DINGO

Dynamique-Instabilité Génétique et Oncogenèse

DNA Damage Response, Innate Immunity & Inflammation

June 21-22, 2018, Amphitheater C. Burg,

Institut Curie, Paris

Acknowledgements

The organizers thank all the speakers for their kind participation and all sponsors for their supports in organizing this meeting.

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Thursday June 21st

14.00 Welcome-Opening session

14.15 Session I *Chairs: P. Pasero & S. Lambert*

14.15 Keynote speaker: Michelle DEBATISSE (30'+ 5')

Respective roles of replication and transcription in common fragile site instability

14.50 Filippo ROSSELLI (20' + 5')

From G1 to G1: how the Fanconi anemia proteins safeguard duplication and transmission of the genetic information from the mother to the daughter cells

15.15 Jean-Sébastien HOFFMANN (20' + 5')

Replication stress modifies the DNA replication Timing program of the next cell generation

15.40 Coffee Break (35')

16.10 Session I *Chairs: A. Constantinou & A. Puisieux*

16.10 Keynote speaker: Jean GAUTIER (30'+ 5')

Mechanisms of MYC-driven genome instability

16.35 Christophe GINESTIER (20' + 5')

Breast cancer stem cells resist to DNA replication stress by promoting homologous recombination

17.00 Angelos CONSTANTINOU (20' + 5')

FANCM anchors to chromatin a pyrimidine catabolism enzyme required to eliminate an endogenous source of interference with DNA replication

Friday June 22nd

9.00 Session II *Chairs: P. Pasero & A. Puisieux*

9.00 Keynote Speaker: Peter McHUGH (30'+ 5')
Role of TRF2 in heterochromatic replication

9.35 Evelyne SEGAL (20' + 5')
Epigenetic regulation of telomerase

10.00 Vincent GELI (20' + 5')
Role of the Set1-Complex during replication stress

10.25 Benoit ARCANGIOLI (20' + 5')
The lysine-specific demethylase, Lsd1, limits segmental duplications between repeated elements

10.50 Coffee Break (40')

11.30 Session III *Chairs: V. Geli & MH Stern*

11.30 Keynote Speaker: Madalena TARSOUNAS (30'+ 5')
BRCA1 and BRCA2 tumour suppressors protect against endogenous acetaldehyde toxicity

12.05 Hélène GAILLARD (20' + 5')
New roles for the yeast Nup84 complex in nucleotide excision repair and replication of damaged DNA

12.30 Pierre-Henri GAILLARD (20' + 5')
SLX4 struggles with SUMO to maintain genome stability

12.55 Lunch 1h35'

14.30 Session VII *Chairs: S. Lambert & B. Lopez*

14.30 Keynote speaker: James LUPSKI (30'+ 5')
Mechanisms underlying structural variant formation in genomic disorders and cancer

15.05 Marc-Henri STERN (20' + 5')
Molecular Signatures of Genome Instabilities

15.30 Jean SOULIER (20' + 5')
Step-wise transformation towards leukemia in Fanconi anemia

15.55-16.15 Conclusion

SPEAKERS ABSTRACTS

Molecular Virology

Monsef Benkirane

Institute of Human Genetics, CNRS & University of Montpellier, Montpellier, FR.

The cross-talks between DNA damage response and the innate immune system

Nelson Ongondo Gekara

The Laboratory for Molecular Infection Medicine Sweden (MIMS),Umeå University, Sweden.

The DNA Damage Response (DDR) that senses threats to our genome, and the immune system - the inherent ability to sense and respond to infections, both function as surveillance systems essential for the preservation of organismal homeostasis. Emerging evidence indicates that these two systems are interdependent. But how these two systems are cross-regulated is less clear. During this session, I will discuss some of our published and unpublished data on the cross-talks between these two biologicals systems and the regulatory molecules involved.

The p21-mTert knock-In mouse: an in vivo model of senescence bypass via the conditional expression of telomerase

Vincent Géli

Marielle BREAU[1], Dmitri CHURIKOV[2], Frederic JOURQUIN[2], Serge BAUWENS[3], Remy CASTELLANO[2], Ignacio FLORES[4], Eric GILSON[3], Serge ADNOT[1], Vincent GELI[2]

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4 Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain.

The cyclin-dependent kinase inhibitor p21Cdk1a is a key regulator of cellular arrest in response to telomere dysfunction and DNA damage. We aimed to create a fine-tuned regulatory loop allowing expression of telomerase that would avoid replicative senescence in cells in which telomeres are damaged. To this purpose, we have created a knock-in mouse model in which a cassette encoding mCherry-2A-mTert (telomerase) has been inserted after the first exon of p21 (p21+/p21-mTert). Strikingly, we show that expression of telomerase driven by the p21 promoter abolishes senescence of pulmonary artery smooth muscle cells that were isolated from lungs and cultured in vitro. The ability of p21-mTert cells to escape senescence in culture correlates with increase telomerase mRNA and the reduced number of very short telomeres that were detected using the TESLA technique recently published the group of J. Shay. Although we cannot exclude a non-telomeric effect of telomerase overexpression, these results suggest that telomerase could act as a repair enzyme that heals damaged telomeres. We further asked whether the p21-dependent expression of telomerase counteracted the development of lung emphysema in mice exposed to hypoxia. P21-mTert expression decreased the number of senescent cells in the lung parenchyma and prevented emphysema in mice experiencing hypoxia. Importantly, we found a weak but positive correlation between the load of short telomeres and emphysema. Collectively, these results indicate that the p21-mTert mouse is protected from lung dysfunction related to cellular senescence. This mouse also exhibits a number of amazing phenotypes that will be discussed.

Genome-embedded ribonucleotides link innate immunity with genome instability.

Andrew Jackson

MRC Human Genetics Unit, the University of Edinburgh, UK.

Aicardi-Goutières syndrome (AGS) is a monogenic inflammatory microcephalic disorder that mimics *in utero* viral infections such as CMV, Rubella and Zika. Three of the AGS disease genes encode Ribonuclease H2, a key genome surveillance enzyme essential for removal of genome-embedded ribonucleotides, the most common aberrant nucleotides (>1,000,000/cell) in the mammalian genome. By investigating the links between genome instability with activation of innate immunity in this condition, this has led us to propose a cell-intrinsic immune surveillance mechanism detecting a range of neoplasia-inducing processes.

Negative regulation of spurious STING activation: impact on tumorigenesis

Nadine Laguette

Institute of Human Genetics, CNRS & University of Montpellier, Montpellier, FR.

Chronic inflammation favors tumorigenesis, negatively influencing patient prognosis. Yet, the underlying molecular mechanisms are poorly understood. In recent years, it has been described that a potent trigger for tumor-associated inflammatory responses are cytosolic nucleic acids. The latter are recognized through the cGAS-STING pathway to sustain chronic inflammation. However, the outcome of this inflammatory response varies depending on several parameters, including the type of nucleic acid involved, the cell type from which the tumor originates and the tumor microenvironment. Furthermore, while activators of STING have been described, no small molecule inhibitor of this pathway has been described as of today. We have identified a pathway that regulates the inflammatory response in the presence of cytosolic nucleic acids. We will discuss the implication of this finding for our current understanding of the STING-cGAS pathway and tumor immunology.

In vivo disruption of the ssDNA/Rad51 filament triggers autoimmune inflammation response and senescence in young mice

Bernard Lopez, Gabriel de Matos Rodrigues, Emmanuelle Martini, and Gabriel Livera.

Institut Gustave Roussy, Villeguif, FR.

Homologous Recombination (HR) is an evolutionary conserved DNA repair process that plays an essential role in genome plasticity. Consistently with the role of HR in genome stability maintenance, the inactivation of many genes involved in HR confer cancer predisposition. The main roles of HR are DNA double strand break repair and the protection and resumption of arrested replication forks. RAD51 is the pivotal actor of HR: it forms the active species of HR, i.e. an ordered filament on resected single strand DNA, ssDNA/RAD51, that performs search of homology and DNA strand exchange with an intact homologous duplex DNA(and which is therefore the actual "enzyme). However, in contrast with yeast, RAD51 is an essential gene in mammals, and its germ line inactivation leads to embryonic lethality in mice (E5-E7). Therefore, the knowledge on the role(s) and impact(s) of the active species of HR, i.e. the ssDNA/RAD51 filament *in vivo* is compromised. To overcome this problem, we generated two transgenic murine models carrying either the wild-type mouse RAD51 (MmRAD51) or a dominant negative form of RAD51 (SMRAD51), expressed under inducible promoters (Dox dependent). Importantly, SMRAD51 specifically poisons the structured ssDNA/RAD51 filament, resulting in inhibition of HR. Ubiquitous expression of the SMRAD51 transgene induces growth defects and death when expressed in young mice, but not in adults. This data is consistent with the working hypothesis that replication dynamics should be affected by HR inactivation. In contrast, the expression of the wild-type MmRAD51 did generate any growth defect or mice death neither in young nor in adult mice.Particularly, the ectopic expression of SMRAD51 (starting 14 days after birth) induced a complete growth arrest and hair loss on young mice exposed to DOX for 12 additional days (injection every 2 days). A prolonged expression of SMRAD51 induced mice death, while arrest of DOX injection allow mice to recover. Analyses of the skin, the most accessible phenotype, reveal hair bubs and fat loss. Moreover, consistent with an inflammatory response in the skin, we observed an increased expression of IL6, ccl2, cxcl1 and IL1ß cytokines and lymphocytes and macrophages invasion after SMRAD51 expression. Preliminary data on other tissue, suggest that the *in vivo* disrution of the ssDNA/RAD51 filament preferentially affect tissues cantaining proliferating cells. In MEFs generated from SMRad51 and MmRad51 embryos, we observed that SMRad51 expression, but not MmRad51, activates a spontaneous DNA damage response cell growth decrease and increased senescence. Herein we have developed a new mouse model that allows to conditionally disrupt the active species of HR, i.e. the ssDNA/RAD51 filament *in vivo*. A deep knowledge on the molecular mechanisms of HR and the consequences of their inactivation *in vivo* should bring new insights into the functions of HR during development and its possible implications in cancer etiology.

Lentiviral Vpr and Vpx impact on host genome integrity

Florence Margottin Goguet

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To evade host immune defenses, HIV-1 and HIV-2 have evolved auxiliary proteins alike Vpr from HIV-1 or Vpx from HIV-2/SIVsmm, which target cell restriction factors and DNA repair proteins. Both Vpr and Vpx act on a similar mode by hijacking the same ubiquitin ligase to inactivate specific proteins. For example, on the one hand, HIV-1 Vpr induces the degradation of HLTF, a DNA translocase that maintains genome integrity during DNA replication. Nonetheless, the role of Vpr-mediated HLTF degradation is presently unknown. On the other hand, HIV-2 Vpx antagonizes SAMHD1, which inhibits synthesis of viral DNA by lowering the nucleotide pool. In addition, we recently identified a new Vpx cellular target involved in the epigenetic silencing of DNA mobile retrotransposons, the HUSH complex. As for SAMHD1 inactivation, Vpx binds HUSH and induces its proteasomal degradation in HIV-2 infected cells, through the recruitment of the DCAF1 ubiquitin ligase adaptor. As a consequence, Vpx is able to reactivate HIV-1 latent proviruses in a model of latency. By revealing an epigenetic regulator as a potential restriction factor counteracted by Vpx, our results provide a molecular link between intrinsic immunity and epigenetic control. They also point to a new mechanism by which the virus may impact host genomic expression integrity.

Connecting the repair of therapy-induced DNA damage and interferon response

Peter McHugh

Department of Oncology, Institute of Molecular Medicine, University of Oxford, UK.

Radiotherapy and chemotherapy are effective treatment methods for many types of cancer, but tumour resistance is common. We have recently discovered that these DNA damaging forms of cancer therapy lead to the release of single-stranded DNA (ssDNA) fragments from the cell nucleus into the cytosol of cancer cells, engaging this innate immune response.
Furthermore, anti-viral type I interferon (IFN) signaling is induced by these treatments in cancer cells. The expression of a set of interferon-stimulated genes that comprises the 'IFN-related DNA damage resistance signature' (IRDS) correlates strongly with resistance to radiotherapy and chemotherapy across many tumour types. While classically, during viral infection, the presence of foreign DNA in the cytoplasm of host cells initiates type-I IFN production as part of the innate immune system, how self DNA is released from the nucleus of cancer cells during therapy to induce IFN signaling remains poorly understood, although our work has revealed a role for a number of enzymes required for DNA end-resection. This work suggests that interplay with the immune and inflammatory systems during DNA damaging therapy is critical for successful of cancer treatment. While revealing the mechanistic basis of this, we also hope to identify new targets and key biomarkers for therapeutic response.

A novel mechanism linking fork processing to inflammation

Philippe Pasero

Institute of Human Genetics, CNRS & University of Montpellier, Montpellier, FR.

SAMHD1 was previously characterized as a dNTPase that protects cells from viral infections. Mutations in SAMHD1 are implicated in cancer development and in a severe congenital inflammatory disease known as Aicardi–Goutières syndrome. The mechanism by which SAMHD1 protects against cancer and chronic inflammation is unknown. Here we show that SAMHD1 promotes degradation of nascent DNA at stalled replication forks in human cell lines by stimulating the exonuclease activity of MRE11. This function activates the ATR–CHK1 checkpoint and allows the forks to restart replication. In SAMHD1-depleted cells, single-stranded DNA fragments are released from stalled forks and accumulate in the cytosol, where they activate the cGAS–STING pathway to induce expression of pro-inflammatory type I interferons. SAMHD1 is thus an important player in the replication stress response, which prevents chronic inflammation by limiting the release of single-stranded DNA from stalled replication forks.

Identification of novel signaling pathway involved in IR-elicited macrophage reprogramming

Dr. Jean-Luc PERFETTINI[1-4]

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The efficacy of conventional anticancer treatments (such as chemotherapy and radiotherapy), targeted drugs, anti-angiogenic agents and immune checkpoint blockers partly depends on several biological functions exerted by tumor-associated macrophages. Although targeting macrophage immune functions has recently emerged as the greatest promise to improve anticancer treatments, the molecular mechanisms involved in the ionizing radiation-mediated activation of macrophages remain elusive. Recently, we identified a novel signalling pathway that involves DNA damage responses (DDR) and is required for ionizing radiation-elicited macrophage activation. In this context, we proposed that the therapeutic modulation of macrophage-specific DDR should help for the improvement of radiotherapy effectiveness through the re-programming of tumor infiltrating macrophages.

NLRP3, a stress sensor at the interface between immunity and genome integrity.

Virginie Petrilli

Centre de Recherche en Cancérologie de Lyon U1052 UMR5286.

The DNA damage response (DDR) is essential to preserve genomic integrity and acts as an anti-cancer barrier. The ATM pathway orchestrates the cellular response to DNA double strand breaks (DSB), and its attenuation has been reported during tumorigenesis. I will show that NLRP3, a pattern recognition receptor involved in the formation of the inflammasome complex, is a crucial actor of the DDR to DNA DSB, independently of its inflammasome activity. In addition, our results suggest that *NLRP3* operates as a lung tumor suppressor gene.

SLFN11 blocks stressed replication forks independently of ATR

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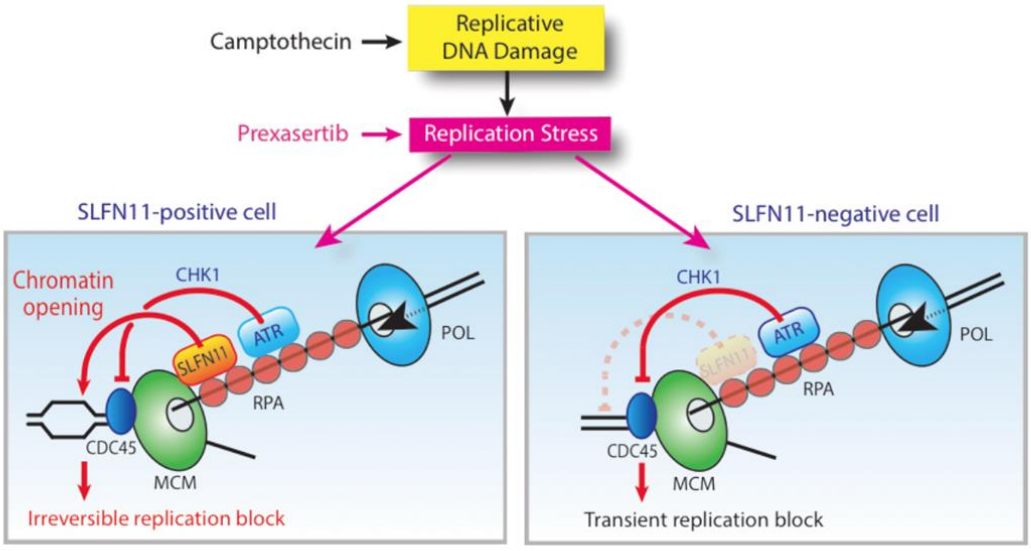
SLFN11, an interferon inducible gene, sensitizes cancer cells to a broad range of widely used DNA-targeted therapies that damage DNA replication: topoisomerase inhibitors (camptothecin, topotecan, irinotecan, etoposide, doxorubicin), alkylating agents (temozolomide, cisplatin, carboplatin), DNA synthesis inhibitors (hydroxyurea and gemcitabine) and PARP inhibitors (olaparib, niraparib,rucaparib and talazoparib). Here we show that, in response to replication stress by camptothecin, SLFN11 tightly binds chromatin via RPA1, interacts with the replication helicase component MCM3, and blocks replication persistently. Unlike ATR, SLFN11 neither interferes with the loading of CDC45 and PCNA nor inhibits the initiation of DNA replication but selectively blocks fork progression. Whole-genome replication origin mapping (nascent strand DNAseq) and accessible-chromatin mapping (ATAC-seq) reveal that SLFN11 induces chromatin opening near replication initiation sites in response to replication stress. The ATPase domain of SLFN11 is required for chromatin opening, replication block and cell death but not for chromatin binding. Induction of replication stress by the CHK1 inhibitor praxesertib also recruits SLFN11 to nascent DNA where CDC45 and PCNA accumulate. We conclude that SLFN11 is recruited to stressed replication forks carrying extended RPA filaments where it blocks replication by opening chromatin.

Model for replication block by SLFN11



SLFN11 binds to stressed replication forks through RPA, and arrests replication by blocking the replicative helicase complex

In the absence of SLFN11 (≃ 50% cancer cell lines: HeLa, U2OS, HCT116, RKO, MCF7, MDA-MB231…), ATR-CHK1 transiently arrests replication to allow DNA repair

Murai, J.…Pommier, Y. 2018 Mol Cell

Epigenetic regulation of heterochromatin in myeloid cells

Derek Rookhuizen

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Several lines of evidence suggest that cyclic GMP-AMP synthase (cGAS), a known sensor of viral DNA, has endogenous ligands. Ablation of *cGAS* in *Trex1 (DNAseIII)*-defective mice, a model for a human interferonopathy, Aicardi-Goutières syndrome, attenuates the severity of this auto-inflammatory disease, linking cGAS to endogenous DNA ligands (Gao D, 2013; Gray et al., 2015). Previous studies in *Trex1*$^{-/-}$ mice, suggest that transposable element (TE)-derived DNA may drive inflammation (Stetson et al., 2008). However, it is still unknown if TE-derived DNA may bind to cGAS and how sensing of endogenous DNA is restricted in normal conditions. Here we show that during inflammatory responses in myeloid cells, members of the HP1 heterochromatin pathway are rapidly modulated to derepress the expression of TE transcription and increase cytosolic levels of TE-derived DNA. Genetic inactivation of the histone methyltransferase *Suv39h1* further increases the amount of TE-derived cytosolic DNA and exacerbates the cGAS-dependent production of type I interferon. Immunoprecipitation of cGAS-binding DNA identifies specific TE families as endogenous ligands for cytosolic DNA sensing. We conclude that epigenetic de-repression of TE expression directly impacts on innate sensing of TE-DNA by cytosolic cGAS.

Thrombopoietin, interferon signaling and hematopoietic stem cell genomic stability

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Blood cell homeostasis is critically dependent on the activity of hematopoietic stem cells (HSCs) that continuously replenish the hematopoietic system, including the HSC compartment itself. Maintenance of HSC integrity throughout life is therefore crucial. HSCs are particularly sensitive to low-dose irradiation. Long time after irradiation HSCs display DNA damage, decreased repopulating ability, and skewing of their differentiation potential towards the production of myeloid cells at the expense of lymphoid lineages. These alterations are similar to those observed with aging. They promote the onset of a variety of hematopoietic malignancies. In addition, the reduced ability of HSCs to generate immune-competent B and T lymphocytes is a critical factor involved in the decline in adaptive immune function which likely contributes to many disorders through enhanced infections and inflammatory response. Thus, understanding the molecular mechanisms controlling HSC genomic stability is necessary to modulate these adverse effects.

The HSC fate is regulated by the bone marrow niche environment and by a variety of growth factors and interactions that tune the balance between quiescence, proliferation and differentiation. Much remains to be elucidated on how cytokine/environmental signals integrate the DNA damage responses in HSCs and regulate the long-term residual HSC defects and the risk of transformation following radiotherapy. One of this niche factor, thrombopoietin (TPO), is a master regulator of HSC self-renewal, being required for the maintenance a platelet/myeloid-primed HSC population at the apex of the HSC hierarchy. We have shown that TPO directly controls the HSC intrinsic DNA repair machinery by increasing DNA-PK-dependent Non Homologous End-Joining-mediated repair efficiency, thus ensuring HSC chromosomal integrity and limiting residual injury after exposure to DNA damaging agents. We also found that TPO triggers and anti-viral innate immune gene response, specifically in HSCs. This is due to TPO ability to activate both STAT1 and STAT2 transcription factors and mimic IFN-type I signaling. TPO-induced IFN signaling is required to restrain retrotransposon expression and mobilization in HSCs. HSCs express unexpectedly high levels of many retrotransposons, including recent LINE-1 family members. Their expression and mobilization further increase upon irradiation. Using reverse transcriptase inhibitors we demonstrated that LINE-1 retrotransposition is involved in irradiation-induced persistent DNA damage and HSC loss of function. These data reinforce the links between DNA damage, retrotransposons and anti-viral immunity. They show that IFN signaling can also have protective functions and may explain why chronic IFN-I treatment and STAT1/2 induction were shown to induce chemotherapy resistance in certain cancers.

POSTERS
ABSTRACTS