Myf-5, myogenin and Myf-6) and late myogenic protein MHC (myosin heavy chain), through p38 MAP kinase/ERK pathway modulation. Metformin (Met) is a first-line anti diabetic therapy. Our previous data suggested that Met induces ERK pathway activation and MHC synthesis in vitro muscle model (C2C12).
Aim of this work is to confirm in vitro and to study in vivo in the rodent model the action of Met during myogenesis. In particular we studied muscle proteosynthesis and morphologic characteristics in the late phase of muscle differentiation. Cells were incubated after 72h of differentiation, with 400 μM Met for 4, 8 and 24 hours. We used a positive control with 0.1 nM insulin added to medium and a negative control in which Met and insulin were not added.
MRFs protein contents, evaluated by Western Blot and Immunofluorescence studies, were higher in cells treated with Met. Furthermore, Met treatment is able to increase cell mass and fusion competence indicating that Met may regulate myogenesis and fibers hypertrophy.
To test those results in vivo, we investigated the action of Met on exercise performance in adult C57BL6 mice. Mice were injected intra abdominally with Met (250 mg/kg) and the control mice with 0.9% saline for 30 days. An endurance performance treadmill running test made at the beginning and at the end of this study revealed that Met treated mice exhibit an enhanced performance respect to the control mice (VO2max ml/kg $^{0.75}$ x min: Met 14.41 ± 1.5 respect to control 12.6 ± 2.8).
Our findings show a novel therapeutic indication of metformin for muscle hypotrophy in chronic wasting diseases.

P50

Role of insulin growth factor binding protein 2 in rhabdomyosarcoma

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Rhabdomyosarcoma (RMS) is the most frequent sarcoma of childhood with two major subtypes: embryonal (ERMS) and alveolar (ARMS). ARMS are characterized, in about 75% of cases, by specific chromosomal translocations that involve PAX and FKHR genes.
It has already demonstrated that PAX3-FKHR positive ARMS possess a significantly different gene expression signature compared to PAX3-FKHR negative tumors.
Our results suggested that IGFBP2 is one of the most interesting overexpressed genes in translocation negative RMS. To better understand the role of IGFBP2 in RMS we have studied mRNA and protein expression level in 8 RMS cell lines confirming the different expression pattern between fusion-positive and fusion-negative RMS tumor.
Furthermore, we have investigated the localization and distribution of the protein in IGFBP2 overexpressing RMS cell lines, revealing that IGFBP2 is highly concentrated in Golgi apparatus that appears condensed in a juxtanuclear position and without its typical stack organization.
IGFBP2 plays an important role in several cancers and its expression is correlated with the invasivness and higher tumorigenicity grade. Silencing of IGFBP2 in RMS cell cultures followed by a transcriptome analysis using microarray technology revealed that genes of cell cycle and regulation of actin cytoskeleton pathways are significantly deregulated.
Subsequent FACS analysis and in vitro migration and invasion assay have confirmed that cells with reduced levels of IGFBP2 have an evident G0/G1 phase arrest and a decrease of migration and invasiveness capability. To analyze the role of IGFBP2 in vivo and in the clinical settings we have assessed the expression levels in tumor specimens and in serum of patients and controls. Our results show that IGFBP2 is more abundant in ERMS patients and it is localized preferentially in Golgi apparatus. Finally, serum levels of IGFBP2 in RMS patients show a positive correlation with high tumor grade.

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High Resolution Melting: an efficient tool in mutational screening of DMP1 and MEPE genes

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Mutation scanning using high-resolution melting (HRM) is an effective and sensitive method that can replace gel-based methods and dHPLC to detect sequence variations. HRM increases simplicity and improves turn around time without compromising assay sensitivity and accuracy. HRM is a closed-tube assay without the requirement of any post-PCR handling. A saturating DNA binding dye is introduced during DNA amplification enabling differentiation of PCR products based on their dissociation behavior as they are subjected to increasing temperature.
The *DMP1* (MIM *600980) and *MEPE* (MIM *605912) genes play a crucial role in mineralization and mutations in *DMP1* have been described as a cause of hypophosphatemic rickets (HYP).
We setup the mutation scanning of *DMP1* and *MEPE* genes by HRM on the HR-1 Instrument (Idaho Technology) to perform molecular analysis in a pediatric cohort of 12 HYP patients. DNA were isolated on the QIAcube (Qiagen). The primer design was performed using the LightScanner Primer Design Software v.1.0 (Idaho Technology). *DMP1* (composed by 5 exons) and *MEPE* (4 exons) were both divided in 9 amplicons. The complete coding region of the *DMP1* and *MEPE* was amplified from genomic DNA using Quantimix Easy HRM (Biotools) in presence of LCGreen Plus dye (Idaho Technology). A potentially weak point of HRM is the detection of homozygous variants. Because *DMP1* and *MEPE* have a recessive pattern of inheritance, to generate heteroduplexes and ensure the detection of all homozygous variants we mixed the proband DNA with a wild-type DNA (1:1 ratio) in the PCR reaction. Finally, to genotype our patients and 50 healthy subjects for the c.A205T polymorphism in *DMP1* we performed SNP genotyping on an amplicon build on the specific polymorphism site.
In our experience HRM, for its ease of use, rate of analysis, sensitivity, specificity and low cost is an optimal tool that can be used for high-throughput mutation screening and genotyping both for research and molecular diagnostic.

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Role of the prolyl-isomerase PIN1 in the oncogenic functions of the NOTCH1 intracellular domain

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Recently, a crucial role in regulating signaling pathways has emerged for the prolyl-isomerase Pin1. This enzyme binds to and catalyzes cis/trans isomerization of prolines on phosphorylated S/P or T/P motifs in many proteins, thereby transducing phosphorylation signaling in conformational changes that profoundly affect their functions. We have recently established that Pin1 activity is crucial in controlling DNA damage checkpoint pathways, by regulating the functional activation of p53 tumor suppressor and p73. However, Pin1 is also an important modulator of cell proliferation and other cellular events, and is involved in many diseases, such as cancer. More recently we have demonstrated that the prolyl-isomerase Pin1 interacts also with Notch1, thereby affecting the activation of the Notch1 pathway. Pin1 potentiates Notch1 cleavage by gamma-secretase, leading to an increase of the active intracellular domain and ultimately enhancing Notch1 transcriptional and tumorigenic activity. Moreover, we found that Notch1 directly induces transcription of Pin1, generating a positive loop. Interestingly, in human breast cancers we observed a strong correlation between Pin1 over-expression and high levels of activated Notch1 (N1ICD). Several findings suggest that the stability of N1ICD could be an important regulator of intracellular signalling thresholds and that abrogation of the Notch1 degradation machinery could predispose cells for transformation. The E3 ubiquitin-ligase Fbw7, a potent tumor suppressor, targets nuclear N1ICD for proteasomal degradation, thus leading to suppression of the Notch signal. Intriguingly, Pin1, together with proline-directed kinases, has been shown to regulate the stability of several Fbw7 targets, such as c-Myc, cyclin E and c-jun. Data about ongoing research on the role of Pin1 in the mechanisms regulating N1ICD stability and degradation by Fbw7 will be presented.

P53

Cell adaptation to activated FGFR3 includes Sprouty4 up regulation to inhibit the receptor-mediated ERKs activation from the endoplasmic reticulum.

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The kinase activity of the thanatophoric dysplasia type II- fibroblast growth factor receptor 3 mutant (TDII-FGFR3) hampers its maturation. As a consequence, the immature receptor activates extracellular regulated kinases (ERKs) from the endoplasmic reticulum (ER), which leads to apoptosis. On the other hand, in stable TDII-FGFR3 cells receptor biosynthesis is restored and ERKs are activated from the cell surface. To identify potential mediators of cell adaptation to the activated receptor we investigated gene products that are differently regulated in TDII and wilde-type FGFR3 cells. cDNA rappresentional difference analysis reveals Sprouty4 up regulation in the TDII-FGFR3 cells. Interestingly, Sprouty4 inhibits the TDII-FGFR3-mediated ERKs activation from the ER, but fails to supress ERKs activation from cell surface. We conclude that cell adaptation to activated FGFR3 include Sprouty4 activity, which silences the premature receptor signaling and suppress apoptosis.

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