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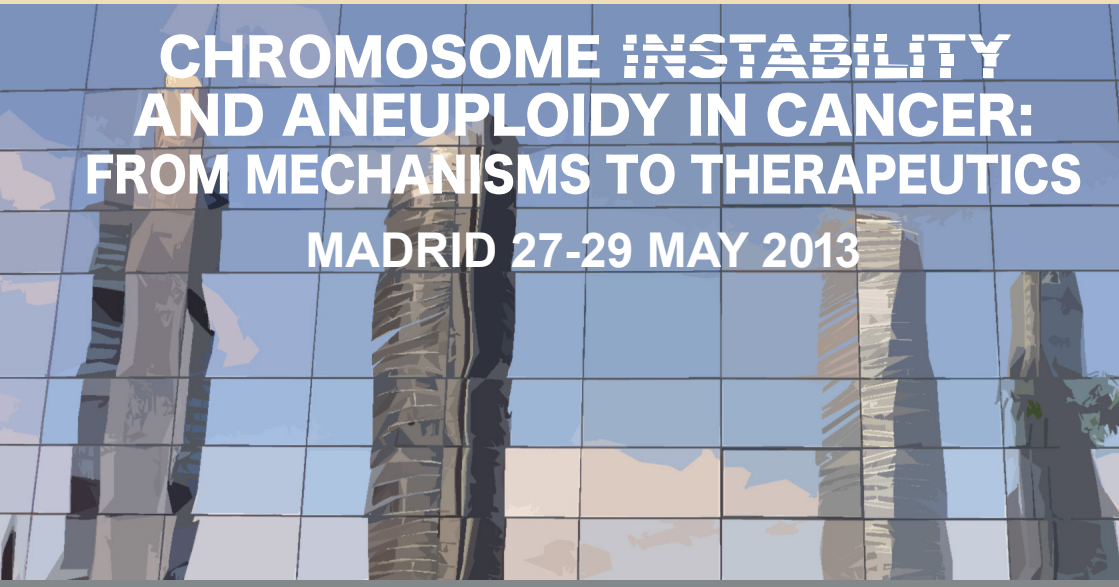
CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER: FROM MECHANISMS TO THERAPEUTICS



MADRID 27-29 MAY 2013

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CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER: FROM MECHANISMS TO THERAPEUTICS

MADRID 27-29 MAY 2013

SUMMARY

DETAILED PROGRAMME

May 27th **Session I: Mechanisms of aneuploidy generation**
Chair: *Robert Benezra*

Session II: Mechanisms of aneuploidy generation II
Chair: *Ana Losada*

May 28th **Session III: Modeling aneuploidy in mouse**
Chair: *Marcos Malumbres*

Session IV: The effects of aneuploidy. A therapeutic opportunity?
Chair: *Geert Kops*

May 29th **Session V: CIN genes in human cancer**
Chair: *René Medema*

Session I: Mechanisms of aneuploidy generation
Chair: *Robert Benezra*

9:30	**Welcome Address**
9:45 - 10:15	*Mechanisms of chromosomal instability in human tumor cells* **Duane Compton**, Geisel School of Medicine at Dartmouth, Hanover, USA
10:15 - 10:45	*Aneuploidy as a cause of chromosome mis-segregation* **Daniela Cimini**, Virginia Tech, Blacksburg, USA
10:45 - 11:05	*Connections between mitosis and DNA replication** **Rob Wolthuis**, The Netherlands Cancer Institute (NKI-AVL), Amsterdam, The Netherlands
11:05 - 11:40	*Coffee break*
11:40 - 12:10	*Poles take center stage: centrosome function and genome stability* **Fanni Gergely**, Cancer Research UK Cambridge Research Institute, UK
12:10 - 12:30	*Multiple functions for murine Cep63 in development** **Travis Stracker**, IRB Barcelona, Spain
12:30 - 12:50	*TCTP is a new POLO kinase substrate required for mitotic spindle assembly** **Alvaro Tavares**, University of Algarve, Faro, Portugal
12:50 - 13:10	*Resolution of DNA bridges at the midbody** **Oscar Fernández-Capetillo**, CNIO, Madrid, Spain
13:10 - 14:30	*Lunch*

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Session II: Mechanisms of aneuploidy generation II

Chair: *Ana Losada*

14:30 - 15:00 *Chromosome repair and segregation mechanisms in tumour suppression and cancer therapy*
Ashok Venkitaraman, University of Cambridge and the Medical Research Council Cancer Cell Unit, UK

15:00 - 15:30 *Integration of microtubule attachment and mitotic checkpoint signaling at the outer kinetochore*
Geert Kops, University Medical Center Utrecht, The Netherlands

15:30 - 15:50 *Catenation in metaphase engages distinctive SAC silencing requiring PKCε revealing a unique therapeutic window**
Nicola Brownlow, Cancer Research UK, London Research Institute, UK

15:50 - 16:50 *Coffee break and poster session*

16:50 - 17:20 *In vitro evolution to study genome stability and cancer*
David Pellman, Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA

17:20 - 17:40 *Uniform response to aneuploidy in human cells**
Zuzana Storchová, Max Planck Institute of Biochemistry, Martinsried, Germany

17:40 - 18:00 *Aurora B and PP2A antagonistically regulate the chromosomal targeting and activity of PP1/Repo-Man in mitosis**
Mathieu Bollen, University of Leuven, Belgium

18:00 - 18:20 *Aneuploidy-induced delaminating cells drive tumorigenesis in Drosophila epithelia**
Marco Milán, IRB Barcelona, Spain

18:20 *Poster Session*

Session III: Modeling aneuploidy in mouse

Chair: *Marcos Malumbres*

9:30 - 10:00 *Guarding the Genome: Centromeres, Centrosomes, Aneuploidy and Tumorigenesis*
Don Cleveland, Ludwig Institute for Cancer Research, University of California, San Diego, USA

10:00 - 10:30 *Modeling the cancer biology of whole-chromosome instability in the mouse*
Robert Benezra, Memorial Sloan-kettering Cancer Center, New York, USA

10:30 - 10:50 *Consequences of Mad2 induced chromosome instability in breast cancer**
Rocío Sotillo, EMBL-Monterotondo, Rome, Italy

10:50 - 11:20 *Group picture and Coffee break*

11:20 - 11:50 *The consequences of cohesin dysfunction in mouse cells*
Ana Losada, CNIO, Madrid, Spain

11:50 - 12:10 *Separase haploinsufficiency sensitizes mice to carcinogen-induced skin tumorigenesis**
Alberto M. Pendás, CIC Salamanca, Spain

12:10 - 12:40 *Aneuploidy in Health, Disease and Aging*
Jan Van Deursen, Mayo Clinic, Rochester, USA

12:40 - 13:00 *Spindle checkpoint deficiency is tolerated by murine epidermal cells but not hair follicle stem cells**
Floris Foijer, European Institute for the Biology of Ageing, Groningen, The Netherlands

13:00 - 14:15 *Lunch*

Session IV: The effects of aneuploidy. A therapeutic opportunity?

Chair: *Geert Kops*

14:15 - 14:45 *Aneuploidy and karyotype dynamics in cellular adaptation - lessons from the budding yeast*
Rong Li, Stowers Inst. for Medical Research, Kansas City, USA

14:45 - 15:15 *Cellular consequences of aneuploidy*
Angelika Amon, Koch Institute for Integrative Cancer Research, Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, USA

15:15 - 15:45 *Drugging Chromosome Instability to Target Cancer*
René Medema, The Netherlands Cancer Institute, Amsterdam, The Netherlands

15:45 - 16:45 *Coffee break and poster session*

16:45 - 17:15 *Targeting Mitotic Exit for Cancer Therapy*
Randall King, Harvard Medical School, Boston, USA

17:15 - 17:45 *Non-Genetic Heterogeneity in Response to Anti-Mitotic Chemotherapeutics*
Stephen Taylor, University of Manchester, UK

17:45 - 18:15 *Modeling Mitosis for Cancer Therapy*
Marcos Malumbres, CNIO, Madrid, Spain

18:15 *Poster Session*

Session V: CIN genes in human cancer

Chair: *René Medema*

9:30 - 10:00 *Chromosome Instability and Synthetic Lethality in Yeast and Cancer*
Philip Hieter, University of British Columbia, Vancouver, Canada

10:00 - 10:30 *Deciphering Mechanisms of Intratumour Heterogeneity*
Charles Swanton, Cancer Research UK, London Research Institute and UCL Hospitals/Cancer Institute, UK

10:30 - 10:50 *POT1 mutations cause telomere dysfunction in chronic lymphocytic leukemia**
Miguel Foronda, CNIO, Madrid, Spain

10:50 - 11:10 *CEP57 links an altered tumor microenvironment to mitotic infidelity in prostate cancer**
Stefan Duensing, University of Heidelberg, Germany

11:10 - 11:40 *Coffee break*

11:40 - 12:10 *Dissecting origin and impact of genomic rearrangements in cancer*
Jan Korbel, EMBL, Heidelberg, Germany

12:10 - 12:40 *Identification of a Genetic Cause of Aneuploidy in Human Cancer*
Todd Waldman, Georgetown University School of Medicine, Lombardi Comprehensive Cancer Center, Washington, USA

12:40 - 13:00 *Exome sequencing of urothelial bladder cancer shows that STAG2 inactivation is a common somatic event not associated with aneuploidy**
Cristina Balbás-Martínez, CNIO, Madrid, Spain

13:00 *Final Remarks*

**Short talk selected from the applications received*

Session I

Mechanisms of aneuploidy generation

Chair: Robert Benezra

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Duane Compton

Mechanisms of chromosomal instability in human tumor cells

Most solid tumors are aneuploid and many frequently mis-segregate whole chromosomes in a phenomenon called chromosomal instability (CIN). CIN positively correlates with poor patient prognosis indicating that reduced mitotic fidelity contributes to cancer progression by increasing genetic diversity among tumor cells. To determine the mechanism leading to the loss of mitotic fidelity in CIN, we used live cell imaging and clonal cell analyses to evaluate chromosome segregation in chromosomally stable and unstable human cells. These experiments revealed that the most common mitotic defect in tumor cells with CIN is the persistence of erroneous attachments of chromosomes to spindle microtubules (i.e. merotely). In normal diploid cells erroneous attachments arise spontaneously and are efficiently corrected to preserve genomic stability. However, kinetochore-microtubule attachments in cancer cells with CIN are inherently more stable that those in normal diploid cells. The observed differences in attachment stability account for the persistence of mal-attachments into anaphase, where they cause chromosome mis-segregation. Importantly, decreasing the stability of kinetochore-microtubule attachments by overexpression of the kinesin-13 proteins MCAK or Kif2b suppresses CIN and restores faithful chromosome segregation fidelity to aneuploid cancer cells. These data identify that cancer cells have a diminished capacity to correct erroneous kinetochore-microtubule attachments and ongoing work is revealing that there are numerous molecular targets that undermine the attachment stability of kinetochore-microtubules which accounts for the widespread occurrence of CIN in tumors.

Duane Compton
Geisel School of Medicine at Dartmouth, Hanover, USA

Daniela Cimini

Aneuploidy as a cause of chromosome mis-segregation

Cancer cells display distinct aneuploid karyotypes (i.e., abnormal chromosome numbers) and typically mis-segregate chromosomes at high rates, a phenotype referred to as chromosomal *in*stability (CIN). While it is readily apparent how chromosome mis-segregation can cause aneuploidy, the effect aneuploidy has on chromosome segregation is unclear. To test the effects of aneuploidy on chromosome segregation and cellular phenotype we utilized the colorectal cancer cell line DLD1 (2n=46) and variants of this line containing defined artificial trisomies for chromosomes 7 and 13 (DLD1+7 and DLD1+13, respectively). We found that DLD1+7 and DLD1+13 cells displayed higher rates of chromosome mis-segregation compared to the parental cell line. Furthermore, we found that cells with trisomy 13 display a distinctive cytokinesis failure phenotype. Interestingly, chromosome 13 encodes for *SPG20* (Spastic Paraplegia 20, or Spartin), a gene involved in cytokinesis completion. We showed that up-regulation of *SPG20*, brought about by trisomy 13, is both required and sufficient for the cytokinesis failure phenotype. Indeed, overexpression of Spartin in DLD1 cells reproduced the cytokinesis failure phenotype observed in DLD1+13 cells. We further determined that Spartin overexpression prevented localization of Spastin, another cytokinesis protein, at the midbody. Finally, we showed that siRNA-mediated Spartin knock down rescued both the cytokinesis failure defect and Spastin midbody localization in the DLD1+13 cell line. Overall, our study shows that aneuploidy per se induces chromosome mis-segregation in cancer cells. Moreover, our data indicate that different aneuploidies can yield distinct cellular phenotypes/bahaviors that are driven by up-regulation of specific genes encoded on the aneuploid chromosome.

Daniela Cimini
Virginia Tech, Blacksburg, USA

Short Talk

Rob Wolthuis

Connections between mitosis and DNA replication

Linda Clijsters, Erik Voets, Janneke Ogink, Veronica Delgado, **Rob Wolthuis**

Division of Cell Biology (B5), The Netherlands Cancer Institute / Antoni van Leeuwenhoek Hospital (NKI-AVL), Amsterdam, The Netherlands.

To protect ploidy and genomic integrity, initiation of a new round of DNA replication should be restricted until after completion of the previous mitosis. DNA replication depends on a preceding licensing event by Cdt1 and Cdc6. In animal cells, re-licensing after S-phase but before mitosis is blocked by the timely appearance of Cdt1-inhibitor geminin and by increasing mitotic cyclin activity, but how these two inhibitory pathways are scheduled in the cell cycle, and are feed-back controlled in preventing premature S-phase, is largely unknown. We investigated the roles of mitotic progression, the spindle checkpoint, and S-phase onset in the events that control DNA replication. We found that the spindle checkpoint and APC/C-Cdc20 schedule the degradation of geminin before that of the APC/C-Cdh1 substrate and critical pre-RC component, Cdc6. High APC/C-Cdc20 activity was permissive for licensing while high APC/C-Cdh1 activity reduced licensing competence right after mitosis. The spindle checkpoint, by protecting against geminin disappearance, also supported S-phase timing by directing the appearance of Cdt1, largely absent from S and G2 phase cells, and stabilizing it during prophase and prometaphase. We conclude that the spindle checkpoint, APC/C-Cdc20, and APC/C-Cdh1 act successively to ensure that the disappearance of licensing inhibitory factors coincides exactly with a peak of Cdt1 and Cdc6 in telophase. Whereas cell cycle entry from quiescence requires Cdc6 re-synthesis, our results indicate that proliferating cells can use a window of time in mitosis, before Cdc6 is degraded, as an earlier opportunity to direct S-phase. In RNAi-screens, we are now analyzing the molecular mechanisms by which misregulation of cyclin-Cdk1 and APC/C activity can threaten genomic stability.

Rob Wolthius
The Netherlands Cancer Institute (NKI-AVL), Amsterdam, The Netherlands

Mechanisms of aneuploidy generation

Fanni Gergely

Poles take center stage: centrosome function and genome stability

Mutations in numerous genes coding for centrosomal proteins cause primary recessive microcephaly (MCPH), a condition that results in reduced brain size. Like DNA, the centrosome duplicates once during a normal cell cycle. We have previously described a homozygous MCPH-causing mutation in the human CEP63 gene and demonstrated a requirement for CEP63 in centrosome duplication. CEP63 interacts with CEP152, a conserved centrosome duplication factor that is also mutated in MCPH. Thus, the CEP152-CEP63 protein complex is essential for controlling centrosome numbers, a role that seems especially important for human cerebral cortex growth. However, mutations in CEP152 and another of its binding partner, CPAP, also give rise to Seckel syndrome, a congenital disorder characterised by short stature, bone malformations and microcephaly. It is therefore feasible that MCPH and Seckel syndrome are caused by the same cellular defect, and may in fact represent a disease continuum. Nonetheless, the link between centrosome dysfunction and the etiology of these genetic conditions is elusive. We propose that centrosome abnormalities cause mitotic defects and aneuploidy *in vivo*, and these constitute the cellular cause of both MCPH and Seckel syndrome. With the aim of identifying a common underlying mechanism, I will present our most recent data regarding the roles of MCPH proteins and intact centrosomes in DNA damage signalling, cell cycle progression and genome stability.

Fanni Gergely
Cancer Research UK Cambridge Research Institute, UK

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Mechanisms of aneuploidy generation

Short Talk

Álvaro Tavares

TCTP is a new POLO kinase substrate required for mitotic spindle assembly

Cell Cycle and Cancer Biology Lab, Regenerative Medicine Program,
Departamento de Ciências Biomédicas e Medicina, Universidade do Algarve, Portugal.

Polo kinase has multiple functions throughout cell division. The diversity of these functions may rely not only on the different intracellular localization of the Polo protein kinase but also on that of its substrates. On a search for Drosophila Polo substrates associated with mitotic chromosomes we have isolated the Translationally Controlled Tumor Protein (TCTP). TCTP has been involved in several cellular processes, including cell cycle progression, malignant transformation and to have anti-apoptotic activity. But no mitotic function has been described so far. We have found that TCTP associates with chromatin and spindle specifically during M-phase, and this association is dependent on Polo kinase activity. We show that TCTP contributes for the timely assembly of the mitotic spindle and downregulation of TCTP results in a severe delay of the spindle assembly. Furthermore, we show that downregulation of TCTP in systems where the centrosomal pathway for spindle assembly is compromised prevents the formation of mitotic spindles. For example, while TCTP and CNN Drosophila mutants are viable, TCTP/CNN double mutant flies show a complete loss of embryonic viability due to an incapacity to form mitotic spindles during the embryonic divisions. Likewise, double TCTP/Plk4 double RNAi in cultured cells result in severe chromosome mislaignment, a phenotype not observed in any of the individual RNAis. Finally, we show that TCTP affects microtubule stability, both in interphase and mitosis. Thus, the requirement for TCTP during mitosis seems to increase with loss of centrosome function and is dependent on Polo kinase activity. We propose that, early in mitosis and after Polo phosphorylation, TCTP associates with chromatin, where it promotes microtubule stabilization thus contributing to timely mitotic spindle assembly.

Álvaro Tavares
Universidade do Algarve, Faro, Portugal

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Mechanisms of aneuploidy generation

Short Talk

Oscar Fernández-Capetillo

Resolution of DNA bridges at the midbody

Juan Luis Rodriguez-Barbancho, Maria Nieto and **Oscar Fernández-Capetillo**

The midbody is a cytological structure that is involved in the latest steps of cytokinesis. To date, a role for the midbody in genome maintenance is not known. Here we show a localized activation of the DDR in midbodies that persist between seemingly independent interphase nuclei. The observed pattern is consistent with a central DNA cleavage, with ssDNA binding proteins like RPA being surrounded by chromatin binding molecules such as 53BP1. DNA bridges connecting both nuclei are observed at some of these structures, which can be induced by genetic and chemical conditions that promote the formation of anaphase bridges. Inhibition of Plk1 or SMC2 depletion abrogates these structures and promotes the accumulation of binucleated cells. We propose that these structures represent the latest step in the resolution of DNA bridges that persist beyond mitosis, and suggest an active role for the midbody-localized Plk1 in promoting a controlled resolution of DNA bridges.

Oscar Fernández-Capetillo
CNIO, Madrid, Spain

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Session II

Modeling aneuploidy generation II

Chair: Ana Losada

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Ashok Venkitaraman

Chromosome repair and segregation mechanisms in tumour suppression and cancer therapy

Instability in the structure and number of chromosomes is a hallmark of epithelial malignancies arising from defects in the normal mechanisms that control chromosome repair and segregation. We study these mechanisms to better understand the pathogenesis of cancer, and to exploit this understanding in improved approaches for therapy. One focus concerns germline mutations affecting the *BRCA2* gene, which predispose to cancers of the breast, pancreas and other organs. We have shown that BRCA2 disruption causes defects in chromosome structure (through an essential role in the regulation of RAD51 recombinase during DNA repair by homologous recombination) as well as to aneuploidy (through defects in chromosome segregation whose mechanism remains uncertain). We have developed a genetically engineered mouse model that recapitulates features of tumour predisposition associated with *BRCA2* mutations. It unexpectedly demonstrates that BRCA2 heterozygosity suffices for tumourigenesis in mice and men, with implications for the use of targeted therapies in the clinic. A second focus concerns the mechanisms of resistance to anti-mitotic chemotherapeutic agents such as taxanes. Taxanes activate the spindle assembly checkpoint (SAC) to arrest anaphase onset, but taxane-exposed cells eventually undergo slippage to exit mitosis. The therapeutic efficacy of taxanes depends on whether slippage after SAC arrest culminates in continued cell survival, or in death by apoptosis. However, the mechanisms that determine these outcomes remain unclear; we have explored them using functional genomic approaches. One critical determinant we have identified is UBE2S, a ubiquitin E2 ligase whose depletion markedly prolongs drug-induced mitotic arrest, but has a relatively small effect on normal mitotic progression. Biochemical studies suggest that UBE2S acts to elongate ubiquitin chains on APC/C substrates that have been initiated by E2 proteins such as UBCH5 or UBCH10, suggesting a two-step mechanism for the activity of the human APC/C E3 ligase that is involved in the cellular response to anti-mitotic therapies.

Ashok Venkitaraman
University of Cambridge and the Medical Research Council Cancer Cell Unit, UK

Mechanisms of aneuploidy generation II

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Geert Kops

Integration of microtubule attachment and mitotic checkpoint signaling at the outer kinetochore

Mathijs Vleugel[1], Saskia JE Suijkerbuijk[1], Eelco C. Tromer[1,2], Manja Omerzu[1], Wilco Nijenhuis[1], Berend Snel[2], and **Geert J.P.L. Kops[1]**

[1]Dept of Medical Oncology and Dept of Molecular Cancer Research, UMC Utrecht, The Netherlands
[2]Theoretical Biology and Bioinformatics, Department of Biology, Utrecht University, The Netherlands

Chromosome biorientation and mitotic checkpoint signaling are two processes that are essential for fidelity of chromosome segregation in mitosis. The outer kinetochore scaffold KNL1/CASC5 plays a central role in both processes, as it is a functional substrate of multiple biorientation and checkpoint kinases, recruits several phosphatases, directly binds microtubules and localizes the paralogs BUBR1 and BUB1 that perform various roles in the checkpoint and in biorientation. Our recent studies on evolution and function of the KNL1-BUB-kinase/phosphatase network have provided insights into how microtubule attachment, the mitotic checkpoint and regulation of these processes are integrated at kinetochores. KNL1 was identified as an oncogenic fusion partner of the MLL gene, and mutations in BUBR1 cause the hereditary cancer predisposition syndrome MVA (mosaic variegated aneuploidy). Understanding the KNL1-BUB network will therefore contribute to understanding how mutations in this network promote chromosomal instability.

Geert Kops
Molecular Cancer Research, University Medical Center Utrecht, The Netherlands

Short Talk

Nicola Brownlow

Catenation in metaphase engages distinctive SAC silencing requiring PKCε revealing a unique therapeutic window

Nicola Brownlow, Tanya Pike, Daniel Zicha, Ken Blight, Peter Parker.

Protein Phosphorylation laboratory, Cancer Research UK London Research Institute and King's College London, UK

Mechanisms that monitor correct chromosome segregation during mitosis are vital to maintenance of genome stability. The spindle assembly checkpoint (SAC) maintains inhibition of the anaphase promoting complex until correct attachment and biorientation of sister chromatids is complete. Once this is achieved the cell is ready to complete chromosome disjunction before commitment to physical separation of sister chromatids in anaphase. Disjunction requires degradation of cohesin and completion of decatenation. Here we describe a regulatory pathway that ensures that chromosome disjunction is complete before anaphase onset, protecting the dividing cell from anaphase chromosome bridging and unequal chromosome segregation to daughter cells. This regulatory pathway is triggered by DNA catenation in metaphase and is regulated independently of the G2 catenation checkpoint. We have defined key players in the checkpoint and show here that PKCε is essential to trigger a unique spindle checkpoint silencing process that modulates the kinetochore microtubule streaming activity of the RZZ complex. This process is invoked after initial satisfaction of the SAC at the metaphase to anaphase boundary and results in a characteristic kinetochore retention of a subset of checkpoint components. We describe how this pathway is an essential failsafe mechanism protecting cells from entering anaphase with excess catenation when the G2 catenation checkpoint fails and therefore that this checkpoint is particularly important in cancer cells that have a damaged or absent G2 catenation checkpoint. These cells display an emergent dependence on PKCε to prevent catastrophic cell division and therefore reveal a therapeutic target with selectivity for transformed cells.

Nicola Brownlow
Cancer Research UK London Research Institute, UK

Mechanisms of aneuploidy generation II

David Pellman

In vitro evolution to study genome stability and cancer

Activation of p53 serves as a barrier to the proliferation of polyploid and aneuploid cells. I will discuss new approaches, using in vitro evolution, to define mechanisms enabling human cells to spontaneously bypass p53.

David Pellman
Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Short Talk

Zuzana Storchová

Uniform response to aneuploidy in human cells

Milena Dürrbaum1,3, Anastasia Yurievna Kuznetsova1, Verena Passerini1, Silvia Stingele1, Gabriele Stoehr2, **Zuzana Storchová1,3**

1- Group Maintenance of Genome Stability, Max Planck Institute for Biochemistry, Martinsried, Germany
2- Department of Proteomics and Signal Transduction, Max Planck Institute for Biochemistry, Martinsried, Germany
3- Center for Integrated Protein Science Munich, Ludwig-Maximilian-University Munich, Germany

Aneuploidy, a karyotype deviating from multiples of a haploid chromosome set, affects the physiology of eukaryotes. In humans, aneuploidy is linked to pathological defects such as developmental abnormalities, mental retardation or cancer, but the underlying mechanisms remain elusive. There are many different types and origins of aneuploidy, but whether there is a uniform cellular response to all types of aneuploidy in human cells remains unknown. Here we evaluate the transcription profiles of twelve model trisomic and tetrasomic cell lines and two cell lines with complex aneuploid karyotypes. We identify a characteristic aneuploidy response pattern defined by up-regulation of genes linked to endoplasmic reticulum, Golgi apparatus and lysosomes, and down-regulation of DNA replication and transcription as well as ribosomes. Strikingly, complex aneuploidy elicits the same transcriptional changes as trisomy. To uncover the triggers of the response we compared the profiles with transcription changes in human cells subjected to stress conditions. Interestingly, we found an overlap only with the response to treatment with the autophagy inhibitor bafilomycin A1. Finally, we identified 23 genes whose expression is significantly altered in all aneuploids and which may thus serve as aneuploidy markers. Our analysis shows that despite the variability in chromosome content, aneuploidy triggers uniform transcriptional response in human cells. A common response independent of the type of aneuploidy might be exploited as a novel target for cancer therapy. Moreover, the potential aneuploidy markers identified in our analysis might represent novel biomarkers to assess the malignant potential of a tumor.

Zuzana Storchová
Max Planck Institute of Biochemistry, Martinsried, Germany

Mechanisms of aneuploidy generation II

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Short Talk

Mathieu Bollen

Aurora B and PP2A antagonistically regulate the chromosomal targeting and activity of PP1/Repo-Man in mitosis

Junbin Qian, Monique Beullens, Bart Lesage and **Mathieu Bollen**

University of Leuven, Department of Cellular and Molecular Medicine, Laboratory of Biosignaling & Therapeutics, Belgium

Aurora B is the catalytic subunit of the chromosomal passenger complex (CPC) and coordinates essential mitotic events through phosphorylation of key regulatory proteins. In prometaphase the CPC is enriched at the centromeres and regulates chromosome segregation. The centromeric targeting of the CPC involves the phosphorylation of histone H3 at Thr3 (H3T3ph) by Haspin, which creates a docking site for the CPC subunit Survivin. PP1/Repo-Man acts antagonistically to Haspin and dephosphorylates H3T3ph at the chromosome arms during (pro) metaphase. PP1/Repo-Man does not dephosphorylate centromeric H3T3ph until anaphase but the underlying mechanism is not known. Here, we show that Aurora B phosphorylates Repo-Man at Ser893, which prevents its binding to core histones and the dephosphorylation of H3T3ph by associated PP1. We also identify the B56-containing PP2A phosphatase holoenzyme as a mitotic interactor of Repo-Man that dephosphorylates Ser893, enabling the dynamic targeting and dephosphorylation of H3T3ph by PP1. Thus, Repo-Man associated PP1 and PP2A collaborate to oppose Aurora B by distinct mechanisms. We propose that the antagonistic regulation of Repo-Man by Aurora B and PP2A is an essential component of the selective centromeric targeting of the CPC during prometaphase.

Mathieu Bollen
University of Leuven, Belgium

Mechanisms of aneuploidy generation II

Short Talk

Marco Milán

Aneuploidy-induced delaminating cells drive tumorigenesis in drosophila epithelia

Andrés Dekanty, Lara Barrio, Mariana Muzzopappa,and **Marco Milán**

ICREA and IRB Barcelona, Spain

Genomic instability has been observed in essentially all sporadic carcinomas. Here we use Drosophila epithelial cells to address the role of chromosomal instability in cancer development as they have provided useful model systems to elucidate the molecular mechanisms underlying tumorigenic growth. We first show that chromosomal instability leads to an apoptotic response. Interestingly, this response is p53 independent, as opposed to mammalian cells, and depends on the activation of the c-Jun N-terminal kinase (JNK) signaling cascade. When prevented from undergoing programmed cell death, chromosomal instability induces neoplasic overgrowth. These tumor-like tissues are able to grow extensively and metastasize when transplanted into the abdomen of adult hosts. Detailed analysis of the tumors allows us to identify a delaminating cell population as the critical one in driving tumorigenesis. Cells loose their apical-basal polarity, mis-localize DE-Cadherin and delaminate from the main epithelium. A JNK dependent transcriptional program is activated specifically in delaminating cells and drives non-autonomous tissue overgrowth, basement membrane degradation and invasiveness. These findings unravel a general and rapid tumorigenic potential of genomic instability, as opposed to its proposed role as a source of mutability to select specific tumor-prone aneuploid cells, and open new avenues towards the understanding of the role of genomic instability in human cancer.

Marco Milán
IRB Barcelona, Spain

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Session III

Modeling aneuploidy in mouse

Chair: Marcos Malumbres

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Don Cleveland

Guarding the genome: centromeres, centrosomes, aneuploidy and tumorigenesis

Noting a correlation of aneuploidy with tumorigenesis, more than 100 years ago Boveri proposed aneuploidy as a cause of tumorigenesis. Boveri's hypothesis is now being tested in mice in two ways. First, unattached centromeres are also responsible for the mitotic checkpoint, the major cell cycle control mechanism that acts to maintain diploid chromosome content. Prevention of premature onset of anaphase requires a set of components that act at centromeres to generate a diffusible "wait anaphase" inhibitor. The mitotic checkpoint can be weakened in mice by reduction of CENP-E, a highly processive, centromere-associated kinesin required for stable capture by each centromere of the correct number of spindle microtubules. Reduction in CENP-E produces near diploid aneuploidy from missegregation of whole chromosomes. Second, increased expression of the Plk4 kinase has been used to produce centrosome amplification, thereby generating multipolar spindles that produce more robust chromosome missegregation. Increasing whole chromosomal aneuploidy has revealed that whole chromosomal aneuploidy can act oncogenically, but depending on the preceding genetic damage chromosomal instability can also play a previously unsuspected role in preventing tumorigenesis.

Don Cleveland
Ludwig Institute for Cancer Research, University of California, San Diego, USA

Robert Benezra

Modeling the cancer biology of whole-chromosome instability in the mouse

Pascal H.G. Duijf[1], Rosario Thomas[2], Daniel Marks, Juan-Manuel M. Schvartzman[1], Rocio Sotillo[1], Nicholas D. Socci[3], Courtney Coker[1], and **Robert Benezra[1]**

[1]Cancer Biology and Genetics Program, [2]BCMB Program of Cornell University, [3]Computational Biology Center, Memorial Sloan-Kettering Cancer Center, New York, USA.

We have shown recently that the loss of the tumor supressors p53 or Rb leads to the upregulation of Mad2, an essential component of the mitotic checkpoint (MC) pathway. Mad2 overexpression by itself both in vitro and in animal models leads to mitotic delay, whole chromosome instability (W-CIN) and DNA damage, thereby establishing a hard-wired connection between tumor initiation and reduced apoptosis with mitotic stress and increased mutation rates. This process has been referred to as "oncogene-induced mitotic stress" (OIMS). Myc overexpression may also contribute to OIMS as it can directly activate the expression of both Mad2 and BubR1 (another component of the MC pathway) and the consequences of dual overexpression are being explored. Mad2 overexpression and the incipient CIN and DNA damage enhances the tumor growth of Kras induced lung tumor growth and importantly leads to dramatically enhanced relapse rates after Kras inhibition suggestion that OIMS may contribute to drug resistance in a variety of tumor types.

In order to further explore W-CIN in human cancers, we examined whether chromosome gains or losses are equally distributed across the human genome in 19,000 tumors catalogued in the Mittleman database. We find that chromosomes are lost significantly more frequently than gained and that there is a strong inverse correlation between the frequency of chromosome loss and size. Rarer gains show no correlation with size or gene number suggestion that proteotoxic stress may not be a major barrier to tumor progression in humans. Finally, we are utilizing a system for inter-chromatid recombination to induce chromosome specific losses and are examining the effects on immortalization, transformation and metabolic changes both *in vitro* and in animal models.

Robert Benezra
Memorial Sloan-Kettering Cancer Center, New York, USA

Rocío Sotillo

Short Talk

Consequences of Mad2 induced chromosome instability in breast cancer

Konstantina Rowald, Martina Mantovan, Cristina Aguirre, Martin Jechlinger, **Rocio Sotillo***
European Molecular Biology Laboratory, Mouse Biology Unit, Monterotondo, Italy

Chromosome instability (CIN) is a hallmark of solid tumors. Mad2 is frequently upregulated in human tumors and its overexpression in a transgenic mouse leads to aneuploid tumors.
To determine whether Mad2 overexpression could accelerate lung tumor formation driven by a classical oncogene, we bred Mad2 transgenic mice with mice expressing rtTA-responsive murine oncogenic K-RasG12D. We observed that tumor appearance is enhanced when Mad2 and KrasG12D are co-expressed, resulting in a decrease in survival. We next tested if Mad2 accelerates tumorigenesis in a different epithelial tissue. Using a similar strategy we crossed Mad2 and Kras transgenic mice with mice expressing the rtTA under the MMVT promoter to achieve transgene expression in mammary epithelial cells. We have observed that mammary tumor development is significantly delayed when Mad2 is co-expressed together with Kras, compared to Kras mice alone. These results suggest that aneuploidy can play a dual role as a tumor promoter and as tumor suppressor depending on the cellular context.
Resistance of certain tumors to targeted therapy might be elicited by mechanisms that cause chromosomal instability (CIN) and therefore induced additional genetic changes in a subset of tumor cells. In fact, we have recently shown that CIN facilitates escape from Kras oncogene addiction in a mouse model of lung adenocarcinoma and increases tumor relapse. Similarly, we have observed that mammary tumor relapse is increased in mice that had expressed Mad2 and Kras, despite their delay in primary tumor formation. These results argue that even successful targeted therapy of a known driving oncogene might fail in the clinic as a result of instability in the primary tumor. We are now investigating the mechanism of how CIN promotes tumor relapse using mouse genetic strategies in combination with innovative 3D in vitro culture systems to recapitulate acquired resistance and to follow single cells during tumor initiation and regression.

Rocío Sotillo
EMBL-Monterotondo, Rome, Italy

Modeling aneuploidy in mouse

Ana Losada

The consequences of cohesin dysfunction in mouse cells

Cohesin is a ring shaped complex that mediates sister chromatid cohesion and participates in the organization of interphase chromatin through DNA looping. In this way, it contributes to accurate DNA repair and chromosome segregation, to efficient DNA replication and to transcriptional regulation. Cohesin consists of Smc1, Smc3, Rad21/Scc1 and SA/Scc3. Three additional proteins known as Pds5, Wapl and Sororin bind cohesin and modulate its dynamic association with chromatin. Vertebrate cells have two versions of the SA cohesin subunit, SA1 and SA2, and two versions of Pds5, Pds5A and Pds5B. We have generated mice carrying null alleles for the genes encoding SA1, Pds5A and Pds5B, and shown that each of them is embryonic lethal in homozygosity. Cells derived from null embryos are being characterized in order to understand the specific functions of the different cohesin complexes. We have learnt that cohesin-SA1 is essential for telomere cohesion and in its absence telomere replication is faulty and leads to chromosome missegregation. Cohesin-SA1 plays also a major role in gene regulation. Importantly, SA1 haploinsufficiency promotes pancreatic cancer development and we are currently exploring the underlying reasons. In the case of the Pds5 proteins, we have found a specific role of Pds5B in centromeric cohesion. Our most recent results using these mouse models will be presented.

Ana Losada
CNIO, Madrid, Spain

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Short Talk

Alberto M. Pendás

Separase haploinsufficiency sensitizes mice to carcinogen-induced skin tumorigenesis

Gutiérrez-Caballero, C1; * Llano, E1,2; Herrán, Y1 , García-Tuñón I1, de Alava, E;
Sánchez-Martín3, M; and **Pendás, AM*1**

1- Instituto de Biología Molecular y Celular del Cáncer (CSIC-USAL). Campus Miguel de Unamuno, Salamanca, Spain.
2- Departamento de Fisiología, Campus Miguel de Unamuno, Salamanca, Spain.
3- Departamento de Medicina, Campus Miguel de Unamuno, Salamanca, Spain.

In order to maintain genome stability, the genetic material must be equally distributed between the two daughter cells during mitosis. To ensure this faithful segregation, sister chromatids are held together until the onset of anaphase by an evolutionarily conserved complex called cohesin. This proteinaceous complex is constituted by four subunits: two members of the family of structural maintenance of chromosome proteins (SMC1α and SMC3), one kleisin subunit (Scc1/RAD21), and a HEAT repeat domain protein (SA1/STAG1 and SA2/STAG2). At the onset of anaphase, chromatid segregation is enabled by cleavage of the centromeric cohesins at their Scc1/RAD21 subunit by the unique cisteín protease separase. Dysregulation of separase has been reported in human cancers suggesting a direct or indirect role in the transformation process. In order to evaluate in vivo the involvement of separase in carcinogenesis, we have developed a genetically engineered mouse model of separase. We conclude that separase haploinsufficiency has no effect on the proliferation properties, immortalization or senescence of embryonic fibroblasts, suggesting that haploinsufficiency of separase is sufficient for normal cell cycle progression in vivo. However, the decrease of separase facilitates cell transformation by E1A and Ras oncogenes. To ascertain the in vivo consequences of this observation, Separase haploinsufficient mice were subjected to a two stage skin carcinogenesis model (TPA/DMBA) and showed a drastic sensitization to carcinogen-induced skin tumors (rapid initiation and progression) in comparison with their wild type controls. These mice constitute a new experimental model to study the direct impact of separase downregulation in the mechanism of papilloma initiation and development.

Alberto M. Pendás
CIC Salamanca, Spain

Jan Van Deursen

Aneuploidy in health, disease and aging

Aneuploidy, an aberrant number of chromosomes, has been recognized as a feature of human malignancies for over a century but compelling evidence for causality was largely lacking until mouse models for chromosome number instability were utilized. These *in vivo* studies have not only uncovered important new insights into the extremely complex aneuploidy-cancer relationship, but also into the molecular mechanisms underlying proper and aberrant chromosome segregation. A series of diverse mouse models for the mitotic checkpoint protein BubR1 have provided evidence for a provocative novel link between aneuploidization and the development of age-related pathologies which will be at the center of the talk.

Jan Van Deursen
Mayo Clinic, Rochester, USA

Floris Foijer

Short Talk

Spindle checkpoint deficiency is tolerated by murine epidermal cells but not hair follicle stem cells

Floris Foijer1, Tia DiTommaso2, Giacomo Donati3, Katta Hautaviita4, Stephanie Xie5 Emma Heath3, Ian Smyth2, Fiona M Watt3, Peter K Sorger5 and Allan Bradley4

1- European Institute for the Biology of Aging (ERIBA), University of Groningen, Groningen, the Netherlands
2- Department of Biochemistry and Molecular Biology, Monash University, Clayton, Australia
3- Centre for Stem cells and Regenerative Medicine King's College London, Guy's Hospital, London, United Kingdom
4- Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, UK
5- Department of Systems Biology, Harvard Medical School, Boston, Massachusetts, USA

Whole chromosome instability (wCIN) is a condition detrimental for the fitness and survival of normal cells, but also a hallmark of cancer cells. We are interested in the consequences of wCIN for tissue and somatic stem cell integrity and the relationship between wCIN, ageing and cancer. One way to provoke wCIN in the mouse is by inactivating this checkpoint., For this purpose, we developed mouse models in which we can inactivate the spindle assembly checkpoint (SAC) in a conditional fashion, allowing us to provoke wCIN in tissues of choice during embryogenesis or in the adult.

To selectively induce wCIN and aneuploidy in the skin, we generated a conditional allele for Mad2, an essential component of the SAC signaling cascade, which was employed to inactivate the SAC in mouse epidermis. Whereas Mad2 deficiency results in rapid cell death in vitro, we found that despite dramatic aneuploidy, in vivo SAC inactivation is tolerated by the interfollicular epidermal (IFE) cells (basal and stratified cells in the skin). So these mice have a functional skin albeit without any hair. Consistent with aneuploid cell state, Mad2 deficient IFE cells in this epidermis exhibit abnormal transcription of metabolic genes and express high levels of p19ARF, suggestive of accelerated skin ageing. Conversely, the hair follicle bulge stem cells that are responsible for hair growth are completely absent, even though rudimentary hair follicles are continuously present, explaining the loss of hair in SAC-deficient skin.

Our results indicate that hair follicle stem cells are intrinsically more sensitive to chromosomal instability than (more differentiated) IFE cells. We are currently investigating the mechanisms that underlie this differential response, and whether similar differential responses also occur in other tissues. By understanding the molecular responses to aneuploidy, we hope to uncover pathways that can be employed to specifically target aneuploid cell progeny in cancer.

Floris foijer
European Institute for the Biology of Ageing, Groningen, The Netherlands

Modeling aneuploidy in mouse

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Session IV

The effects of aneuploidy. A therapeutic opportunity?

Chair: Geert Kops

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Rong Li

Aneuploidy and karyotype dynamics in cellular adaptation – lessons from the budding yeast

We use the budding yeast *Saccharomyces cerevisiae* as the experimental model to gain fundamental insights into how aneuploidy, as a frequently observed genome variation in eukaryotes, affects gene expression, chromosome stability and cellular adaptation. We have developed several new methods to generate isogenic aneuploid strains and to follow karyotype changes in populations exposed to different types of stress. These analyses led us to obtain several observations on the relationship between aneuploidy and cellular stress, and the profound impact of chromosome copy number changes on gene expression. I will discuss our recent results on the mechanism by which aneuploidy modulates gene expression and genome stability and a possible approach to control aneuploid populations by harnessing karyotype dynamics.

Rong Li
Stowers Institute for Medical Research, Kansas City, USA

The effects of aneuploidy. A therapeutic opportunity?

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Angelika Amon

Cellular Consequences of Aneuploidy

Stefano Santaguida, Ana Oromendia, Yun-Chi Tang, Jason Sheltzer and **Angelika Amon**

Koch Institute for Integrative Cancer Research, Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, USA

Aneuploidy is a hallmark of cancer. Understanding how aneuploidy impacts cell physiology is thus vital for understanding the principles underlying tumor formation. We developed yeast and mouse models to study the effects of aneuploidy on cell physiology. Our analyses revealed that the condition causes chromosome-specific phenotypes, and, remarkably, phenotypes shared by many different aneuploid yeast and mouse cells, which we collectively call the aneuploidy-associated stresses. Among, these stresses, proteotoxic stress caused by aneuploidy-induced proteomic changes appear especially prominent. We will discuss how aneuploidy affects protein quality control systems in both yeast and mammalian cells and elicits. Furthermore, we will discuss the discovery that aneuploidy leads to a conserved gene expression signature that is indicative of slow growth and cellular stress.
We will also discuss the paradox that despite the adverse effects of aneuploidy on cell physiology, tumor cells, who are characterized by high proliferative potential, are highly aneuploid. Our data indicate that whole chromosome aneuploidy causes further genome instability, providing a potential explanation for how the condition contributes to tumorigenesis.

Angelika Amon
Koch Institute for Integrative Cancer Research, Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, USA

René Medema

Drugging Chromosome Instability to Target Cancer

Aniek Janssen[1], Ritan Maia[1], Andre Koch[1], Marja van der Burg[2], Karoly Szuhai[2], Geert J.P.L. Kops[3] and **René H. Medema[1].**

[1]Division of Cell Biology, Netherlands Cancer Institute, Amsterdam.
[2]Department of Molecular Cell Biology, Leiden University Medical Center, Leiden.
[3]Department of Molecular Cancer Research University Medical Center Utrecht, Utrecht, The Netherlands.

Various types of chromosomal aberrations, including numerical (aneuploidy) and structural (e.g. translocations, deletions), are commonly found in human tumors and are linked to tumorigenesis. Aneuploidy is a direct consequence of chromosome segregation errors in mitosis, while structural aberrations are caused by improperly repaired DNA breaks. We have recently shown that chromosome segregation errors can also result in structural chromosome aberrations. Chromosomes that missegregate are frequently damaged during cytokinesis, triggering a DNA double strand break response in the respective daughter cells involving ATM, Chk2 and p53. Importantly, we found that these double strand breaks can lead to unbalanced translocations in the daughter cells. Our data uncover a previously unrecognized mechanism to generate chromosomal aberrations and provide novel insights on the role of whole-chromosome instability in tumorigenesis. Our recent efforts to exploit this mechanism to selectively inhibit proliferation of tumor cells will be discussed.

Talk under the auspices of AICR

René Medema
The Netherlands Cancer Institute, Amsterdam, The Netherlands

The effects of aneuploidy. A therapeutic opportunity?

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Randall King

Targeting Mitotic Exit for Cancer Therapy

Microtubule inhibitors, such as taxanes, represent one of the most widely used treatments for cancer. Despite their widespread use, we still do not fully understand how taxanes selectively kill cancer cells. One hypothesis is that taxanes arrest cancer cells in mitosis, an inherently pro-apoptotic state. By perturbing microtubule function, taxanes activate the spindle assembly checkpoint, which delays exit from mitosis. Because a primary function of the SAC is to inhibit the ubiquitin ligase activity of the Anaphase-Promoting Complex/Cyclosome (APC), direct APC inhibitors might induce mitotic arrest without toxicities such as neuropathy associated with microtubule perturbation. Furthermore, taxane-treated cells can exit mitosis before dying, through a process of “mitotic slippage” that is a result of incomplete inhibition of the APC by the SAC. Direct inhibition of APC may therefore be a more effective way of inducing mitotic arrest than stimulating the SAC. Using a phenotypic screen in *Xenopus* cell cycle extracts, we identified TAME (tosyl-L-arginine methyl ester) as an inhibitor of the APC. TAME binds to the APC and prevents its activation by Cdc20 and Cdh1. Furthermore, we developed a cell-permeable derivative, proTAME, which causes a surprisingly robust mitotic arrest. In contrast, SAC-activating compounds such as microtubule inhibitors do not suppress APC activity as completely. As a result, cells rely on continued protein synthesis to maintain mitotic arrest, providing an explanation for the known variability in cellular response to microtubule inhibitors. Here I will present an update on our work to understand the mechanism by which TAME inhibits the APC and blocks mitotic exit in cells, as well as discuss new approaches to inhibit APC-dependent proteolysis and mitotic exit.

Randall King
Harvard Medical School, Boston, USA

Stephen Taylor

Non-Genetic Heterogeneity in Response to Anti-Mitotic Chemotherapeutics

Compounds targeting mitosis are widely used as chemotherapy agents: in the UK, ~70% of women with ovarian cancer are prescribed the microtubule stabilizer Taxol. While unclear exactly how microtubule toxins exert anti-tumour effects in patients, interfering with microtubule dynamics during mitosis activates the spindle checkpoint causing prolonged mitotic arrest. Cells either then undergo "*death in mitosis*" (DiM), or "*slippage*", i.e. they return to interphase without dividing. What dictates the balance between these two fates is unclear. Also, genetically identical cells can undergo either fate, indicating that this phenomenon is subject to non-genetic heterogeneity. Previously, we proposed a new model whereby DiM vs. slippage is determined by two competing networks, one activating cell death pathways, the other protecting cyclin B1 from degradation [Gascoigne & Taylor, 2008]. These networks work in opposite directions; while death signals become stronger, cyclin B1 levels fall, and both networks have thresholds so that cell fate is dictated by whichever threshold is breached first. To test this model, we performed a genome-wide siRNA screen, using a line that predominantly undergoes DiM. Cells were transfected, challenged with a lethal dose of Taxol and viability measured 48 hours later. Primary hits were re-screened using independent siRNAs, yielding a subset for more detailed analysis. This yielded several siRNAs that specifically inhibit DiM: cells enter mitosis normally and arrest for as long as controls. However, now DiM is inhibited in favour of slippage, demonstrating the existence of genes that control mitotic cell fate. One of these hits is a transcription factor and the talk will focus on our latest data characterising how this transcription factor regulates mitotic cell fate. Moreover, this transcription factor may also provide an excellent candidate to account for the non-genetic heterogeneity that is observed following a prolonged mitotic arrest.

Stephen Taylor
University of Manchester, UK

The effects of aneuploidy. A therapeutic opportunity?

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Marcos Malumbres

Modeling Mitosis for Cancer Therapy

Multiple anti-mitotic therapies are currently evaluated in preclinical and clinical studies for cancer treatment. Inhibition of major mitotic kinases and kinesins has resulted in encouraging therapeutic benefits in preclinical models. However, the clinical activity of inhibitors against these proteins is modest as best. Some problems associated to these trials include the essential role of these proteins for proliferation of both tumor and normal cells, and further therapeutic strategies will benefit from the analysis of the mitotic stress generated by oncogenic signals. In addition, the success of current strategies are limited by the low mitotic index of human tumors and mitotic slippage, a process that drives the exit from mitosis even in the presence of these inhibitors. The Anaphase-promoting Complex/Cyclosome (APC/C) is an E3 ubiquitin ligase required for degradation of cyclin B1 and securin and mitotic exit. In the absence of APC/C activity, cells arrest in metaphase and they are unable to exit mitosis, suggesting that this complex is essential for mitotic slippage. In these conditions, cyclin B1 accumulates and Cdk1 activity remains high until cells die. The modulation of mitotic cell death (MCD) can therefore be exploited as a strategy to increase the efficacy of current chemotherapeutic agents. However, an accurate and consistent definition of MCD is still lacking. MCD is known to exhibit features that resemble the apoptotic pathway (caspase activation and cytochrome c release) but caspase-independent pathways may also be involved. Work in our laboratory is focused to investigate the physiological relevance ofmajor mitotic kinases such as members of the Aurora or Polo-like kinase families as well as new enzymatic activities with therapeutic potential. Understanding the relevance of these targets and their therapeutic value in preclinical models will contribute to improve the efficacy of therapeutic strategies in cancer.

Marcos Malumbres
CNIO, Madrid, Spain

Session V

CIN genes in human cancer

Chair: René Medema

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Philip Hieter

Chromosome Instability and Synthetic Lethality in Yeast and Cancer

Philip Hieter, Melanie Bailey, Nigel O'Neil, Derek van Pel and Peter Stirling

Michael Smith Laboratories and Department of Medical Genetics, University of British Columbia, Vancouver, Canada

Genes that maintain chromosome stability (CIN genes) are conserved in eukaryotes and are often somatically mutated in cancer. We have been identifying synthetic lethal partner genes, in yeast synthetic lethal (SL) interaction networks, that are highly connected with sets of CIN genes somatically mutated in cancer. This identifies hub proteins and processes that are candidate targets for synthetic lethal killing of cancer cells with defined CIN gene somatic mutations. One hub process in these networks is DNA replication. The protein product of FEN1 (encoding flap endonuclease) was used as a target for small-molecule inhibitor screening using a fluorescence-based assay for enzyme activity. Inhibitors of FEN1 activity in vitro were shown to selectively inhibit the proliferation of cultured cancer cells carrying inactivating mutations in CDC4, or knockdown or inhibition of MRE11A, two genes frequently mutated in a variety of cancers. Analysis of synthetic lethal interactions with cohesin gene mutations, a class of somatic mutation found in several tumor types, found that cohesin mutants require the function of genes that mediate replication fork progression. PARP1 has roles in the DNA damage response but also the restart of stalled replication forks. We found that cohesin mutants exhibited synthetic lethal interactions with PARP mutants in C. *elegans*, and demonstrated that this interaction is conserved in human cells by showing that PARP inhibitors reduce the viability of cultured human cells depleted for cohesin components. Solomon et al (Science 333:1039-43, 2011) have shown that the cohesin subunit gene, STAG2, is recurrently mutated in glioblastoma, Ewing's sarcoma, and melanoma tumors. Using matched glioblastoma cell lines containing either a truncated STAG2 or wild-type STAG2 knock-in, we found that STAG2 mutants undergo significantly decreased proliferation in the presence of PARP inhibition, suggesting that PARP inhibitors may be effective in treating cancers carrying somatic mutations in cohesin genes.

Philip Hieter
University of British Columbia, Vancouver, Canada

Charles Swanton

Deciphering Mechanisms of Intratumour Heterogeneity

The majority of metastastic solid tumours remain incurable. In-depth analysis of tumour genomes is revealing evidence for branched evolution and cancer subclonal spatial and temporal intratumour heterogeneity. Consistent with the role of tumour diversity as a substrate for tumour evolution, drivers of intratumour and intercellular heterogeneity such as chromosomal instability (CIN) are associated with drug resistance and poor clinical outcome. However, despite increasing knowledge of tumour diversity, there is limited insight into the mechanisms driving genomic instability and tumour heterogeneity and the processes that shape cancer genome evolution over time and space. We have used an integrative functional genomics and bioinformatics approach to define candidate regulators of CIN. We have found evidence for ordered regions of consistent genomic loss amongst chaotic chromosomally unstable colorectal cancer genomes. A chromosome segregation error RNA interference screen of genes encoded within these regions of consistent genomic loss in aneuploid tumours revealed three candidate suppressors of chromosomal instability. Stable silencing of these three candidate genes initiates CIN in diploid cells. Through analysis of genetically defined aneuploid colorectal cancer cells and functional validation of the three candidate CIN suppressor genes, we provide evidence for a pre-mitotic cause of chromosome segregation errors in colorectal cancer driven by DNA replication stress. Consistent with these observations, we find evidence that chromosome segregation errors in chromosomally unstable colorectal cancer cells, or enforced instability in diploid cells following CIN-suppressor gene silencing, can be attenuated by exogeneous nucleoside addition. These data raise the possibility that tumour diversity might be limited through the attenuation of DNA replication stress.

Charles Swanton
Cancer Research UK,
London Research Institute and UCL Hospitals/Cancer Institute, UK

Short Talk

Miguel Foronda Álvaro

POT1 mutations cause telomere dysfunction in chronic lymphocytic leukemia

Ramsay AJ, Quesada V, **Foronda M**, Conde L, Martínez-Trillos A, Villamor N, Rodríguez D, Kwarciak A, Garabaya C, Gallardo M, López-Guerra M, López-Guillermo A, Puente XS, Blasco MA, Campo E, López-Otín C.

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Hospital Clínic, Universitat de Barcelona, Institut d'Investigacions Biomèdiques, Barcelona, Spain

Chronic lymphocytic leukemia (CLL) is the most frequent leukemia in adults. We have analyzed exome sequencing data from 127 individuals with CLL and Sanger sequencing data from 214 additional affected individuals, identifying recurrent somatic mutations in POT1 (encoding protection of telomeres 1) in 3.5% of the cases, with the frequency reaching 9% when only individuals without IGHV@ mutations were considered. POT1 encodes a component of the shelterin complex and is the first member of this telomeric structure found to be mutated in human cancer. Somatic mutation of POT1 primarily occurs in gene regions encoding the two oligonucleotide-/oligosaccharide-binding (OB) folds and affects key residues required to bind telomeric DNA. POT1-mutated CLL cells have numerous telomeric and chromosomal abnormalities that suggest that POT1 mutations favor the acquisition of the malignant features of CLL cells. The identification of POT1 as a new frequently mutated gene in CLL may facilitate novel approaches for the clinical management of this disease.

Miguel Foronda
CNIO, Madrid, Spain

Short Talk

Stefan Duensing

CEP57 links an altered tumor microenvironment to mitotic infidelity in prostate cancer

Nina Korzeniewski(1), Yanis Tolstov(1), Rolando Cuevas(2), **Stefan Duensing(1)**

1- University of Heidelberg School of Medicine, Section of Molecular Urooncology, Heidelberg, Germany
2- University of Pittsburgh Cancer Institute, Pittsburgh, PA, USA

It is widely accepted that the tumor microenvironment contributes importantly to malignant progression. Malignant progression is commonly associated with chromosomal instability but whether and how microenvironmental factors can impact on chromosome segregation is largely unknown. One of the most frequently altered growth factors in human cancer is FGF-2 (basic FGF) and many cancers that overexpress FGF-2 are also frequently aneuploid. This includes prostate cancer, in which over 90% of metastatic tumors were found to be aneuploid. A link between FGF-2 signaling and chromosomal instability, however, has so far been elusive. Using an siRNA screen, we identified CEP57 as a novel regulator of centriole duplication. Overexpression of CEP57 was found to rapidly uncouple centriole duplication from the cell division cycle and to stimulate cell division errors. Intriguingly, CEP57 is an intracellular trafficking protein for the 18 kD isoform of FGF-2 and required to transport FGF-2 to the nucleus in a microtubule-dependent manner. Stimulation of LNCaP prostate cancer cells, which express FGF receptor 1 (FGFR1), with recombinant FGF-2 led to a rapid induction of centriole overduplication as well as mitotic defects in a CEP57- and FGFR1-dependent manner. CEP57 and FGF-2 were both found to be overexpressed in a substantial proportion of prostate cancers.
Taken together, our results provide unexpected evidence that cancer genomes can be shaped by the impact of microenvironmental factors on mitotic fidelity. In the case of FGF-2, this event was mediated by CEP57 and FGFR1. Our findings furthermore provide a rationale for the use of FGFR-targeted therapies in prostate cancer to suppress aneuploidization and malignant progression.

Stefan Duensing
University of Heidelberg, Germany

Jan Korbel

Dissecting Origin and Impact of Genomic Rearrangements in Cancer

My presentation will cover research from our group at the European Molecular Biology Laboratory in Heidelberg. Our research group is developing and applying approaches from computational biology and functional genomics to decipher the impact of and the molecular mechanisms underlying structural variations in the genome, specifically such occurring during the development of cancer. Chromothripsis scars the genome when localized chromosome shattering and repair occurs in a one-off catastrophe. Outcomes of this process are detectable as massive DNA rearrangements affecting one or a few chromosomes. While recent findings from our group and others suggest a crucial role of chromothripsis in cancer development, the reproducible inference of this process remains challenging, requiring that cataclysmic one-off rearrangements be distinguished from localized lesions that occur progressively. Recently we have developed new conceptual criteria for the inference of chromothripsis, based on ruling out the alternative hypothesis that stepwise rearrangements occurred. In addition to our work on chromothripsis I will present data from our group's efforts to decipher the earliest molecular lesions occurring in prostate cancer. I will provide evidence that prostate cancer in young men is formed by a molecular mechanism, involving androgen-driven chromosomal DNA rearrangements, distinct from the molecular mechanism by which prostate cancer arises in elderly men.

Jan Korbel
EMBL, Heidelberg, Germany

CIN genes in human cancer

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Todd Waldman

Identification of a Genetic Cause of Aneuploidy in Human Cancer

In the vast majority of human tumors the molecular basis of chromosomal instability and the aneuploidy it produces remains unknown. We have identified a clue to the mechanistic origins of aneuploidy through integrative genomic analyses of human tumors. A diverse range of tumor types were found to harbor deletions or inactivating mutations of STAG2, a gene encoding a subunit of the cohesin complex, which regulates the separation of sister chromatids during cell division. Because STAG2 is on the X chromosome, its inactivation requires only a single mutational event. Studying a near-diploid human cell line with a stable karyotype, we found that targeted inactivation of STAG2 led to chromatid cohesion defects and aneuploidy, whereas in two aneuploid human glioblastoma cell lines, targeted correction of the endogenous mutant alleles of STAG2 led to enhanced chromosomal stability. This study demonstrates that genetic disruption of cohesin is a genetic cause of aneuploidy in human cancer. Recent unpublished findings regarding the role of STAG2 in the pathogenesis of human cancer will be also presented.

Todd Waldman
Georgetown University School of Medicine,
Lombardi Comprehensive Cancer Center, Washington, USA

CIN genes in human cancer

Short Talk

Cristina Balbás-Martínez

Exome sequencing of urothelial bladder cancer shows that STAG2 inactivation is a common somatic event not associated with aneuploidy

Cristina Balbás-Martínez1, Ana Sagrera1, Enrique Carrillo-de-Santa-Pau1, Julie Earl1,2, Mirari Márquez1, Miguel Vazquez1, Francesc Castro-Giner3, Sergi Beltran3, Mònica Bayés3, Alfredo Carrato2, Juan C. Cigudosa1, Orlando Domínguez1, Marta Gut3, Jesús Herranz1, Núria Juanpere4, Manolis Kogevinas 5-8, Xavier Langa1, Elena López-Knowles6, José A. Lorente9, Josep Lloreta4,9, David G. Pisano3, Laia Richart1, Daniel Rico1, Rocío N. Salgado1, Adonina Tardón10, Stephen Chanock11, Simon Heath3, Alfonso Valencia1, Ana Losada1, Ivo Gut3, Núria Malats1, Francisco X Real1,9

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2- Hospital Ramón y Cajal, Madrid, Spain
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4- Hospital del Mar, Barcelona, Spain
5- Centre de Recerca d'Epidemiologia Ambiental, Barcelona, Spain
6- IMIM, Barcelona, Spain
7- CIBER Epidemiología y Salud Pública, Barcelona, Spain
8- National School of Public Health, Athens, Greece
9- Universitat Pompeu Fabra, Barcelona, Spain
10- Universidad de Oviedo, Oviedo, Spain
11- Translational Genomics Laboratory, NCI, Bethesda, US

Urothelial bladder cancer (UBC) is a heterogeneous entity; at least two genetic pathways are involved in its development. FGFR3 is the main oncogene involved in non muscle-invasive, genomically stable, UBC; activating mutations are associated with good prognosis. The p53 and RB pathways are commonly inactivated in muscle-invasive tumors that are genomically unstable.

We used massive parallel sequencing to identify new genes involved in UBC. Exome sequencing (n=17), followed by a prevalence screen (n=48), identified 11 damaging somatic STAG2 mutations (17% of cases), most of which led to loss of protein expression, analyzed using immunohistochemistry. In one tumor, STAG2 loss was attributable to a genomic deletion. We analyzed STAG2 expression in tissue microarrays from a well-annotated UBC series. Loss of STAG2 expression was found in 29% of 671 tumors and it was significantly associated with low stage (P= 5.7 x10-15), and low grade (P= 2x10-15).

STAG2 encodes a subunit of cohesin, a complex involved in sister chromatid cohesion that is essential for adequate chromosome segregation. STAG2 mutations in human cancers have recently been proposed to cause aneuploidy. This is at odds with our finding that STAG2 expression is lost in low grade UBC. We examined changes in chromosome numbers using arrays in 23 low grade/stage tumors. Of 11 tumors with STAG2 loss, 9 lacked aneuploidy and the remaining 2 had lost one copy of chromosome 9. Similarly, 9/12 cases retaining STAG2 expression failed to display aneuploidy. Silencing STAG2 in UBC cell lines did not yield consistent differences in chromosome number per metaphase. STAG2 knockdown resulted in reduced colony formation in several cell lines, supporting the notion that it can regulate cell proliferation.

STAG2 is a novel, significantly mutated, UBC gene. It is involved in tumor development through mechanisms different from aneuploidy. These studies should provide insights into non-canonical functions of cohesins.

Cristina Balbás-Martínez
CNIO, Madrid, Spain

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

SPEAKERS' BIOGRAPHIES

Chromosome Instability and Aneuploidy in Cancer

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Duane Compton

Geisel School of Medicine at Dartmouth, Hanover, USA

Dr. Compton earned his PhD at U.T.M.D. Anderson Cancer Center in Houston Texas where his work focused on assembling a physical map of human chromosome 11 that pinpointed the location of the tumor suppressor gene for Wilms' Tumor. He performed postdoctoral work at Johns Hopkins Medical School where he led studies that identified important structural proteins within the mitotic spindle. He started his independent laboratory at Dartmouth in 1993 where he has explored mechanisms responsible for spindle architecture and chromosome movement. His current work focuses on determining the causes of chromosomal instability in human tumor cells.

Daniela Cimini

Virginia Tech, Blacksburg, USA

Dr. Daniela Cimini is currently an Associate Professor in the Department of Biological Sciences at Virginia Tech (USA). She obtained a PhD degree in Genetics and Molecular Biology from the University of Rome "La Sapienza" (Italy) under the guidance of Dr. Francesca Degrassi. She joined the lab of Dr. Ted Salmon at the University of North Carolina at Chapel Hill (USA) for her postodoctoral training. Since starting her lab at Virginia Tech her research has focused on two major areas: the mechanics and dynamics of mitosis and the mitotic apparatus; and the causes and consequences of aneuploidy in vertebrate cells.

Fanni Gergely

Cancer Research UK Cambridge Research Institute, UK

Dr. Fanni Gergely, originally from Hungary, received her undergraduate degree in Natural Sciences from the University of Cambridge, UK. In 2001 she completed her PhD on mechanisms of mitotic spindle assembly in Jordan Raff's laboratory at the Gurdon Institute. Supported by a Royal Society Research Fellowship she investigated calcium channels in Colin Taylor's group before returning to mitosis and centrosome biology for the remainder of her postdoctoral studies. In 2006 as a tenure-track scientist she joined the then newly established CRUK Cambridge Research Institute (CRI), where she received tenure in 2012. Current research in the group focuses on elucidating a link between centrosome dysfunction and developmental diseases.

Ashok Venkitaraman

University of Cambridge and the Medical Research Council Cancer Cell Unit, UK

Professor Ashok Venkitaraman holds the Ursula Zoellner Professorship of Cancer Research at the University of Cambridge, and is the Director of the Medical Research Council (MRC) Cancer Cell Unit. He trained in clinical medicine before his Ph.D. and postdoctoral work in molecular cell biology.
Ashok's research interest is in understanding the role played by chromosome instability in cancer pathogenesis, and in exploiting this information for the improvement of clinical interventions, using new enabling technologies.
Ashok is a Fellow of the Academy of Medical Sciences, London, and a Member of the European Molecular Biology Organization, Heidelberg.

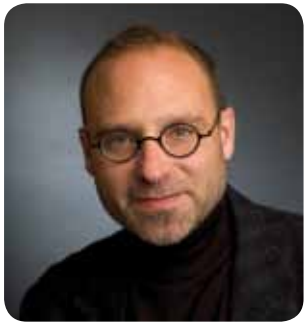
Geert Kops

University Medical Center Utrecht, The Netherlands

Dr. Geert Kops is Professor of Molecular Tumor Cell Biology at the University Medical Center Utrecht, The Netherlands. He obtained his PhD in 2001 at Utrecht University for his investigations into the PI3kinase-PKB/Akt–FOXO pathway and its role in cellular proliferation. He pursued postdoctoral studies in the lab of Don Cleveland at the Ludwig Institute for Cancer Research in La Jolla, California, where he investigated aspects of the mitotic checkpoint. His primary research interests include signaling networks that regulate chromosome segregation in healthy and cancer cells, and the potential use of targeting these networks for cancer therapy.

David Pellman

Dana-Farber Cancer Institute, Harvard Medical School, Boston, USA



Dr. David Pellman is the Margaret M. Dyson Professor of Pediatric Oncology at the Dana-Farber Cancer Institute and the Children's Hospital, Boston. He is also Professor of Cell Biology at Harvard Medical School and an Investigator of the Howard Hughes Medical Institute. He received his undergraduate and medical degrees from the University of Chicago. His internship, residency and fellowship in pediatric oncology were at Children's Hospital and the Dana-Farber Cancer Institute. His postdoctoral fellowship was at the Whitehead Institute at the Massachusetts Institute of Technology. His awards include the Damon Runyon Scholar Award, the Stohlman Scholar Award from the Leukemia and Lymphoma Society of America, and the E. Mead Johnson Award.

Don Cleveland

Ludwig Institute for Cancer Research, University of California, San Diego, USA

Dr. Don Cleveland is Professor and Chair of the Department of Cellular and Molecular Medicine at the University of California at San Diego and a member of the Ludwig Institute for Cancer Research. He has been elected to the US National Academy of Sciences and the Institute of Medicine. He has identified genes and proteins involved in faithful chromosome segregation, including deciphering the signaling cascade of the mitotic checkpoint, the major cell cycle control pathway guarding against chromosome mis-segregation. Aneuploidy produced by chromosome missegregation has long been linked to cancer. Cleveland's efforts have demonstrated that low rates of missegregation drive tumorigenesis, but high rates can suppress it.

Robert Benezra

Memorial Sloan-Kettering Cancer Center, New York, USA

Dr. Robert Benezra received his PhD from Columbia University in 1986 before pursuing postdoctoral work at the Fred Hutchinson Cancer Center with the late Dr. Hal Weintraub. With Weintraub, in 1990 Benezra identified the Id proteins as antagonists of myogenesis and has gone on, in his own laboratory at Memorial Sloan-Kettering Cancer Center, USA to characterize their importance in stem cell and cancer biology. The Benezra laboratory in 1996 also identified the first human mitotic checkpoint gene, hsMad2, as an antagonist of the anaphase promoting complex and continues to explore the role of Mad2 overexpression in mouse models of chromosome instability, cancer progression and relapse.

Ana Losada

CNIO, Madrid, Spain

Dr. Ana Losada obtained her PhD in Biochemistry and Molecular Biology in the laboratory of A. Villasante at the Centro de Biología Molecular "Severo Ochoa" (CSIC-UAM), Madrid. Her research aimed at identifying the DNA sequences that specify the centromeres of higher eukaryotes, using D. melanogaster as a model system. In October 1996 she joined Tatsuya Hirano's group at Cold Spring Harbor Laboratory (New York, USA) as a postdoctoral fellow. Using the Xenopus egg cell-free system, she identified the first cohesin complex from vertebrate cells. The importance of her studies on cohesin and the molecular mechanism of sister chromatid cohesion has been widely recognised in the field of chromosome dynamics. In 2004 she returned to Spain to establish her own research group at the CNIO where she has continued to work on the regulation of chromosome segregation and in the most novel functions of the cohesin complex.

Jan Van Deursen

Mayo Clinic, Rochester, USA

Dr. Jan van Deursen received his Ph.D. in Cell Biology at the University of Nijmegen, The Netherlands in 1994, started his independent career at St Jude Children's research hospital in in 1996, and joined Mayo Clinic as an Associate Professor in 1999. He is currently a Professor of Biochemistry and Molecular Biology, and Pediatrics at Mayo Clinic. He is the Vita Valley Named Professor of Cellular Senescence, Director of the Senescence Program in the Robert and Arlene Kogod Center on Aging, and Chairs the Biochemistry and Molecular Biology Department.

Since joining Mayo Clinic, Dr. van Deursen studies the molecular genetic basis of normal and aberrant chromosome segregation using a combination of in vitro biochemistry approaches with genetic experiments in mice. More recently his lab is exploring the relationship between aneuploidy, senescence and aging.

Rong Li

Stowers Institute for Medical Research, Kansas City, USA

Dr. Rong Li is a cell biologist with a broad interest in the dynamic processes governing cellular behavior and evolution. She obtained her PhD degree at University of California San Francisco. After postdoc training at UC Berkeley, she held a faculty position at Harvard Medical School for ten years. Presently she is an Investigator at the Stowers Institute for Medical Research in Kansas City, Missouri, with an affiliated Professorship at University of Kansas Medical Center.

Angelika Amon

Koch Institute for Integrative Cancer Research, Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, USA

Dr. Amon obtained her PhD in 1994 from the University of Vienna. Dr. Amon joined the faculty of the Department of Biology and the Koch Institute for Integrative Cancer Research at MIT in 1999 and the Howard Hughes Medical Institute in 2000. At the Koch Institute Dr. Amon studies the molecular mechanisms that prevent chromosome mis-segregation and what happens to cells in which these mechanisms fail, leading to a condition known as aneuploidy. As aneuploidy is a key characteristic of cancer, these studies have important implications for our understanding of tumorigenesis.

René Medema

The Netherlands Cancer Institute, Amsterdam, The Netherlands

Prof. Dr. Rene H. Medema has worked in the cell cycle field since his postdoctoral training in the laboratory of Prof. Dr. R.A. Weinberg (Whitehead Institute, Cambridge, MA). His work as an independent group leader at the University Medical Center Utrecht and the Netherlands Cancer Institute has always focused on the control of cell division. The specific research lines in his group have addressed transcriptional regulation of the cell cycle, the function of cell cycle checkpoints, and chromosome segregation.

His group has made several key contributions to the general understanding of control of the cell cycle by Forkhead transcription factors (Medema et al, 2000, Nature **404**:p.782; Laoukili et al., 2005, Nat. Cell Biol. **7**:p126; Alvarez-Fernandez et al., 2010, EMBO Rep. **11**:p.452) and the DNA damage checkpoint (Smits et al, 2000, Nat. Cell Biol. **2**:p.672; van Vugt et al., 2004, Mol. Cell **15**:p.799; Macurek et al., 2008, Nature **455**:p.119; Lindqvist et al., 2009, EMBO J. **28**:p.3196). In addition, work from his group has provided more insight on the role of motor proteins in spindle assembly (Tanenbaum et al., 2006, EMBO J. **25**:p.45; Tanenbaum et al., 2009, Curr.Biol. **19**:p.1703; Tanenbaum et al., 2011, Curr.Biol. **21**:p.1356) and the consequences of chromosome missegregation on the genomic stability and viability of a tumor cell (Janssen et al., 2011, PNAS **106**:p.19108; Janssen et al., 2011, Science **333**:p.1895).

Rene Medema is currently scientific director of the Netherlands Cancer Institute and professor of Experimental Oncology at the University Medical Center Utrecht. He was elected EMBO member in 2007. He is member of the editorial boards of Cancer Research, Oncogene, EMBO Reports and BBA Reviews on Cancer. He also serves on the Scientific Council of the Dutch Cancer Society since 2009.

Randall King

Harvard Medical School, Boston, USA

Dr. Randall King, M.D., Ph.D. is Associate Professor of Cell Biology, Harvard Medical School. He received an undergraduate degree in Chemistry from Carleton College, a PhD in Biochemistry from UCSF, and his MD from Harvard Medical School. His work at the interface of chemistry and biology has identified small molecules that target the Anaphase-Promoting Complex and proteasome-associated deubiquitinating enzymes. His lab is interested in the development of these molecules for treatment of cancer and neurodegenerative disease.

Stephen Taylor

University of Manchester, UK

After undergraduate studies in Manchester, Dr. Stephen moved to Oxford to pursue his PhD with Ed Southern, working on human centromere function. In 1995, he moved to Harvard Medical School where he worked with Frank McKeon, discovering several mammalian spindle checkpoint components. In 1998, Stephen moved back to Manchester to set up his own lab, funded by personal Fellowships from BBSRC and then Cancer Research UK. In 2009 he was promoted to Professor of Cell Biology, and in 2010 he was elected to Academia Europaea.

SPEAKERS' BIOGRAPHIES

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

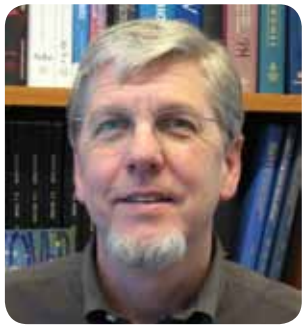
Marcos Malumbres

CNIO, Madrid, Spain

Dr. Marcos Malumbres research is focused in the effect of oncogenes in cell cycle control and proliferation, and the therapeutic opportunities derived from the control of the cell division cycle. After postdoctoral training at the New York University Medical Center and the CNIO, he obtained a Scientist position at the CSIC, and in 2004 he decided to stay at the CNIO to lead the Cell Division and Cancer Group. In 2005, he received the Beckman-Coulter Award for Young Scientists. In the last years, his laboratory has characterized the in vivo relevance of major cell cycle regulators such as Cdks, Aurora, Polo-like kinases or some microRNAs using cellular or genetic models in mammals. His current interests include the analysis of the therapeutic effect of inhibiting mitotic exit in tumors, understanding cell cyle regulation in pluripotent cells, and the generation of new therapeutic proposals based on these processes.

Philip Hieter

University of British Columbia, Vancouver, Canada



Dr. Philip Hieter is a Professor in the Michael Smith Laboratories at the University of British Columbia. He received his Ph.D. in biochemistry from Johns Hopkins University in 1981, trained as a postdoctoral fellow at Stanford, and was a faculty member at the Johns Hopkins University School of Medicine from 1985 -1997. He moved to the University of British Columbia in 1997, and served as Director of the Michael Smith Laboratories until 2008. Phil served as President of the Genetics Society of America in 2011. He is an elected Fellow of the Royal Society of Canada and the American Academy of Arts and Sciences.

Charles Swanton

Cancer Research UK, London Research Institute and UCL Hospitals/Cancer Institute, UK

Dr. Charles Swanton completed his PhD in 1998 at the Imperial Cancer Research Fund Laboratories on the UCL MBPhD programme before completing his medical oncology and Cancer Research UK funded post-doctoral clinician scientist training in 2008. Charles was appointed Medical Research Council and Cancer Research UK senior clinical research fellow and Group Leader of the Translational Cancer Therapeutics laboratory at the CR-UK London Research Institute in 2008. He combines his laboratory research with clinical duties focussed on biological mechanisms of drug resistance in cancer medicine. Charles worked as a consultant medical oncologist at the Royal Marsden Hospital with an interest in early phase drug development for the treatment of specific subtypes of metastatic solid tumours (2008-2011). Charles was made Fellow of the Royal College of Physicians in April 2011 and appointed to the Chair in Personalised Cancer Medicine at the University College London Cancer Institute in November 2011. Charles is a Consultant Medical Oncologist at UCL Hospitals and conducts his laboratory research at the CR-UK London Research Institute and UCL Cancer Institute focussing on personalised cancer medicine through an understanding of mechanisms of drug resistance, intratumour heterogeneity and genomic instability.

Jan Korbel

EMBL, Heidelberg, Germany

I obtained my PhD in Molecular Biology with a focus on Computational Biology from EMBL Heidelberg and from the Humboldt University Berlin in 2005. In 2005-2007, I joined Dr. Mark Gerstein's group at Yale University as a postdoc. The main focus of my work at Yale, in close collaboration with Dr. Michael Snyder's group, and of subsequent independent research at EMBL (since 2008), has been research on genomic structural variation using massively parallel DNA sequencing. Our group is currently involved in several projects in the context of the International Cancer Genome Consortium, where our research focuses on identifying cause and consequence of complex DNA rearrangements in cancer.

Todd Waldman

Georgetown University School of Medicine,
Lombardi Comprehensive Cancer Center, Washington, USA

Dr. Todd Waldman is an Associate Professor of Oncology at the Lombardi Comprehensive Cancer Center, Georgetown University School of Medicine. He grew up in Bethesda, Maryland and attended Yale University, where he received a BS in Molecular Biophysics and Biochemistry in 1991. He then moved to Baltimore, where he attended Johns Hopkins School of Medicine and received an MD/PhD in 1997. His doctoral research focused on developing novel methods for gene targeting in cultured human cells, for which he was awarded the Michael Shanoff Research Prize for the best PhD thesis at Johns Hopkins Medical School. After a brief post-doctoral stint at Hopkins, he moved back home to Washington, DC and began his career at the Lombardi Cancer Center as an Assistant Professor of Oncology. He was promoted to Associate Professor with tenure in 2005. His current research focuses on identifying the genetic basis of aneuploidy in human cancer.

POSTER SESSION

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

Pilar Alonso Lecue

Mitosis-differentiation checkpoint and genomic instability in skin carcinoma cells

Pilar Alonso-Lecue1, Ana Valtuille3, Francisco Mazorra2,3, Juan Ramón Sanz1,2, Alberto Gandarillas1.

1- Cell Cycle, Stem Cell Fate and Cancer Lab. FMDV-IFIMAV, Santander, Spain
2- HUMV, Santander, Spain
3- Clínica Mompía, Santander, Spain

Basal Cell carcinoma (BCC) and Squamous cell carcinoma (SCC) are the main forms of nonmelanoma skin cancer and the most frequent human malignancies. SCC has worse prognostic than BCC and is associated with a higher risk of metastasis. Paradoxically SCC but not BCC usually retains an altered component of squamous differentiation. Ectopic expression of protooncogene MYC or the DNA replication regulator Cyclin E in normal skin keratinocytes, induces DNA damage, mitosis failure and terminal differentiation. This is a response to a novel mitosis-differentiation checkpoint and to replication stress. In seek of understanding for their different degree of malignancy, we have studied the alterations of this checkpoint in BCC and SCC cells before and after constitutive overexpression of Cyclin E-GFP. The results suggest that SCC and BCC cells are fundamentally different in their degree of genomic instability.

Pilar Alonso Lecue
FMDV-IFIMAV, Santander, Spain

Mónica Álvarez-Fernández

Physiological role and therapeutic implications of mammalian Greatwall

Mónica Álvarez-Fernández, Ruth Sánchez-Martínez, Belén Sanz, Marta Cañamero and Marcos Malumbres

Cell Division and Cancer Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Protein phosphorylation is an essential mechanism to control progression throughout the different phases of the cell cycle. Different kinases, including Cdks, Polo and Aurora kinases are critical regulators of mitotic progression. During the last years, a new cell cycle kinase, known as Greatwall in Drosophila and Xenopus, and Mastl in mammals, has emerged as an important regulator of cell division. In order to explore the physiological roles of this kinase, we have generated a knockout mouse model for Mastl. Acute ablation of Mastl results in embryonic lethality, indicating that Mastl activity is essential for embryonic development. Deletion in young mice, by using an inducible knockout model, leads to significant defects in proliferation in different tissues. At the cellular level, these defects are accompanied by multiple mitotic alterations, including DNA condensation problems and chromosome segregation defects. Importantly, Mastl is overexpressed in several human tumors and its depletion also leads to impaired proliferation of human tumor cell lines, which places Mastl as a potential new target for cancer therapy.

Mónica Álvarez-Fernández
CNIO, Madrid, Spain

Marin Barisic

Kinetochore motors dominate over polar ejection forces to bias chromosome motion towards the cell equator

Marin Barisic, Stephan Geley and Helder Maiato

Institute for Molecular and Cellular Biology, Porto, Portugal
Faculty of Medicine, University of Porto, Porto, Portugal
Biocenter, Innsbruck Medical University, Innsbruck, Austria

Chromosome alignment towards the equator is a hallmark of cell division in metazoans that ensures the accurate segregation of genetic material. Dispersed chromosomes are initially brought close to the poles via lateral microtubule attachments mediated by the kinetochore motor Dynein. This force is then counteracted by the kinetochore motor CENP-E, which tracks chromosomes towards microtubule plus-ends, while chromokinesins mediate “polar ejection forces” (PEFs) that expels chromosome arms away from spindle poles. However, how exactly these distinct motor activities are coordinated to bias chromosome motion towards the cell equator instead of the cell periphery remains unknown. In this study we used laser microsurgery to separate chromosome arms from the respective kinetochores in living human cells, combined with RNA interference and small molecule inhibitors to specifically abrogate the function of each motor. We show that microtubule plus end-directed activity of CENP-E at kinetochores is dominant over chromokinesins and critical to direct chromosome motion towards the cell equator. This must be coordinated with the microtubule minus end-directed activity of Dynein that prevents the formation of stable end-on microtubule-kinetochore attachments near the pole, which would otherwise move chromosome towards the cell periphery due to PEFs. Thus, opposite kinetochore motor activities dominate over PEFs to bias chromosome motion towards the cell equator.

Marin Barisic
Institute for Molecular and Cell Biology (IBMC), Porto, Portugal

Daniel Booth

The role of KIF4A in microtubule organisation and midbody formation

Daniel Booth, Kumiko Samejima & William C. Earnshaw

Wellcome Trust Centre for Cell Biology, University of Edinburgh

The spindle is a microtubule-based assembly that directs the segregation of chromosomes into two daughter cells. During anaphase and through to cytokinesis, anti-parallel microtubules of the spindle midzone undergo a programme of repositioning, from relatively loosely associated individual microtubules, into a highly organised and compact midbody. The midbody acts as a docking site for numerous proteins essential for the finale of cell division, abscission. Midbody formation is orchestrated by the recruitment of several key midbody components in a spatio-temporal dependant manner. Failure in cytokinesis can lead to tetraploidy, an event that can promote further aneuploidy and potentially tumourigenesis.
KIF4A is a plus-end directed kinesin motor protein with a dynamic localization pattern: It is nuclear during interphase, and during mitosis it localizes to the chromosome axes, midzone microtubules and midbody. Previous studies have revealed functions for KIF4A in chromosome condensation, chromosome segregation and spindle organisation. The purpose of this study was to further characterise the role of KIF4A during late mitosis and cytokinesis. We have established a KIF4A conditional knockout in chicken DT40 cells, in which expression of KIF4A is rapidly suppressed through the addition of doxycycline to culture media. Using both fluorescence and correlative light electron microscopy (CLEM) we have analysed KIF4A depleted cells, in which frequent abscission failure was observed. It is likely that this phenotype was a result of several structural defects identified, including: a loss of midbody microtubule organisation, an abnormal spindle length, lagging chromosomes, chromatin bridges, and mislocalisation of other midbody proteins. These results help to further define the role for KIF4A in the regulation of midzone and midbody microtubule organisation and dynamics.

Daniel Booth
University of Edinburgh, United Kingdom

Rebecca Burrell

DNA replication stress and loss of chromosome 18q in chromosomally unstable colorectal cancer

Rebecca A. Burrell(1), Sarah E. McClelland(1), David Endesfelder(1,2), Petra Groth (3), Marie-Christine Weller(3), Nadeem Shaikh(1), Eva Gronroos(1), Enric Domingo (4), Sally M. Dewhurst(1), Nnennaya Kanu (1), Su Kit Chew (1,5), Andrew J. Rowan (1), Michael Howell (1), Maik Kschischo (2), Ian Tomlinson(4), Thomas Helleday(3), Jiri Bartek (6,7), Charles Swanton (1,5)

1- Cancer Research UK London Research Institute, London, UK
2- University of Applied Sciences, Remagen, Germany
3- Science for Life Laboratory, Div. Translational Medicine & Chemical Biology, Dept. Med. Biochem. & Biophysics, Karolinska Institutet
4- Molecular & Population Genetics & NIHR Biomedical Research Centre, Wellcome Trust Centre for Human Genetics, Oxford, UK
5- UCL Cancer Institute, Paul O'Gorman Building, London, UK
6- Danish Cancer Society Research Center, Copenhagen, Denmark
7- Inst. of Molecular & Translational Medicine, Palacky University Olomouc, CZ

Chromosomal instability (CIN) results in an increased rate of change of chromosome number and structure. CIN generates intratumour heterogeneity, is observed in most solid tumours and is associated with both poor prognosis and drug resistance. Therefore understanding a mechanistic basis for CIN is crucial. CIN is associated with chromosome segregation errors at anaphase. We have recently found evidence for a link between DNA replication stress and defective chromosome segregation in colorectal cancer. CIN+ colorectal cancer cells displayed impaired replication fork progression and evidence of increased DNA replication stress relative to CIN- cells, exhibiting high levels of DNA damage in early mitosis and 53BP1-positive nuclear bodies in G1 cells. In keeping with these observations, structural chromosome abnormalities were responsible for the majority of chromosome missegregation in mitosis. We identified three new CINsuppressor genes (PIGN (also known as MCD4), RKHD2 (MEX3C) and ZNF516 (KIAA0222) encoded on chromosome 18q, which is subject to frequent copy number loss in CIN+ colorectal cancer. 18q loss frequently occurred during the transition from pre-malignant adenoma to invasive carcinoma, linking DNA replication stress and CIN to the onset of aggressive disease. Silencing each of these novel CIN-suppressor genes leads to DNA replication stress, structural chromosome abnormalities and chromosome missegregation. To alleviate DNA damage associated with replication stress, cells were supplemented with nucleosides, which reduced the frequency of chromosome segregation errors both after CIN-suppressor gene silencing in diploid cells, and in CIN+ cells that have lost chromosome 18q. On-going examination of replication defects observed in CIN+ colorectal cancer cells will be described, together with further functional characterisation of PIGN, MEX3C and ZNF516, which should provide important mechanistic insights into chromosomal instability in colorectal cancer.

Rebecca Burell
Cancer Research UK London Research Institute, United Kingdom

Guillermo de Cárcer

New physiological roles for Polo-like Kinase 1 in vivo

Guillermo de Cárcer, Paulina A. Wachowicz, Eva Porlan, Carlos Marugán, Marina P. Lopez, Marcos Malumbres

Spanish National Cancer Research Centre (CNIO), Madrid, Spain

During the last 20 years, Polo-like Kinase 1 (Plk1) has been demonstrated to be an essential mitotic regulator. It drives mitosis from the very beginning controlling centrosome maturation, to the very end regulating cytokinesis. Interestingly, more recent data has shown that Plk1 is also implicated in non mitotic roles such DNA damage signaling, replication machinery, and gene transcription. Plk1 is also considered an oncogene, although there is no formal demonstration in vivo for this. In order to shed light on Plk1 physiological roles during animal development, we have generated three different Plk1 genetic mouse models: a classical constitutive knock-out, a conditional knock-out, and a Plk1 overexpression knock-in.
As expected, Plk1 deficiency leads to embryonic lethality at the morula stage, with a wide mitotic arrest. Surprisingly, Plk1 haploinsufficiency leads to unexpected phenotypes related with cardiovascular and neuronal function. In addition, Plk1 overexpression in mice leads to animal death in a dose dependent manner, and this overexpression seems to be tumor prone. The functional relevance of Plk1 in cardiovascular and neuronal physiology, as well as in tumor development, will be discussed.

Guillermo de Cárcer
CNIO, Madrid, Spain

Sally Dewhurst

Tetraploidy is permissive for Chromosomal Instability (CIN) and accelerates cancer genome evolution

Sally M Dewhurst, Nicholas McGranahan, Rebecca A Burrell, Andrew J Rowan, Eva Gronroos & Charles Swanton

London Research Institute, Cancer Research UK, UCL Cancer Institute, London, UK

Tetraploid cells are commonly observed in multiple types of malignancy, even though polyploidy is seen relatively rarely in mammalian somatic cells. The tetraploid state has also long been proposed as an intermediate stage en route to the aneuploidy that is a hallmark of most tumours. We have used an isogenic system of diploid and tetraploid clones to examine the effects of tetraploidy on long-term genome evolution. A small sub-population of tetraploid cells exists in the near-diploid colon cancer cell line HCT-116, and it was possible to continuously passage the few tetraploid cells that survived single-cell cloning. As expected based on previous work, tetraploid clones exhibit increased segregation errors compared to diploids, but tetraploids clones specifically were able to tolerate and propagate these chromosome segregation errors based on live-cell imaging studies. Furthermore tetraploid clones exhibited a significant increase in structural chromosome abnormalities, such as acentric and dicentric chromosome fragments. Using SNP6.0 CGH arrays we have observed an increase in genome complexity over time specifically in tetraploid clones, whilst the isogenic diploid clones remain stable. Crucially, some specific genomic losses only observed in tetraploid clones are significantly correlated with increasing genome instability (wGII) in tumours. This cell-line system demonstrates that an increasingly unstable genome can develop specifically on the background of tetraploidy, and our system is recapitulating the genomic changes observed in highly chromosomally unstable (CIN) tumours. The genomic regions we have identified could be crucial in identifying CIN tolerance mechanisms in tumours.

Sally Dewhurst
London Research Institute, United Kingdom

Elena Doménech

Preventing mitotic slippage through the modulation of mitotic cell death pathways

Elena Doménech, Marcos Malumbres

Cell Division and Cancer Group, Spanish National Cancer Research Centre (CNIO), Madrid, Spain

Targeting the spindle assembly with spindle poisons, such as taxol, leads to a prometaphase delay that is often followed by cell death in mitosis. Cells often escape from the mitotic arrest due to progressive degradation of cyclin B1 despite the unsatisfaction of the mitotic checkpoint, a process known as mitotic slippage. Mitotic slippage is a potential source of aneuploidy and can be considered as one of the major mechanisms of resistance to current anti-mitotic poisons. Our group has recently generated a genetic model in which the Anaphase-promoting Complex (APC/C) cofactor Cdc20 can be eliminated from cells or live animals (Manchado et al., Cancer Cell 18,641-654, 2010). Cdc20-null cells arrest in metaphase and eventually die in mitosis due to the lack of degradation of cyclin B1 and high Cdk1 activity. Using this genetic model, we are now characterizing the molecular requirements for cell death in mitosis. We have developed a strategy to follow individual cell fate by videomicroscopy after mitotic arrest caused by genetic depletion of Cdc20 or chemical inhibition of mitosis. We use H-Ras-transformed Cdc20-conditional knockout cells expressing a histone H2B-GFP reporter as well as an inducible form of the Cre recombinase. Cell death becomes evident in these cells by the use of a soluble dye, To-Pro3, which diffuses into the cells after the permeabilization of the membranes that accompanies cell death. This system allows the interrogation of the consequences of modulating several signaling pathways by using RNA interference or specific chemical inhibitors. As expected, several members of the Bcl2 family of prosurvival factors contribute to inhibit apoptosis in these conditions. Surprisingly, Cdc20-null cells die in mitosis even in conditions in which apoptosis is efficiently prevented. In addition, we have evidences that other caspaseindependent types of cell death may contribute to MCD.

Elena Doménech
CNIO, Madrid, Spain

Manuel Eguren

Identification of new APC/C-Cdh1 substrates and therapeutic implications

Manuel Eguren1, Fernando García2, Mónica Álvarez1, Eusebio Manchado1, Javier Muñoz2 and Marcos Malumbres1

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The Anaphase-Promoting Complex/Cyclosome (APC/C) is an E3-ubiquitin ligase that mediates the ubiquitination of a wide variety of cell cycle proteins. The APC/C is modulated by two cofactors, Cdc20 and Cdh1, which select and target the proper substrates such as kinases and other cell cycle regulators for degradation. We have recently addressed the physiological relevance of the APC/C cofactors using conditional gene-targeted alleles in the mouse. Genetic ablation of Cdh1 during embryogenesis or in adult mice suggests that this protein is not required for the cell cycle although it seems to be necessary to maintain genomic integrity and may also provide specific modulation for differentiation or quiescence in particular cell types. Taking advantage of these genetic models, we have performed a proteomic screen to identify new Cdh1 targets both in cultured cells and in whole brain using quantitative approaches (SILAC and iTRAQ). These screenings suggest that Cdh1 may control multiple pathways that modulate proper progression throughout the cell cycle. In addition, some of the candidates are therapeutic targets in cancer and preliminar experiments suggest that Cdh1 activity may modulate the sensitivity to specific drugs and, therefore, determine the outcome of specific therapeutic approaches.

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Rocío Gómez Lencero

The side effect of Topo IIα inhibition during male mouse meiosis

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DNA type II topoisomerases are enzymes that play essential functions in DNA replication, transcription and repair. DNA Topoisomerase IIα (Topo II) is directly implicated in mitotic chromosome segregation by catalyzing the untanglement of inter-sister catenations and resolving persisting centromere catenations between condensed sister chromatids during the metaphase/anaphase transition. The ability to interfere with Topo II and generate enzyme mediated DNA damage is an effective and approved experimental strategy for cancer chemotherapy. Antitumoral drugs targeting Topo II, such as the epipodophyllotoxin Etoposide (ET, also commonly known as VP-16), include some of the most active chemotherapy agents currently available for the treatment of patients with different neoplastic diseases. Therefore, understanding the profound effects that Topo II inhibitors may cause on cell physiology and subsequently its negative consequences for patient's health is a critical aim in cancer research. We have studied the subcellular distribution of Topo II during male mouse meiosis. Our results show that Topo II is concentrated at the chromocentres during prophase I, below sister kinetochores and at the chromatid axes in metaphase I bivalents, and as a strand that connects sister kinetochore by traversing the inner centromere domain in metaphase II chromosomes. This Topo II connecting strand stretches during early anaphase II when sister chromatids are migrating to opposite poles, and persists after the removal of centromeric cohesin. Approaching the study of the effects of chemotherapy over fertility we have analysed the side effects of the inhibition of Topo II with ET by using drug i.p. concentrations comparable to those used in experimental human therapy. We propose that this poison has an important effect in chromosome segregation that may be responsible for the generation of gametes that carry serious chromosomal aberrations or a temporal impairment of fertility since delays in chromatid segregation in anaphase I and II are commonly observed.

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Alejandra González Loyola

Deciphering the role of Aurora kinase B in aneuploidy and tumor development

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Aneuploidy is a common characteristic of the majority of human tumors (Weaver and Cleveland, 2006). Several animal models with mutations in genes implicated in the mitotic machinery develop tumors, suggesting a causal relationship between mitotic defects and tumor development (Schvartzman et al., 2010). Within these regulators, the Aurora kinase (Aurk) family members are known to be overexpressed in a variety of malignant cancers. Due to the fact that AurkB, the catalytic component of the Chromosomal Passenger Complex, is essential for correcting errors in microtubule attachment, defects in the function and expression of this kinase may result in aneuploidy, making it an attractive target for the development of new anticancer treatments. To analyse whether AurkB overexpression can lead to tumor formation an endogenous, gain-of-function conditional mouse model (iKI) was generated. First, in vitro, we observed that AurkB overexpression affects proliferation and induces aneuploidy in murine embryonic fibroblasts (MEFs). Moreover, mitotic defects were clearly appreciated, such as abnormal interphasic cells (binuclei and micronuclei), anaphases with laggards and extended cytokinesis bridges. In vivo, induction of AurkB expression resulted in reduced lifespan and the development of spontaneous lymphomas and histiocytic sarcomas, accompanied by an increase in weight, steatosis and adipose tissue hypertrophy. We are currently analyzing the possible link between Aurora B and p53 in the development of these tumors. In addition, a karyotypic analysis of Aurora B-overexpressing mice will be carried out to assess the levels of aneuploidy. We also plan to study the cooperative effects of AurkB overexpression with different oncogenes or tumor suppressors.

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The impact of aneuploidy on ulcerative colitis-associated colorectal carcinogenesis

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Background: Ulcerative colitis (UC) patients have an increased risk to develop ulcerative colitis-associated colorectal cancer (UCC). Unfortunately, current surveillance programs show uncertainty in individual risk assessment. Thus, DNA ploidy was investigated for its diagnostic and prognostic potential and microarray analysis applied to unravel target genes of ploidy types.
Methods: 683 mucosal biopsies of ulcerative colitis patients, 257 sporadic colorectal carcinomas (SCCs), 31 UCCs and 122 non-malignant adjacent mucosa were assessed for ploidy and correlated to survival. 135 mucosal biopsies of additional 32 colitis patients were assessed by image cytometry and 33K oligonucleotide microarrays to define differentially expressed genes (DEGs).
Results: Aneuploid cell populations were more frequently in patients with subsequent UCCs ($P = 0.006$) than in UC patients without subsequent UCC. Comparison of the primary malignancies detected aneuploidy to be more frequent in UCCs (100%) than in SCCs (74.6%). Logistic regression yielded aneuploidy (OR, 4.07; 95% CI, 1.46-11.36; $P = 0.007$) to be the strongest independent prognostic factor for R0-resected patients devoid of metastases. Aneuploidy was also more frequent in non-malignant adjacent mucosa of UCCs than SCCs ($P < 0.001$) while no correlation between aneuploidy and dysplastic lesions could be observed. Gene expression changes were more pronounced between normal mucosa and UC than between UC and UCC. 1,749 DEGs distinguished euploid UC and UCCs, while only 15 DEGs differentiated aneuploid UC and UCCs. Genes pivotal for chromosome segregation were differentially regulated along aneuploidy development.
Conclusion: Aneuploidy can aid as independent predictive marker in UC surveillance and as independent prognostic marker of primary colorectal carcinomas. Gene expression signatures of aneuploid UC revealed a malignant-like signature suggesting that these lesions might need to be treated by prophylactic proctocolectomy.

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Nannette Jelluma

CiMKi: timed and local induction of chromosomal instability in the mouse

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We have designed and tested a genetic strategy to conditionally reduce or ablate Mps1 kinase activity in mice. This results in a weakened mitotic checkpoint and chromosomal instability (CIN). This model, named CiMKi (Cre-inducible Mps1 Knock-in) is a Cre-loxP-based knock-in mouse model, in which the mutations D637A (0% kinase activity) or T649A (20% kinase activity) can be introduced in the endogenous Mps1 locus in a timed and local manner. This allows us to control when and where Mps1 activity will be reduced, and to what extent. As expected from our previous work and predicted from our preliminary experiments with the CiMKi-T649A strain, different activities of Mps1 will cause diverse levels of CIN. This model has four major advantages over most previous models for CIN: First, various levels of CIN can be reached by inducing different mutations in the same checkpoint protein and by combining alleles with different mutations. This enables us to compare effects on tumor formation/progression of different levels of CIN. Second, CIN can be induced in specified tissues, allowing the study of tumor formation in various tissues of otherwise healthy mice within the same mouse model, thus enabling comparison of effects between tissues. This makes it possible to study the effects of higher levels of CIN in specified tissues that would be lethal if occurring throughout the body. Third, CIN can be induced in adult mice, allowing induction of levels of CIN that would be lethal if occurring from/during development. Fourth, high levels of CIN (shown to be lethal for tumor cells in vitro) can be induced by controlled inhibition of the checkpoint in an otherwise induced tumor in an adult mouse, to assess the effects hereof regarding tumor regression. This together makes the CiMKi mouse model unique and the first to facilitate studies of whether the mitotic checkpoint and Mps1 could be a promising target for treatment of tumors.

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Lilian Kabeche

Cyclin A degradation controls the transition form prometaphase to metaphase

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The cell cycle is an orderly sequence of events governed by switch-like changes in cellular biochemistry to ensure unidirectionality. In contrast, the transition from prometaphase to metaphase in mitosis is not viewed as a switch-like transition because it has historically been defined only by chromosome congression, which occurs progressively. However, we show that the stability of kinetochoremicrotubule (k-MT) attachments undergoes a switch-like change in RPE-1 cells increasing from an average t1/2 of 1.8 ± 0.5 min in prometaphase to 3.8 ± 0.5 min in metaphase. Similar switch-like changes in k-MT stability also occur in PTK-1 and U2OS cells. To rule out that the variability in our measurements arise from a progressive stabilization of k-MTs during prometaphase, we performed repeated measurements on the same cells using photoactivation of GFP-tubulin. Equivalent k-MT stabilities were obtained for each measurement when the same cell was photoactivated twice during prometaphase (1.7 ± 0.5 min and 1.7 ± 0.4 min). In contrast, photoactivating the same cell twice, once in prometaphase and once in metaphase, yielded different k-MT stability (2.0 ± 0.5 min in prometaphase and 3.9 ± 0.5 min in metaphase). Strikingly, the difference in k-MT stability between prometaphase and metaphase on these single cell analyses was consistently 1.9 ± 0.2 min, in line with the differences obtained from averaging populations of prometaphase and metaphase cells. Thus, the variability in our data using populations of cells reflects cell-to-cell variation and of k-MT during prometaphase. Furthermore, the switch to more stable k-MT attachments in metaphase requires the proteosome-dependent destruction of cyclin A in prometaphase. Thus, our data reveal that the transition from prometaphase to metaphase is governed by a switch-like change in k-MT attachments regulated by the presence of cyclin A.

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Anastasia Kuznetsova

The molecular mechanisms underlying chromosomal instability in aneuploid human cells arising from transient tetraploidy

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Aneuploidy, defined as alterations in both chromosome number and structure, along with chromosomal instability (CIN) are common hallmarks of cancer. Growing evidence suggests that aneuploidy and CIN facilitate carcinogenesis in both mice and humans. One of the routes to CIN can be via an unstable tetraploid intermediate. However, the mechanisms contributing to the development of CIN in the post-tetraploid progeny remain elusive. We examined the progress of human cells after tetraploidization induced by cytokinesis failure in otherwise chromosomally stable and p53-proficient human cells. The post-tetraploid progeny displayed both complex aneuploidy and CIN manifested by the increased frequency of mitotic errors, in particular lagging chromosomes. We could rule out the presence of multiple centrosomes as the sole source of CIN, as the doubled centrosome numbers reduced soon after tetraploidization. Instead, we identified downregulation of several mitotic kinesins, in particular the kinesin-8 family motor protein Kif18A. Accordingly, the post-tetraploid progeny show an altered spindle geometry, which likely reflects changes in microtubule dynamics. Furthermore, we found that the post-tetraploid cells divide in the presence of tensionless attachments. This suggests an altered spindle assembly checkpoint response, possibly accompanied by a defective mitotic error correction. Our work shows for the first time that a single tetraploidization event is sufficient to cause CIN even in p53-proficient human cells. Importantly, our results outline the possible mechanisms that can lead to CIN in the progeny of tetraploid cells.

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Martina Mantovan

Oncogene addiction, CIN and Mad2: a vicious triangle

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Breast cancer is one of the leading causes of death in women. Despite recent efforts to directly target oncogene overexpressing tumor cells, current treatments still cannot completely eradicate this disease. The molecular mechanisms, leading to acquired resistance, are still mostly unknown. Chromosome instability (CIN) is a predominant feature in human cancers and has been associated with metastasis, tumor recurrence and poor patient outcome. Overexpression of the mitotic checkpoint protein Mad2 has been observed in a variety of human tumors, including breast, and is known to be a cause of CIN.
To better understand the role of CIN during mammary tumorigenesis and relapse, we are using doxycycline inducible transgenic mouse models overexpressing Mad2 in combination with different oncogenes such as Kras, Her2 and c-MYC. In all cases we observe a significant delay in primary tumor formation. However, after mimicking targeted therapy there is an increased incidence of tumor relapse when Mad2 was present, compared to mice expressing oncogenes alone. These phenomena were recapitulated in 3D mammary cultures of primary cells. After longterm induction of Mad2 and the corresponding oncogenes we observe an expansion of acinar structures. Compared to controls that express only the oncogene, the co-expression of Mad2 with the respective oncogene results in an increased mitotic index and complete filling of the acini.
3D cultures of primary mammary tumors allow the isolation and expansion of a population of tumor cells that survives targeted therapy. The growth of tumor-like acini, selected for the capacity of proliferating in an oncogene-independent manner, suggests that resistant cells could be already present in primary tumors. These cells might be the potential source of relapse. Comparison between tumors induced by single oncogenes and those additionally overexpressing Mad2 will help us understanding how CIN shapes malignant cells and leads to increased tumor recurrence.

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Balca Mardin

EGF Induced Centrosome Separation Promotes Mitotic Progression and Cell Survival

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Timely and accurate assembly of the mitotic spindle is critical for the faithful segregation of chromosomes and centrosome separation is a key step in this process. The timing of centrosome separation varies dramatically between cell types; however, the mechanisms responsible for these differences and its significance are unclear. Here, we show that activation of epidermal growth factor receptor (EGFR) signaling determines the timing of centrosome separation. Premature separation of centrosomes decreases the requirement for the major mitotic kinesin Eg5 for spindle assembly, accelerates mitosis and decreases the rate of chromosome missegregation. Importantly, EGF stimulation impacts upon centrosome separation and mitotic progression to different degrees in different cell lines. Cells with high EGFR levels fail to arrest in mitosis upon Eg5 inhibition. This has important implications for cancer therapy since cells with high centrosomal response to EGF are more susceptible to combinatorial inhibition of EGFR and Eg5.

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Investigating the Roles of Mad2 in Aneuploidy and Structural Instability

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Chromosome instability (CIN) is comprised of aneuploidy (whole chromosome instability) and structural instability (breaks, deletions, translocations). CIN is frequently found in human cancers, and is associated with poor prognosis. The mechanism of how both types of instability are generated in tumors is incompletely understood. Genes important for the spindle assembly checkpoint (SAC), including Mad2, are frequently overexpressed in cancer and are associated with CIN. Previous work has shown that loss of key tumor suppressors, such as p53 or Rb family proteins leads to overexpression of checkpoint genes. Using Mad2 overexpression as a model in mice, we have found that checkpoint gene overexpression leads to tumorigenesis as well as both aneuploidy and structural instability. How Mad2 overexpression causes both of these phenotypes is incompletely understood. Recent work by others suggests that both aneuploidy and structural instability are possibly tied together and generated simultaneously when chromosome missegregation is induced. Two mechanisms have been proposed: missegregated DNA can become broken when trapped in the cleavage furrow during cytokinesis, or additionally, missegregated DNA can form micronuclei where the DNA inside becomes damaged in subsequent cell cycles. How these missegregation events occur in cancer cells has not been fully elucidated. Imaging of our cell culture models of Mad2 overexpression suggests these cells have a prolonged and abnormal mitosis, with features such as lagging chromosomes, anaphase bridges, cohesion fatigue, cell slippage, and potentially telomere decapping. These phenotypes lead to chromosome missegregation, with some evidence suggesting that both types of missegregation-induced damage are occurring. We propose a model where loss of key tumor suppressors leads to overexpression of SAC genes, which in turn cause an abnormal mitosis ending with chromosome missegregation and subsequent aneuploidy and structural instability.

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Sarah McClelland

Adaptation to Chromosomal Instability-associated replication stress by amplification of DNA replication proteins

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Chromosomal instability (CIN) describes ongoing genomic rearrangements resulting in loss or amplification of whole chromosomes and/or discrete chromosomal regions, leading to extensive intercellular karyotypic heterogeneity. Understanding the molecular mechanisms responsible for the acquisition and propagation of CIN is of particular importance in oncology due to the association of CIN with drug resistance, poor prognosis and tumour relapse. We hypothesised that any genomic regions that are consistently and stably maintained at aberrant DNA copy number in CIN+ cancers compared to CIN- cancers may reflect adaptations necessary for ongoing chromosomal instability, and that these regions would harbour dosage-sensitive genes whose deregulated function promotes initiation or maintenance of CIN. We recently demonstrated a key role for elevated replication stress promoting structural and numerical chromosomal instability and identified three 'CIN-suppressor' genes that are subject to frequent copy number loss in CIN+ cells. Here we identify a set of essential DNA repair genes that are amplified in CIN+ cell lines and tumours, suggesting a putative role in adaptation to elevated DNA replication stress. Pharmacological induction of replication stress in diploid HCT116 cells resulted in upregulation of POLD, RFC and RPA3 protein complexes. Overexpression of single POLD, RFC or RPA complex members induced stabilisation of their constituent complexes and furthermore partially rescued the induction of chromosome segregation errors and prometaphase DNA damage upon aphidicolin or hydroxyurea treatment. Such a mechanism to limit excessive DNA replication stress may be required to maintain tumour chromosomal instability under a threshold, promoting karyotypic diversity whilst maintaining cell viaility and preventing tumour burnout due to excessive CIN.

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Oscar Molina

H3K4me2 is necessary for Kinetochore Assembly and Function

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Mammalian centromeres occupy megabases of high-order arrays of alpha-satellite DNA organized into a specialized chromatin containing the centromere-specific histone CENP-A interspersed with histone H3 bearing modifications including H3K4me2 among others. The aim of this project is to study the effects of H3K4me2 removal from the centromere of a Human Artificial Chromosome (HAC) in order to study its effects on kinetochore assembly and function. The H3K4me2-specific demethylase LSD2 was cloned and afterwards expressed as a tetR-EYFP fusion protein in a HeLa cell line containing a HAC bearing tetracycline operators in its centromeric alphoid-DNA sequence (alphoidtetOHAC). Immunofluorescence (IF) experiments were performed to study the effects of the centromeric tethering of the tetR-EYFP-LSD2 fusion protein on alphoidtetOHAC kinetochore assembly and function.
We observed a significant reduction of H3K4me2 on the alphoidtetOHAC following tethering of the tetR-EYFP-LSD2 construct compared with tethering the control tetR-EYFP (P=0.0005). Afterwards, we studied the effects of LSD2 after two and four days of tethering to the alphoidtetOHAC centromere. In IF experiments we stained for the kinetochore-specific proteins CENP-A or CENP-C. Our results showed a significant reduction of CENP-A and CENP-C levels at the alphoidtetOHAC centromere after four days of tethering (P=0.0181 and P=0.0254, respectively). However, although we could see an increased frequency of mitotic abnormalities correlated with LSD2 tethering to the alphoidtetOHAC, this was not statistically significant at day four after tethering.
Our results show that H3K4me2 is important for kinetochore assembly. Moreover, the reduced levels of CENP-A and CENP-C assembly are accompanied by a slower increase in the frequency of mitotic abnormalities. These results are in agreement with previous results of our lab that suggest that the centromeres contain more CENP-A than is really necessary for kinetochore function.

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Matilde Murga

The mammalian decatenation checkpoint is a response to unreplicated centromeres

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In response to catalyitc inhibitors of topoisomerase II (Topo II), mammalian cells arrest at the G2 stage of the cell cycle. While this arrest can be bypassed with inhibitors of DNA damage-response (DDR) kinases, it occurs in the absence of DNA breakage. This led to the proposal of a “decatenation checkpoint”, which would activate DNA damage-responsive checkpoints in the absence of actual DNA damage. How the decatenation checkpoint is activated remains unknown. We here show that Topo II inhibitors activate a localized Brca1-, Atm- and H2ax-dependent DDR at centromeres (cDDR), which is present in cells that keep unreplicated centromeres at late G2. Abrogation of the cDDR in Topo II inhibitor-treated cells leads to anaphase bridges in which cells remain linked through their centromeres. In addition to their role in centromere replication, we also show that Brca1, Atm and H2ax constitutively suppress centromeric transcription. Our study reveals the long-sought signal that activates the “decatenation checkpoint”, and defines an active role of the DDR in modulating centromere replication and transcription in mammalian cells.

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A localized DDR is activated at the midbody to resolve DNA bridges

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The midbody is a cytological structure found at the end of citokinesis between the two daugther cells, which presents a characteristic concentration of Aurora B kinase. An active role for the midbody in genome maintenance has not been described. We have observed a recruitment of DDR proteins at midbodies that persist beyond mitosis. In some cases, DNA connecting the two daughter cells was detected by DAPI staining along the structure . The observed pattern, with ssDNA-binding protein RPA in the center and 53BP1 flanking it, is consistent with a central cleavage of the DNA bridge and suggests an actual activation of the DDR. We propose a role for the midbody in the resolution of unseggregated DNA remaining after mitosis, in which Aurora B kinase and Plk1 would trigger a localized cut of the DNA to promote its repair and proper segregation.

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Diana Papini

The role of TD-60 in mitosis progression

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The Chromosomal Passenger Complex (CPC) is the major regulator of mitosis and it is composed of the catalytic subunit Aurora B, the inner centromere protein INCENP, Survivin and Borealin/Dasra B. The CPC controls many aspects of mitosis, ranging from chromosome and spindle structure to the correction of kinetochore-microtubule attachments errors, regulation of mitotic progression and completion of cytokinesis (Carmena et al., 2012). Down regulation of either of CPC components results in delocalization of the others and disrupts mitotic progression (Adams et al., 2001 ; Carvalho et al., 2003 ; Lens et al., 2003 ; Gassmann et al., 2004 : Vader et al., 2006) Telophase Disc 60 (TD-60 – also known as RCC2) has been shown to be involved in the completion of cytokinesis through GTPase binding (Mollinari et al., 2003). However its mechanism of action is still unclear. Interestingly, TD-60 has a typical chromosomal passenger localization but it is not part of the complex. TD-60 down-regulation affects the localization of other CPC components in mitosis.
We are interested in understanding the biological function of TD-60 in mitosis, in particular if TD-60 is required for mitosis regulation and progression and also to determine its effect, if any, on core CPC activity.

Key words: Mitosis, CPC, TD-60, chromosomes, cell cycle

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TPX2: a CIN associated protein with tumorigenic properties

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TPX2 (Targeting protein for Xklp2) has been mainly related with proliferating cells. Originally described as the antigen of the proliferation marker Ki-S2, TPX2 is thought to be a microtubule regulator with a critical role in spindle formation and dynamics. Specifically, during mitosis and thanks to the RAN-GTP gradient, TPX2 participates in the chromatin-dependent microtubule nucleation through the activation of the kinase Aurora-A. Interestingly, Aurora-A and TPX2 proteins are over-expressed in a wide range of different tumor types. Moreover, TPX2 over-expression is a marker of worse prognosis and chromosome instability (CIN) in human cancer. However, the causal relationship between TPX2 abnormal expression, CIN and tumorigenesis has not been properly established. We have generated mouse models for the conditional deletion and inducible over-expression of Tpx2 that will allow us to better understand the role of TPX2 in the induction of aneuploidy and tumorigenesis. Here, we show preliminary data from mice and cells from both models that confirm the induction of CIN and tumors upon Tpx2 abnormal expression and the existence of Tpx2 non-mitotic functions.

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Genotypic and functional consequences of aneuploid karyotypes in cancer cells

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Cancer cells exhibit high rates of chromosome mis-segregation, which lead to chromosomal instability (CIN). However, whether aneuploidy per se generates CIN and chromosome-specific functional disturbances is unresolved. To investigate how chromosome segregation is affected by DNA ploidy, DLD-1 and 184B5, a colorectal and a breast cancer cell lines, respectively, were FACS sorted according to DNA content, and subsequent single-cell based diploid and tetraploid clones from the same parental line were established. Firstly, analysis of CIN by karyotyping evaluation and fluorescence in situ hybridization (FISH) showed that tetraploid clones displayed higher rates of chromosome mis-segregation compared to diploid clones. For DLD-1 derived lines, tetraploid clones 8 and 28 had nine subpopulations with a different number of chromosomes (range of 83-94 and 85-93, respectively) compared to the modal number in the parental line. In contrast, diploid clones 1 and 2 had only five and three subpopulations with a number of chromosomes different from the modal number, respectively. FISH experiments for specific cancer-related chromosomes, showed also a higher percentage of cellular subpopulations in the tetraploid clones. Second, tetraploid clones tend to display slower proliferating rates than their diploid counterparts. Likewise, colony formation assays indicated that diploid clones formed larger colonies compared to tetraploid clones, which showed both fewer and lower number of colonies. Third, migratory/invasion capacity measured by wound-healing (scratch) and transwell (Boyden chamber) assays, showed that tetraploid clone 28 exhibited far greater migration capability than clone 1. Nevertheless, when a layer of matrigel was used to assess invasiveness, clone 28 did not exhibit greater penetration capacity. These results are preliminary, however, experiments are being performed to confirm functional differences in vivo as well as the causative mechanism of different CIN rates.

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David Rasnick

Cancer is a Progressive Chromosomal Imbalance Syndrome: The Autocatalyzed Progression of Aneuploidy IS Carcinogenesis

Cancer results from a profound disorganization of cellular activity caused by carcinogen-induced chromosomal imbalance that spontaneously progresses during cell division—independent of gene mutation.
The Chromosomal Imbalance Theory of Cancer
1. Cancer is a progressive somatic aneuploidy syndrome.
2. Carcinogens and spontaneous mitotic errors produce pre-neoplastic chromosomal alterations and aneuploidies.
3. Aneuploidy is the steady source of karyotypic-phenotypic instability.
4. The rate of chromosomal variation (chromosomal instability) is proportional to the degree of chromosomal imbalance.
5. Since chromosomal alterations in cancer cells unbalance thousands of genes, they corrupt teams of proteins, including those that segregate, synthesize and repair chromosomes, simultaneously producing a heterogeneous mix of unique metabolic and cellular phenotypes.
6. A gain in gene dosage over a substantial fraction of the genome is better tolerated than a loss.
7. The survival advantage of the hyperploid cells (Point 6), coupled with the inherent chromosomal instability of aneuploid cells (Points 3–5), leads to the autocatalyzed progression of aneuploidy and cancer with each cell division.
8. The initiation of chromosomal imbalance, coupled with the autocatalyzed progression of aneuploidy during cell division, is NECESSARY and SUFFICIENT to generate cancer on the rare occasions the cells survive.
9. In classical Darwinian terms, selection of viable chromosomal alterations promotes the evolution and spontaneous progression of neoplastic cells. Thus, cancer cells evolve through a self-perpetuating chromosomal disorganization, increasing karyotypic entropy up to a maximum compatible with viability (DNA index ~1.7 and harmonics).

Other Information:
Cancer cells are inherently unstable because chromosomal imbalance massively disrupts cellular activity. The fact that no two cancer cells are identical, coupled with the ever-changing compliment of chromosomes and the resulting quantitative changes in the expression of thousands of genes, means there is no stable list of genes or genetic pathways that either cause or characterize cancer. Consequently, searching for molecular targets to either diagnose or attack cancer with drugs is a Sisyphean task.

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Tania Sánchez Pérez

Delaying mitotic exit down-regulates FLIP expression and strongly sensitizes tumor cells to TRAIL

Tania Sánchez-Pérez (1,2), René H. Medema (2) and Abelardo López-Rivas (1)

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Cell cycle deregulation is a feature of tumor cells. Most of the current therapeutic strategies are based on the perturbation of the cell cycle, especially during mitosis. Anti-mitotic drugs target different mitotic proteins impeding the assembly of a properly bipolar mitotic spindle which triggers mitotic checkpoint activation, mitotic arrest and eventually cell death. However, sometimes, after several hours of mitotic arrest, cells go out of mitosis by "slippage". This failure to die during mitotic arrest seems to be the main mechanism of resistance to these treatments. Recently, in an attempt to avoid the process of slippage, targeting mitotic exit has been proposed as a better strategy to kill tumor cells. In this study we show that treatments that induce mitotic checkpoint activation and mitotic arrest down-regulate FLIP levels and sensitize several tumor cell lines to TRAIL-induced apoptosis. We also demonstrate that in the absence of mitotic checkpoint activation, mitotic arrest induced either by Cdc20 knockdown or over-expression of non-degradable Cyclin B is sufficient to induce both FLIP down-regulation and sensitivity to TRAIL. Interestingly, our data suggest that a combination of anti-mitotic drugs targeting Cyclin B degradation and TRAIL might prevent mitotic slippage and allow tumor cells to reach the threshold for apoptosis induction, facilitating tumor suppression.

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Garry Sedgwick

Examining the Mad2 template model in vivo

Garry G Sedgwick, Diasuke Izawa, Werner Streicher, Jakob Nilsson
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During early mitosis the spindle assembly checkpoint (SAC) prevents sister chromatid separation until all chromosomes are properly bi-oriented. The SAC delays mitotic progression by inhibiting the anaphase promoting complex/cyclosome (APC/C) co-activator Cdc20 through the incorporation of Cdc20 into an inhibitory complex known as the mitotic checkpoint complex (MCC), which contains BubR1, Mad2 and Bub3. The Mad2 protein can adopt two extreme conformations referred to as open Mad2 (O-Mad2) or closed Mad2 (C-Mad2) the latter being the active conformation. The first step in the formation of the MCC is the binding of C-Mad2 to Cdc20 and the "template model" proposes that this C-Mad2 is generated at the kinetochore through dimerization of soluble O-Mad2 with C-Mad2 bound to Mad1 and this then converts O-Mad2 into C-Mad2. Although the template model is strongly supported by biochemical experiments certain aspects still await validation from in vivo experiments.
To investigate Mad2 conformations in vivo we have generated monoclonal antibodies specific for either O-Mad2 or C-Mad2 as well as a pan Mad2 antibody. The specificity of each antibody was confirmed using Mad2 mutants locked in their open or closed conformations for immunoprecipitation or isothermal titration calorimeter (ITC) experiments. We used these antibodies to examine the levels of O-Mad2 and C-Mad2 during an active checkpoint and their levels when the SAC was silenced. Interestingly the levels of O-Mad2 and C-Mad2 remain constant, which is supported by a constant association with p31comet. In addition by using size exclusion chromatography followed by immunoprecipitation we show that the majority of C-Mad2 can infact exist in a stable free pool without the requirement for stable binding to its ligands Cdc20 or Mad1. We are currently investigating whether this soluble pool of C-Mad2 can be activated during an active checkpoint.

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Alexey Stepanenko

Constitutive expression of CHI3L1 oncogene promotes chromosome instability in immortalized 293 cells

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- CNRS UMR8126, Université Paris-Sud 11, Institut de Cancérologie Gustave Roussy, Villejuif, France

Most of the experiments detecting transforming ability of genes overexpressed and/or mutated in tumors (oncogenes) were performed using mouse embryonic fibroblasts (MEFs), NIH3T3 mouse fibroblast cell line, human embryonic kidney 293 cell line (HEK293), and human mammary epithelial cell lines (mainly HMECs and MCF10A). These cells are immortalized; they have the abnormal karyotypes, and obtain much stronger ability to grow over one another in cell culture, form colonies in soft agar, and form tumors when injected into immunodeficient rodents after their malignant transformation. Multiple genetic rearrangements, including whole chromosome and gene copy number gains and losses, chromosome translocations, gene mutations are necessary for establishing the malignant cell phenotype.
Previously, we and others have demonstrated that increased CHI3L1 gene expression stimulated cellular mitogenic and proliferative properties, enhanced migration, invasion, radio- and chemoresistance of tumor cells, capacity for anchorage-independent growth in soft agar, promoted tumor growth, and angiogenesis.
In this study we found that constitutive expression of CHI3L1 promoted chromosome instability (CIN) in 293 cells. Modal number of chromosomes in 293_CHI3L1 cells was distinct from that in transfected control 293_pcDNA3.1 and parental 293 cells. Interline whole chromosome heterogeneity was shown by conventional cytogenetics. A number of new distinct marker chromosomes were observed in CHI3L1-expressing cells in two independent experiments. Array comparative genome hybridization (aCGH) revealed significant differences between the spectra of cytoband gains and losses in 293_CHI3L1 and control cells. Thus, here it was established the link between transforming properties of oncogene CHI3L1 and changes in karyotype of 293 cells with stable expression of CHI3L1.

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Catherine Evangeline Symonds

The varying role of Cdk1 in the pluripotent vs. differentiated cell cycle

Catherine E. Symonds, David Santamaria, Mariano Barbacid

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Cyclin-dependent kinase 1 (Cdk1) is essential in the progression of eukaryotic cells through the mitotic cell cycle. It coordinates the phosphorylation of multiple substrates necessary for the cell to enter into mitosis. Despite considerable research on the cell cycle, recent discoveries (i.e. MastL/Greatwall kinase) have demonstrated that our understanding of the molecular control underlying mitotic entry is far from complete. To this end we have generated a Cdk1 conditional mouse model, which will allow for precise genetic analysis of the role of Cdk1 in the mammalian cell cycle. Mice lacking Cdk1 are early embryonic lethal and genetic depletion of a Cdk1 conditional allele in the adult mouse leads to rapid proliferative tissue atrophy and lethality. To further study in vitro we isolated mouse embryonic fibroblasts (MEFs), embryonic stem cells (ES) and induced pluripotent stem cells (iPS) from this mouse model. We have observed in differentiated cell types (MEFs) that when Cdk1 is ablated the cells will permanently arrest at the G2-M transition and undergo endoreplication. This in vitro system will be applied towards a high-throughput screen in order to identify novel cell cycle regulators able to rescue or overcome the phenotype observed in the absence of Cdk1. However, when either ES or iPS cells were depleted of Cdk1, under pluripotent culture conditions, we observed a transient arrest at the G2-M transition followed by apoptosis. This phenotype is strictly dependent on the undifferentiated state, as these same cells, when grown under culture conditions more permissive to differentiation were able to arrest and undergo endoreplication. We are currently investigating if other progenitor cells, including cancer stem cells will behave similarly. If indeed ablation of Cdk1 in all stem cells is lethal, targeting Cdk1 inhibitors to these cellular tissue compartments could potentially be a relevant therapeutic strategy for hyperproliferative diseases, such as cancer.

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Rozario Thomas

Creation of an aneuploidy model to study the effects on tumorigenesis

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Whole chromosome aneuploidy is a hallmark of most human tumors. One of the major hurdles in addressing if aneuploidy is a cause or consequence of tumorigenesis has been the generation of appropriate aneuploidy models. Current models rely heavily on altering the levels of various spindle assembly checkpoint (SAC) components to generate the aneuploidy. Since SAC components have been implicated in roles outside of mitosis such as, transcriptional repression, apoptosis and premature aging, it is unclear if the tumor phenotypes observed in these models are solely a result of aneuploidy or other cellular perturbations. To study whether aneuploidy alone can cause/promote tumorigenesis, we are using a Cre recombinase-mediated chromosome loss strategy to individually delete mouse chromosomes 10 or 14. The model involves a pair of oppositely oriented loxP sites in one homolog of a chromosome. Cre recombinase induction results in dicentric and acentric chromosome fragments in half the mitosis population, both of which will be subsequently lost. We have chosen to model the effects of chromosome losses since we have shown recently that losses are much more common than gains in human tumors. Preliminary data using two kinds of tamoxifen inducible mouse models – a ubiquitous Cre recombinase and one that is expressed in adult stem cells, show clear loss of markers present on chromosomes 10 and 14 implying the formation of aneuploidy. In addition, immortalized mouse embryonic fibroblasts from these animals also display a clear loss of chromosomes 10 and 14 upon treatment with 4-hydroxy tamoxifen. Preliminary data reveal that these cells have a growth disadvantage and we are in the process of analyzing them with respect to other changes seen in MEFs bearing trisomies. In conclusion, our system for generating aneuploidy can be used to induce losses of specific chromosomes in vitro and in vivo and may shed light on the early consequences of aneuploidy in mammalian cells.

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Giulia Vargiu

Kinetochore structure dissection. A Super-Resolution Microscopy issue

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2- Instituto IMDEA Nanociencia, Madrid, Spain
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The kinetochore is a complex proteinaceous structure that assembles at the primary constriction of sister chromatids. Kinetochores provide a key role during mitosis where they direct chromosome segregation. Errors in this process can lead to aneuploidy; the unequal distribution of chromosomes between the daughter cells. Recently, growing interest has been focused on Constitutive Centromere Associated Network (CCAN) components, which are required for the formation of a scaffold involved in kinetochore assembly. Therefore much effort has been put in trying to understand both their distribution and their role in kinetochore functions. In the last decade, novel super-resolution microscopy techniques have been developed. Amongst these, Photo-Activated Localization Microscopy (PALM) has been recently applied to study the organization of extended fibers of kinetochore chromatin. On linearized chromatin fibers, PALM allows us to localize, with a resolution of approximately 37 nm, single molecules of Dronpa, a fluorescent protein, fused to the N-terminal region of CENP-A relative to the position of other antigens labelled with Alexa-tagged antibodies.
In our study we exploit the high homologous recombination efficiency in DT40 Blymphoma chicken cell line, which has allowed us to obtain conditional knockouts of several CCAN components. These knockouts have been used to study the localization of several CENPs and post-translational modifications of histones relative to Dronpa:CENP-A on unfolded centromere chromatin fibres by PALM. This system represents a unique and reliable model to visualize, at high resolution, how CENPs and epigenetic histone marks change in composition and distribution along the chromatin fibre following the deletion of different CCAN components.

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University of Edinburgh, United Kingdom

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER: FROM MECHANISMS TO THERAPEUTICS

Previous CNIO Frontiers Meetings
and CNIO Cancer Conferences

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

2012

ALLOSTERIC REGULATION OF CELL SIGNALLING

17/09/2012 - 19/09/2012

Organisers: Francesco Gervasio, Ermanno Gherardi, Daniel Lietha, Giulio Superti-Furga

2011

RECAPTURING PLURIPOTENCY: LINKS BETWEEN CELLULAR REPROGRAMMING AND CANCER

07/11/2011 - 09/11/2011

Organisers: Maria A. Blasco, Konrad Hochedlinger, Manuel Serrano, Inder Verma

CANCEROMATICS II : MULTILEVEL INTERPRETATION OF CANCER GENOME

28/03/2011 - 30/03/2011

Organisers: Søren Brunak, Stephen Chanock, Núria Malats, Chris Sander, Alfonso Valencia

BREAST CANCER

07/02/2011 - 09/02/2011

Organisers: Joaquín Arribas, José Baselga, Miguel Ángel Piris, Lajos Pusztai and Jorge Reis-Filho

2010

CANCER PHARMACOGENETICS: PERSONALIZING MEDICINE

22/11/2010 - 24/11/2010

Organisers: Javier Benítez, William E. Evans, Miguel Martín and Magnus Ingelman-Sundberg

MOLECULAR CANCER THERAPEUTICS

08/03/2010 - 10/03/2010

Organisers: Gail Eckhardt, Roy S. Herbst and Manuel Hidalgo

2009

THE ENERGY OF CANCER

02/11/2009 - 04/11/2009

Organisers: Toren Finkel, David M. Sabatini, Manuel Serrano and David A. Sinclair

CANCER-OM-ATICS : MULTILEVEL INTERPRETATION OF CANCER GENOME DATA

06/07/2009 - 08/07/2009

Organisers: Søren Brunak, Núria Malats, Chris Sander and Alfonso Valencia

STEM CELLS AND CANCER

23/02/2009 - 25/02/2009

Organisers: Elaine Fuchs, Maria A. Blasco, Eduard Batlle and Mirna Pérez-Moreno

CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

2008

SIGNALLING UPSTREAM OF mTOR

03/11/2008 - 05/11/2008

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STRUCTURE AND MECHANISMS OF ESSENTIAL COMPLEXES FOR CELL SURVIVAL

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2007

LINKS BETWEEN CANCER, REPLICATION STRESS AND GENOMIC INTEGRITY

05/11/2007 - 07/11/2007

Organisers: Oskar Fernández-Capetillo, Jiri Lukas, Juan Méndez and André Nussenzweig

MYC AND THE TRANSCRIPTIONAL CONTROL OF PROLIFERATION AND ONCOGENESIS

11/06/2007 - 13/06/2007

Organisers: Robert N. Eisenman, Martin Eilers and Javier León

MOLECULAR MECHANISMS IN LYMPHOID NEOPLASM

19/02/2007 - 21/02/2007

Organisers: Elias Campo, Riccardo Dalla-Favera, Elaine S. Jaffe and Miguel Angel Piris

2006

TELOMERES AND TELOMERASE-CNIO / JOSÉF STEINER CANCER CONFERENCE

13/11/2006 - 15/11/2006

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MEDICINAL CHEMISTRY IN ONCOLOGY

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Organisers: Fernando Albericio, James R. Bischoff, Carlos García-Echeverria and Andrew Mortlock

INFLAMMATION AND CANCER

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PTEN AND THE AKT ROUTE

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2005

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2004

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Organisers: Amparo Cano, Hans Clevers, José Palacios and Franz Van Roy

STRUCTURAL BIOLOGY OF CANCER TARGETS

27/09/2004 - 29/09/2004

Organisers: Ernest Laue, Guillermo Montoya and Alfred Wittinghofer

2004
CNIO Cancer Conferences

2003

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01/12/2003 - 03/12/2003

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16/06/2003 - 18/06/2003

Organisers: Juan Carlos Lacal, Channing Der and Shuh Narumiya

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17/03/2003 - 19/03/2003

Organisers: Amancio Carnero and David H. Beach

2003
CNIO Cancer Conferences

2002

MECHANISMS OF INVASION AND METASTASIS

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THE CELL CYCLE AND CANCER

30/09/2002 - 02/10/2002

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CANCER EPIGENETICS : DNA METHYLATION AND CHROMATIN

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CHROMOSOME INSTABILITY AND ANEUPLOIDY IN CANCER

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14/SEP
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deCODE Genetics, Reykjavik, Ireland

21/SEP
Cristóbal Belda
University Hospital Madrid Sanchinarro, Spain

05/OCT
Geoffrey Wahl
The Salk Institute for Biological Studies, La Jolla, USA

19/OCT
Eamonn Maher
University of Birmingham, UK

26/OCT
George Thomas
IDIBELL, Barcelona, Spain

16/NOV
Dan Littman
Skirball Institute of Biomolecular Medicine, New York, USA

23/NOV
Paul Nurse
Cancer Research UK, London, UK

30/NOV
Keith Baggerly
The University of Texas, M. D. Anderson Cancer Center, Houston, USA

14/DEC
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21/DEC
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2013

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18/JAN
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25/JAN
Peer Bork
EMBL, Heidelberg, Germany

01/FEB
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04/FEB
Thomas Jenuwein
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08/FEB
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15/FEB
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08/MAR
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15/MAR
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22/MAR
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05/APR
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12/APR
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29/APR
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17/MAY
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Howard Hughes Medical Institute, Stanford University, Stanford, USA

24/MAY
Bruno Amati
IEOM-IEO Campus, Milan, Italy

31/MAY
John Blenis
Harvard Medical School, Boston, USA

07/JUN
Carl Djerassi
Stanford University, Stanford, USA

14/JUN
Victor Velculescu
The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, USA

21/JUN
Helen Blau
Stanford University School of Medicine, Stanford, USA

24/JUN
Pier Paolo Pandolfi
Beth Israel Deaconess Medical Center, Boston, USA

28/JUN
Luis Paz-Ares
Virgen del Rocío University Hospital, Seville, Spain

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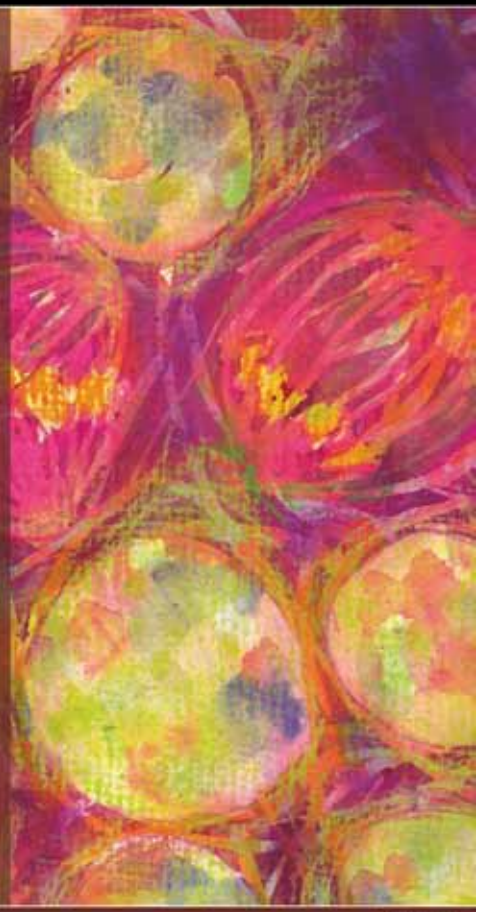

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